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### The species status of the viviparous sea snakes

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Sphenodon punctatus  
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# Book of Abstracts **Oral Presentations**

**WCH9**   
**9<sup>TH</sup> WORLD CONGRESS OF HERPETOLOGY**  
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**Victory against all odds: the successful conservation story of the Admirable Red-Belly Toad in Brazil**

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The Admirable Red-Belly Toad (*Melanophryniscus admirabilis*) is a microendemic species and one of the most threatened amphibians in Brazil. The only known site, in the southern Brazilian Atlantic Forest, is embedded in a small forest fragment surrounded by a heavily deforested agricultural landscape. Our work in the region was initially motivated by concern about the true geographic range and abundance of the Admirable Red-Belly Toad, which grew into a sense of conservation urgency, due to the very small size of the very threatened known population. Since 2010, we have studied its natural history, biology, ecology and genetics, aiming to its conservation. At that time, there was also a plan to build a small hydroelectric power plant upstream of the site. Through a multi-institutional effort, we managed to revert the government authorization to build the plant and, in the process, evaluated the risk category of Admirable Red-Belly Toad as Critically Endangered in global and regional red lists. It was the first time in Brazilian history that an amphibian prevented the construction of a big enterprise. Nevertheless, there are still many threats to the species. Currently, to ensure the maintenance of the population, our challenges are to create a protected area and to apply a long-term project involving local communities to replace their agro-chemical-intensive monocultures of tobacco and soybean with the production of native (and locally much consumed) Yerba-Mate in agroforestry systems. We believe this change in local production will benefit both local people and the entire population of the species.

### **The challenges of conservation management for the Nationally Critical Chesterfield skink**

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The Nationally Critical Chesterfield skink is currently known from a single coastal site <1 ha in extent north of Hokitika on the South Island's West Coast. The species was already in a critical state when it was discovered in the 1990s. Taxonomic uncertainty (now resolved) delayed management and further habitat was lost in the intervening period. A research programme began in 2016 aiming to understand the species' life history traits and threats to its persistence and to experimentally test predator exclusion fencing as a recovery strategy. Whilst agents of decline are not clearly identified, observations of injuries suggest rodent predation may be a factor in their decline along with habitat degradation. Early in 2018 the population was estimated to be only 200 individuals. In 2018 the destructive Cyclone Fehi resulted in the loss of c. 1/3 of the coastal buffering habitat and the whole area was extensively washed over and damaged.

As a result, the programme underwent a major change in direction. A captive population was established for insurance and the proposed experimental fence was abandoned due to the vulnerability to storm damage. A predator exclusion fence within the known historical range, away from storm damage is recommended, but options are limited on this privately-owned land. The wild population remains at risk from storm events and the species' future is still precarious with extinction a real possibility.

**Microclimate, more than phylogeny, drives interspecific variation in heat tolerance within a subtropical anuran community.**

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Studies on species responses to climate change have relied primarily on coarse-scale climatic data (e.g., > 1-km<sup>2</sup>), but individual-level processes determining some of these responses occur in the domain of the microclimate, i.e., the climate in spatiotemporal scales relevant to individuals. Herein we investigated the interspecific variation in heat tolerance within an anuran community in a small area (~ 1-km<sup>2</sup>) of a subtropical forest ecosystem, the Brazilian Atlantic Forest. We collected tadpoles of seven anuran species in a set of waterbodies differing in physical structure, temporality and vegetation cover, and measured their Critical Thermal Maximum (CTmax) in the field. Under an information-theoretical framework, we analyzed the extent to which species' CTmax was related to microclimatic or macroclimatic temperatures and phylogeny. We found that microclimates experienced by tadpoles differ considerably from their common macroclimate: waterbodies in open areas amplified thermal extremes, whereas waterbodies inside the forest were more thermally stable and buffered macroclimatic conditions. Furthermore, species' CTmax was tightly related to the microclimatic maximum temperature (Tmax) and less to the macroclimate or phylogeny. Thus, the most heat-tolerant species occupy open temporary ponds that reached the highest Tmax, whereas the less heat-tolerant species inhabit permanent ponds and streams inside the forest. Since the environmental and physiological variation within this anuran community is compatible with the previously reported across macroecological gradients, we highlight the role of the microclimate in shaping physiological tolerances in anuran larvae.

**Anuran larvae exhibit lower upper thermal limits and warming tolerance than a natural predator.**

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Warming tolerance (WT), the difference between the Critical Thermal Maximum (CT<sub>max</sub>) and habitat temperature (Thab), is a widely used metric to estimate individual vulnerability to acute warming. However, a conceptual expansion of WT to the population level requires more than averaging individual values, and certainly should consider inter-individual variation in both CT<sub>max</sub> and microclimate exposure. Also, the biotic environment, a critical aspect affecting population dynamics, remains overlooked in assessments of species' vulnerability to warming. Herein we tested the hypothesis that average CT<sub>max</sub> of two anuran species is lower than that of a natural predator (dragonfly naiads), and inter-individual variation in CT<sub>max</sub> differ among taxa. We collected both tadpoles and dragonfly naiads in the same pond, estimated their CT<sub>max</sub> in the field using two experimental heating rates (0.1 and 1°C/min), and measured Thab both at macroclimatic (local weather station) and microclimatic scales (HOBO dataloggers). The CT<sub>max</sub>, and consequently WT, of *Dendropsophus minutus* and *Rhinella icterica* was in average more than 2°C lower than in dragonfly naiads. Also, the inter-individual variation in CT<sub>max</sub> and WT of *D. minutus* and dragonfly naiads were almost two-fold that of *R. icterica*. Furthermore, WT values were overestimated by about 8.6°C when using the macroclimatic Thab. Finally, heating rate effects were quite small on averages CT<sub>max</sub> and WT and did not affect their variances. Overall, larvae of both anuran species are more vulnerable to an acute warming than their predator, which could put them at greater predation risk as microclimatic temperatures approach their CT<sub>max</sub>.

## First report of extracranial calcified endolymph in *Liopholis* skinks (Scincidae) and its relationship with burrowing

**AGUILAR R<sup>1,2</sup>**

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Calcified endolymph (CE) is a crystallised form of calcium carbonate found in the endolymphatic sacs within the braincase of many squamates. In some species of geckos, agamids and iguanians the CE is extensively developed, expanding outside of the cranium and into the nuchal region. Among geckos where it is considered the plesiomorphic condition, expanded CE is thought to act as a reservoir of calcium for egg shell deposition, although it has also been found in male lizards (*Anolis*), juveniles (*Eurydactyloides*), species laying parchment-shelled eggs (iguanians), and live-bearers (*Cophotis*). Alternative hypotheses include associations with orientation and equilibrium in arboreal lizards and a source of calcium for yolk production and skeletal growth, suggesting independent functions in different groups. Here we report for first time the presence of extracranial CE in *Liopholis* skinks (Scincidae), based on X-ray computed tomography (CT) scans of 87 fluid-preserved specimens. CE was present in 6 of the 22 sampled species, with 3 exhibiting intraspecific variability with no consistent ontogenetic pattern. We found no relationship between the volume of CE and individual snout-vent length. However, using a published molecular phylogeny of skinks, we found a significant evolutionary relationship between burrowing and the extent of CE. This suggests a functional, possibly protective role in fossorial or semi-fossorial species. Broader sampling needed to further test this association is currently being conducted in the scope of a Monash/Museums Victoria Linkage Project aiming to CT scan every species of Australian lizard.

**Species Delimitation, Phylogenetic Relationship and Biogeography of *Hemiphyllodactylus* (Reptilia: Gekkonidae) in Yunnan Karsts**

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Karsts outcrops provide a unique habitat to many animals and plants due to the specialist requirements, making karsts a hotspot for endemism and biodiversity. Recent studies in the karstic area in South China has shown that China has a lot more hidden species. However, Chinese karsts have undergone unprecedented overexploitation caused by quarrying and tourism. In order to develop appropriate protection, a clearer understanding of karst diversity patterns is needed to guide conservation efforts. Geckos show high levels of endemism and recently many new species have been described from karst ecosystems. *Hemiphyllodactylus* is a genus of the Gekkonidae family that consists of lowland and upland species. The upland species are restricted to mainland Asia which does not have any lowland species. Given the discontinuous and patchy karsts in Yunnan, South China, as well as structural and microclimate differences, it is likely that the region has fragmented populations of *Hemiphyllodactylus*. This fragmentation leads to isolation and lack of gene flow between populations leading to speciation. In this study, we use *Hemiphyllodactylus* as a model to understand the value of karst ecosystems in the Yunnan province. We incorporated biogeographic patterns, genetic divergence, and evolutionary history of *Hemiphyllodactylus* geckos. The preliminary analysis on our initial datasets showing that Yunnan karsts potentially harbor up to 9 undescribed new species based on phylogenetic analysis. We report on the diversity of this group from the karst system in the Yunnan province and how this information can be used to inform conservation strategies for this ecosystem.



### Sex Chromosome Homologies across Dragons (Squamata:Agamidae)

Altmanova M<sup>2</sup>, Gamble T<sup>4</sup>, Kratochvil L<sup>2</sup>, Prasongmaneerut T<sup>3</sup>, Srikulnath K<sup>3</sup>, Alam S<sup>1</sup>, Georges A<sup>1</sup>, Gleeson D<sup>1</sup>, Sarre S<sup>1</sup>, Ezaz T<sup>1</sup>

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There are about 480 species Agamid lizards (Squamata: Agamidae) under six sub-families across Asia, Australasia and Africa. These lizards are mostly oviparous with diverse sex-determination mechanisms (SDM) including genotypic (GSD), temperature dependent sex determination (TSD) and GSD with temperature overrides, as well as parthenogenesis. In terms of sex determination, most of the studies have focused particularly on the species under one sub-family, i.e. Australian species from the sub-family Amphibolurinae and almost nothing is known about the other agamids from other sub-families. Agamid lizards (Squamata: Agamidae) are karyotypically heterogeneous. About one quarter of the agamid species, worldwide, have been karyotyped, which makes them a well-studied group in this respect. However, heteromorphic sex chromosomes have been identified in only five of those karyotyped species, most of which have microchromosomes as cryptic sex chromosomes, while only one species reported to have macrochromosomes as sex chromosomes, and all 5 species have female heterogamety (ZZ/ZW). No XX/XY system has yet been discovered in these lizards and there is not much evidences about transitions between GSD and TSD and among GSD systems (ZW↔ZW).

Here, we examined representative species across the family, at sub-family level. We have conducted a comparative analysis of sex-chromosomes homologies across species, both GSD and TSD as well as of unknown SDM, with *Pogona vitticeps* ZW sex-chromosomes using two BAC clones as hybridization probes in fluorescence in situ hybridization (FISH) to metaphase chromosomes. The results show high conserved synteny across Australian clades and varied levels of syntenies across the Agamid sub-families.

**Seasonal dynamics and calling behaviour of the terrestrial-breeding microhylid *Cophixalus australis***

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We studied the seasonal dynamics and the calling behaviour of male *Cophixalus australis* at a site near Paluma, Queensland, between 1993 and 2014. We used timed recorders to monitor calling behaviour every night at multiple locations during the extended calling season in 1995-96 and the calling seasons between late 2002 and early 2006. We monitored calling behaviour, body size and condition, and the spatial distributions of individual males by visual encounter surveys in those seasons and in 1993-94 and 2013-2014. Calling occurred between August and late March. Males called most nights except during some dry periods or cold conditions. The timing of onset of the calling season varied substantially among years, but the cessation of calling occurred remarkably constantly in mid March. Males called from stereotypical calling perches, usually between 1-2 meters above ground. Calling perches were often occupied on many nights by the same individual, and in some cases the same individual returned to a calling perch across years. Perches were spatially clustered on multiple scales; most were in small local clusters of a few individuals interacting vocally, these in turn were clumped on a larger scale. Males responded to the calls of nearby males by altering the timing of calls to intersperse them. Individual male body condition remained constant or declined slowly over the season, and it appeared that males that commenced calling later were on average smaller. The number and distribution of males remained relatively stable over the period of the study.

## Using social media to promote positive outcomes for amphibians and reptiles

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Social media is a powerful tool to communicate information to large audiences rapidly. However social media can also distract young people from exploring the natural world or getting involved with activities such as hands-on fieldwork. Studies have also linked the over-use of social media to the increased likelihood of mental health problems. By removing direct connections with nature and people, the misuse of social media can contribute to increased anxiety. Equally, without review or regulation it is easy to disseminate misinformation to the masses.

Despite this, social media can also act as a powerful tool for conservationists. Within conservation, too much 'bad news' can cause fatigue with target audiences. It is therefore important to convey positive messages in order to keep audiences engaged. As researchers working with species that are often overlooked by the media, more promotion of good news through social media may improve engagement in herpetological conservation.

Social media can be used to help increase awareness of a situation, deliver relevant information and enhance understanding. By targeting the right audiences (such as young researchers and interested amateurs) social media can also be used to drive behaviour change (e.g. ZSL's #OneLess campaign). There has never been a more vital time to signpost audiences to information on how they can help and what can they do to help prevent global herpetofaunal declines.

**Nest-site selection and hatchling success of box turtles (*Terrapene carolina*) at their northern range limit.**

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<sup>1</sup>Grand Valley State University, <sup>2</sup>University of Otago, <sup>3</sup>USFS

We examined the reproductive behavior and ecology of eastern box turtles (*Terrapene carolina carolina*) at the species' northern range limit in Michigan, USA. Our objectives were to determine which microhabitat factors influence nest-site selection by female box turtles and how the selected microhabitat and surrounding environment affect hatchling success. In June 2013–2016 we collected environmental data from box turtle nest sites within four forest openings. Once hatchlings emerged from nests we collected data through radio-telemetry to create known-fate survival estimates through the first year of life. We used logistic regression to explore relationships between the microhabitat surrounding nests and random control sites and to compare microhabitat variables from successful and unsuccessful nests. We used Kaplan-Meier estimators modified for staggered-entry to create known-fate annual survival estimates for hatchling turtles. Female box turtles selected nest sites with a higher percentage of bare soil and avoided nesting on north facing slopes. The success rate of the observed nests was 50% and the probability of nest success increased with larger clutch sizes and lower percentages of bare soil at selected nests sites. Hatchling survival decreased through the first year of life until reaching 0% survival short of the 1-year mark (day 335 = 0.0). We identified depredation and suboptimal environmental conditions as the main sources of hatchling mortality. Our study highlights the complex relationship between the microhabitat at nest-sites and hatchling survival and sheds light on the precarious position eastern box turtles occupy as the threat of climate change increases.

## Understanding amphibian declines in complex environments

Alton L<sup>1</sup>

<sup>1</sup>*Monash University*

Around the world, hundreds of amphibian populations have suffered rapid declines despite the availability of suitable habitat. Before we understood that a pathogenic chytrid fungus was the proximate cause of these mysterious declines, researchers also entertained the idea that increases in ultraviolet-B radiation (UVBR) due to ozone depletion might be a contributing factor. We now know that exposure to UVBR is detrimental to amphibian health, causing mortality, reduced growth, slowed development rate, developmental malformations, reduced locomotor performance, immunosuppression, and changes in metabolic rate and behaviour. However, studies that examine environmental factors in isolation of others risk under- or overestimating their impact on wildlife since factors can interact either synergistically or antagonistically, respectively. To better understand how UVBR might affect amphibians in the natural world, we have examined the responses of a model amphibian species to UVBR in combination with a range of other environmental factors, including temperature, aquatic oxygen, conspecific density and predation. I will present examples of our research showing how these naturally-occurring environmental challenges alter the impact of UVBR on amphibians. I will also discuss how the interaction between UVBR and temperature may influence disease susceptibility in amphibians. Our research demonstrates that consideration of additional environmental factors together with anthropogenic factors is vital for understanding the contribution of human-induced environmental change to biodiversity loss.

### **Linking physiological and behavioural variation to territory thermal quality and fitness in an African lizard**

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With global climate change, the environmental conditions experienced by natural populations are changing at an unprecedented rate. Much emphasis has been given to developing correlative and mechanistic models to predict species' responses to temperature change. However, most studies include coarse scale environmental data (>1 km), which are generally not ecologically relevant to small ectotherms, such as lizards. These mismatches between the resolution of climate data and lizard ecology (body size, dispersal and home range) can lead to erroneous conclusions about the species' vulnerability to climate change. At fine-scales, the thermal quality of heterogeneous habitats can vary greatly due to differences in soil properties, configuration of the vegetation, and the distribution of rock formations. Thus, there is often substantial variation among individual territories, which has the potential to shape behavioural and physiological responses, and ultimately fitness. However, behaviour and physiology can also drive the habitat quality experienced by individuals. Therefore, it is often difficult to pinpoint the cascade of effects that underlie climate (or operative temperature) as an agent of selection of lizard performance. In this study, we explored the relationships between individual physiological performance, thermoregulatory behaviour, territory thermal quality and fitness of the southern rock agama (*Agama atra*), a lizard endemic to southern Africa, using an array of laboratory and field techniques. By linking intraspecific variation in physiology and behaviour with the spatial heterogeneity of environments and fitness, we aim to reveal mechanisms underlying lizard performance and enable more accurate predictions of responses of organisms to environmental change.

## Camera traps for early detection and rapid response and management of an invasive reptile

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Camera traps are heralded as labor-saving and cost-effective tools for monitoring wildlife, and have found growing application in monitoring invasive species. Camera traps may be useful both in establishing species occupancy on the invasion front and providing information on established populations. The use of camera traps for ectothermic animals that do not trigger camera sensors presents unique challenges. Brown treesnakes were introduced to Guam in the 1940s and substantially negatively impacted the island's native biodiversity. We used data from remotely triggered camera traps facing baited traps to examine the efficacy of camera trapping for detecting snakes and estimating density in a population of known size. Brown treesnakes are not uniquely identifiable in photographs, limiting our analysis to models for data on unmarked animals. We found that cameras "capture" (i.e., detect) snakes 26% more often than traps capture snakes. However, we found that 3 different models for estimating density using unmarked individuals performed poorly, with inaccurate or imprecise density estimates. Estimating density from unmarked animals is challenging, and these data violated several standard assumptions, including the assumption that traps do not change animal behavior. However, baited camera traps may be useful for EDRR on islands at risk of invasion or with credible reports of snakes. While the use of baited camera traps may be useful for establishing snake occupancy, a combination of surveying methods are likely needed to estimate population-level information. Simulation studies can help identify how different monitoring designs will perform in terms of cost and accuracy of estimates.

## Decision analysis for early detection and rapid response: modeling to advance identification of optimal management

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Management of invasive reptiles, especially in an early detection and rapid response (EDRR) context, is an incredibly challenging problem for decision makers. Stakes are high, uncertainty is substantial, delay can have serious consequences, and financial resources are limited. In these settings, managers need a framework to identify and organize the information required to make the best decisions possible. Decision analysis provides a powerful framework for organizing information in these settings and for informing the structure of modeling tools needed to make decisions. We will introduce the concept of decision analysis and demonstrate how it can be useful for identifying optimal management approaches for invasive reptiles. Our case study showcases the development of a model for the identification of an optimal removal schedule that considers the logistical and financial resources required to eradicate an invasive reptile. We applied this framework, post hoc, to a successful eradication of veiled chameleons (*Chamaeleo calyptratus*) on the island of Maui, Hawai'i, and demonstrated how different removal strategies have substantially different costs and success probabilities. Managers of virtually every EDRR effort must grapple with how to minimize costs in the process of responding to the threat of an invasive reptile, and decision analysis can provide the cognitive map required to help managers make the best use of the resources they have available to them. We anticipate that a decision-analytic framework will prove useful for future EDRR and are currently evaluating similar approaches for localized eradication efforts of brown treesnakes (*Boiga irregularis*) in Guam.



### **The importance of scale in amphibian conservation: species responses to fragmentation**

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Conservation of amphibians in a fragmented landscape is complicated by the importance of scale in understanding species' responses. Patch size may result in differences in amphibian occupancy at the level of the overall patch, sites within patches, and substantial variation may exist by taxa and species within taxa. We find that while overall species richness is positively associated with increasing patch size in small vertebrate communities in Southern California, amphibians were sparser within patches likely due to their habitat specificity. Life history traits such as high fecundity and location within the species' climate envelope also influenced amphibian sensitivity to change in patch size, highlighting the complexity of conserving land parcels based solely on overall size. By considering multiple scales of amphibian response to fragmentation, we can identify parcels where target species of conservation occur, the size and complexity of habitat required to contain these species, and which life history traits and range characteristics make them sensitive to fragmentation. Additionally, parcel prioritization should consider the goal of maintaining connectivity and complexity of habitats, and key areas can be highlighted for potentially maximized conservation gains. Managers can then better weigh the economic cost of purchasing parcels based not only on acquisition cost and the resulting patch size, but in light of target species' ecological needs, potential mutual benefit to multiple species, and projected changes to habitats. This will allow for decision optimization and risk assessment of land parcel acquisition under multiple conservation scenarios.

## **Integrative taxonomy of the rock gecko *Cnemaspis siamensis* species complex (Squamata: Gekkonidae) in southern Thailand**

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Southern Thailand is located in a global biodiversity hotspot and serves as the transition zone between the Indochinese and Sundaic biotas. However, the diversity of reptiles in this region, especially geckos remains underestimated due to limitation of surveys. In this study, the diversity of the Rock gecko, the *Cnemaspis siamensis* complex was investigated using morphological and molecular approaches. The morphological and genetic analyses (NADH dehydrogenase subunit 2) revealed hidden diversity within the species complex, containing at least 11 species in Thailand. High morphological similarity was found between the member of *C. siamensis* group whereas the genetic divergence between species of the *C. siamensis* complex is high (17.3–22.4%). The findings in this study suggests that the *C. siamensis* complex from mainland and islands of southern Thailand contains unrecognized species. Differences in microhabitat preferences (karst formation, granitic rocky stream, and vegetation) were found in this species complex. Further research and additional field surveys in unexplored regions are needed to better understand the taxonomy, ecology, distribution, biogeography, and conservation of the *C. siamensis* complex in southern Thailand.

## Present and recent past habitat suitability ranges of northeast Asian treefrogs using maximum entropy models

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Habitat suitability models are used to delineate species distributions, discern species' ecological requirements, and test hypotheses related to niche divergence. In this study, we examine the relationships in ecological niches among the Hylid Treefrog Dryophytes in northeast Asia. Within the Dryophytes genus, three species occur in northeast Asia: *D. japonicus*, *D. immaculatus*, and *D. suweonensis*. *Dryophytes japonicus* has been separated into two species-level clades through phylogenetics, while *D. immaculatus* and *D. suweonensis* have been hypothesized to be synonymous. In this study, we use maximum entropy models to identify current and past distributions for the two clades of *D. japonicus*, *D. immaculatus* and *D. suweonensis* over four time periods: current, Holocene, last glacial maximum, and last interglacial. Models for each clade were trained using current climate and occurrence points then projected to past climates. We then used the nicheOverlap function in the "dismo" R package to test for overlap between *D. immaculatus* and *D. suweonensis*, and between the two clades of *D. japonicus*. Our results show that the current overlap statistics support the existence of four distinct groups based on ecological niches relating to bioclimatic variables. Additionally, the two clades of *D. japonicus* exhibit consistently separate distributions and low levels of niche overlap throughout the past 130 thousand years, supporting an earlier divergence. The results also support greater overlap in suitable area for *D. immaculatus* and *D. suweonensis* during the last interglacial period, which may explain the controversy on the divergence time or latest contact and admixture of the two clades.

**Is ecophysiological variation in lizards better explained by proximate or distal environmental predictors?**

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The mechanisms underlying species distribution are deeply influenced by abiotic factors, mainly due to the interaction between environment and physiology. The environment imposes restrictions which alter ecological functions and physiological tolerance, potentially affecting species' geographic range. Thus, an understanding of the climatic drivers underlying ecophysiological adaptations may reveal ecological limits for species distributions. Although ecophysiological traits often correlate with climate, they ultimately reflect the outcome of organisms interacting with microclimates and their biotic communities. Macrophysiological patterns may reflect direct responses to the physical environment or indirect responses mediated by interspecific interactions. In the former case we might proximate environmental predictors to be most strongly correlated whereas in the latter case coarse climatic predictors may be just as potent explanatory variables. Here, we undertook a comparative analysis of global patterns of ecophysiological variation in lizards across sets of environmental predictors representing macroclimatic, microclimatic and biophysical processes, i.e. ranging from distal to proximate. We found that water- and temperature-related metrics strongly predict lizard physiology, including water loss rates, metabolic rates and thermal physiology. However, we also found that distal, macroclimatic predictors explain similar amounts of ecophysiological variation to proximate, microclimatic or biophysical predictors. Our findings suggest that selective forces acting on ecophysiological traits are not necessarily simple responses to environmental extremes, but likely reflect more indirect causal pathways involving climate-mediated behavior, habitat structure and biotic interactions. Overall, we demonstrate that wider characterizations of lizards' physiology, extending beyond a focus on temperature, is essential to understand the mechanisms underpinning their ecology and distribution.

### **Spatiotemporal variability of paedomorphosis in *Ambystoma talpoideum* across two metapopulations**

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The expression of discrete breeding phenotypes in adults of some pond-breeding salamanders, termed facultative paedomorphosis, has been a model system in evolutionary ecology. While numerous studies have explored the mechanisms that influence phenotypic expression, investigations of how paedomorphosis varies across different spatial and temporal extents in natural populations, as well as the in-situ drivers, have been limited. We examined spatiotemporal variation in paedomorphosis in *Ambystoma talpoideum* (mole salamander) across two separate metapopulations separated by 120 km over a 13-year period. Using minnow traps, we surveyed 19 populations of *A. talpoideum* in Kentucky (KY), USA from 2011-2018, and 18 population in Tennessee (TN), USA from 2006-2015 to estimate the abundance, and spatial and temporal variability (coefficient of variation of abundance across ponds or years) of paedomorphs and overwintering, immature larvae in each pond and year. Overall populations sizes were larger and the prevalence of paedomorphs greater in TN than in KY. Across all ponds and years, over 1000 paedomorphs were captured in TN, whereas only 135 paedomorphs were captured in KY. In contrast, overwintering in an immature larval state was much more prevalent in KY; > 1200 individuals and 90% of all captures in KY were overwintering immature individuals, as opposed to 70% of all captures in TN. In TN, temporal variability in abundance of both life stages was slightly greater than spatial variability (CV across ponds). In KY, spatial and temporal variability were approximately equal, but variability in paedomorphosis was greater than the variability in overwintering as a larva.

### Impacts of increasing phenological variability on salamander demography and pond community dynamics

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Phenological shifts are expected to affect species interactions, in part by influencing which size classes, life stages, or species occur simultaneously in a community. Yet, how changes in phenology beyond shifts to the first, mean or median date of an ontogenetic event are underexplored in their importance to population and community dynamics. We experimentally manipulated variability in breeding phenology of two different pond-breeding salamanders (*Ambystoma annulatum* and *Ambystoma opacum*) to assess its impact on demographic traits and survival. We also tested whether breeding variability would impact lower trophic levels (intermediate salamander predators, anuran tadpoles, zooplankton and periphyton). We used outdoor mesocosms that simulated pond communities, set up across two years for each of our predator species (2017-2018 = *A. annulatum*; 2018-2019 = *A. opacum*). For *A. annulatum*, only variability in body size at metamorphosis was impacted by phenological manipulations, with size variability being greater at higher levels of phenological variability. We also found that the density and size of *A. annulatum* were better predictors of overall survival and diversity of amphibian prey than phenological variability treatments. Overwintering mortality of *A. annulatum* due to pond freezing likely limited the impacts of phenological variability on demography and community structure. For *A. opacum*, which experienced less severe winter conditions, larval body size variability was affected by our phenological manipulation, with body size structure significantly increasing with greater breeding variability. We anticipate such effects will translate to greater rates of cannibalism, resulting in a trophic cascade for lower trophic levels.

### **The Golden mantella frog: a species on the way to recovery?**

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<sup>1</sup>*Madagasikara Voakajy*

Golden mantella was assessed as Critically Endangered on the IUCN Red List since 2004 due to its small range and the ongoing habitat degradation. A conservation strategy was established for the species in 2011. To tackle habitat loss, a protected area was established in 2015 at the area with numerous known ponds: the Mangabe-Ranomena-Sahasarotra reserve. Throughout the protected area creation process (2008-15), we have been monitoring golden mantella, raising people's awareness on its importance, and tackling direct threats such as pond conversion into rice fields and gold mining through law enforcement. These efforts were continued after the protected area was created. Population assessment was done using direct observation within pond and forest. The number of ponds located in Mangabe Reserve has increased from 19 in 2011 to 143 in 2018, 85 of which were used by the species during the 2018-19 breeding season. We found that number of individuals change between ponds but not in relation with their size. However, it's related to habitat quality such the pond and adjacent forest. The average number of golden mantellas encountered during the last monitoring was 18 individuals per pond. This increase in number of ponds occupied by golden mantella can explain its potential way to recovery. The golden mantellas are still under pressure by habitat loss caused by illegal gold mining and slash-and-burn agriculture and 36 % of the ponds we located have been disturbed. Efforts should be continued to protect the species and its habitats through reducing threats.

## **Snakelike forms, a buried diversity: head shape evolved in association with burrowing substrate in fossorial lizards**

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Convergence is ubiquitous to the evolution of biological diversity. Seemingly resembling phenotypes that originated independently are often interpreted as evidence for the strength of natural selection. Elongated limb-reduced phenotypes evolved at least twenty-five times among squamates, frequently in association with fossoriality. Explanations for this recurrent association reside on functional and biomechanical predictions of such phenotype for burrowing performance and subterranean locomotion. Snakelike fossorial lizards use their heads to perforate the soil during head-first locomotion. Despite the recognition of distinct evolutionary trajectories for snakelike phenotypes in fossorial lineages of Squamata, morphological variation in head shape remains relegated in macroevolutionary approaches. In this context, we aimed to characterize morphological diversity in snakelike lizards and determine if head shape evolved in association with the burrowing substrate. We assembled an ecomorphological database comprising thirteen linear measurements of over 200 species of lizards (average of 10 individuals per species), focusing on representatives of different snakelike lineages of lizards distributed around the globe. We then compared distinct ecological regime hypotheses and determined that head shape evolved in association not only with locomotion, but also with burrowing substrate. Our results suggest that head shape is more diverse among snakelike fossorial lizards than usually acknowledged in macroevolutionary approaches, indicating that lineages that occupy similar burrowing substrates converge in shape. We characterize lizard snakelike lineages as a remarkable model for macroevolutionary studies in convergent evolution, and emphasize the importance of determining the role of specific ecomorphological associations in phenotypic evolution – specially among seemingly similar, although diverse, forms.



## Sister newt species facing landscape changes: comparing key landscape features and resilience patterns

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Amphibians, as a group, are broadly characterized by low mobility and limited ecological versatility, making them especially vulnerable to landscape changes. Even though, at a finer taxonomic level, amphibians show a wide range of life-history traits and consequently a wide range of responses to landscape changes. Previous studies, making use of comparative approaches, have explored this relation between life-history traits and responses to landscape changes. However, such studies have been limited in scope, focusing mainly on comparisons at the level of order or genus, while it is known that landscape changes can have distinct effects at much lower levels of interspecific differentiation. Here, we examined two closely related, but ecologically distinct, newt species (*Lissotriton montandoni* and *L. vulgaris*) across a gradient of habitat fragmentation along the Carpathian mountain range. We genotyped > 200 populations, for > 10 000 SNPs, across six independent geographic regions (ca 250 Km<sup>2</sup> each) for each species. Preliminary analyses revealed that functional connectivity at such scales was mainly related to distance, topography and land cover type. Considering this, we applied a landscape genetics approach and identified key forest types underlying functional connectivity in each species, while controlling for topography. Comparisons among independent geographic regions allowed us to account for idiosyncratic factors. Furthermore, by comparing patterns of isolation by resistance among species across a gradient of tree cover fraction (associated with habitat fragmentation) we were able to measure species exposure to landscape changes and compare their resilience.

**Thermal biology of *Pristimantis medemi* along an altitudinal gradient in the Colombian Andes.**

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Natural habitat in the Colombian Andes has been transformed by habitat destruction. The novel thermal landscapes resulting from human modification may threaten regional amphibian diversity, making it critical to understand how species interact with changing temperatures and loss of forest habitat. The frog *Pristimantis medemi* is an abundant species in the eastern Andes, with an elevational range spanning over 2000 m. We assessed the acclimation capacity and interaction between thermal biology and habitat associations of *P. medemi* along an elevation gradient (415-1350 m) around Villavicencio, Colombia. We used a thermal hardening test to confirm that an acclimation response does not vary with altitude, with individuals from both highlands and lowlands showing little evidence of thermal acclimation. From mark-recapture surveys in forest and adjacent clearings conducted at several sites, we established that *P. medemi* do not commonly move between habitats within a site, and that with increasing elevation, a greater proportion of individuals occupy clearings rather than forest. We then assessed the thermal quality of the habitat by comparing thermal preference ( $T_{pref}$ ) to environmental temperature ( $T_{env}$ ) measurements. For this species, we found better thermal quality ( $T_{pref} - T_{env}$ ) in clearings compared to forest at higher elevations, which corresponded to increasing proportions of individuals in high-elevation clearings. Anthropogenic disturbance reduces thermal quality for *P. medemi* in lowlands but can improve thermal quality for this species in highlands. However, this species shows little evidence of short-term acclimation in the face of drastic changes in thermal environments.

### **Warming, green-up, breeding phenology and development in the wood frog: a 20-year study**

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Amphibians are strongly affected by conditions being altered by climate change. Consequently, a number of studies have shown that traits like the onset of breeding have advanced in regions that have warmed. In this study we build on prior work by examining evaluating both breeding onset as well as larval development rate in a dataset covering 40 wetlands and collected over 20 years in Connecticut, USA. We focus on the wood frog (*Rana sylvatica*), a widely distributed North American anuran, that breeds in nonpermanent wetlands within forested ecosystems. Wood frogs are among the first amphibians to emerge in late winter, often traveling over ice and snow to breeding ponds. Larvae must then develop and metamorphose before their wetlands dry in summer. Thus, development is constrained by early-season temperatures at one end and hydroperiod at the other. We evaluate phenological shifts in wood frog habitat and development. We evaluated changes in and around wetlands (water and air temperature, incident solar radiation, and start-of-season (SOS) estimates from a newly developed Landsat phenology algorithm) with larval development estimated from over 16,500 unique specimens. This large dataset indicates that increasing spring temperatures and advancement in SOS of the forest canopy coincide with a contraction of the larval period. However, developmental rates of larvae increased over the same period resulting in little net shift in estimated metamorphic timing. In continuing work, we are evaluating the extent to which phenological shifts represent plasticity or localized adaptation to shifting environmental conditions.

**Revisiting phylogenetic problems of *Huia* (Amphibia, Ranidae): which one is the true *Huia*?**

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The systematics and phylogenetic position of the Asian frogs of the genus *Huia* has been unstable through time and has not been resolved convincingly. Currently, five species (*H. cavitympanum*, *H. masonii*, *H. sumatrana*, *Huia modigliani*, and *H. melasma*) are recognized under this genus, all of them associated with cascading, clear streams and possess gastromyzophorous tadpoles. In extensive fieldwork we compiled broad samples from Java and Sumatra including the hitherto most comprehensive taxon sample of *Huia*. We analyzed mtDNA and nucDNA markers from *Huia* and taxa that are believed to be close relatives (e.g., *Amolops*, *Meristogenys*, and other riparian ranids) in order to evaluate current phylogenetic uncertainties. Confirming the previous hypotheses, *Huia* species were not forming a monophyletic group because the type species (*H. cavitympanum*) was more closely related to *Meristogenys* from Borneo than other species in *Huia*. Our analysis discovered that the genus *Huia* is more diverse than previously thought with at least six distinct lineages from Sumatra and Java and two lineages from Borneo. Furthermore, several morphological characters vary within *Huia* tadpoles from Sumatra, Java, and Borneo. This study gives insight on the phylogenetic systematics of the *Huia* group and hopefully will help to solve its parahyly problem. This study is yet another example of previously underestimated Southeast Asian frog diversity.

**Is individual variation in growth rates relevant to population dynamics of long-lived reptiles?**

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It is well known that individuals vary greatly in their life expectancies and reproduction rates, but the consequences of this variation at the population level remain unclear. Such variation is particularly relevant to reptiles, as subtle environmental differences may lead to pronounced variation in growth rates, which in turn lead to size-related variation in reproduction rates and survival probabilities. Our 45-year data set for snapping turtles in Algonquin Park, Canada, has revealed strong variation in individual growth rates even after sexual maturity, despite such growth being extremely subtle. Integrated modelling of growth, survival and reproduction data has further indicated that this variation in indeterminate growth leads to > 4-fold variation in expected lifetime reproductive output from the time females reach sexual maturity. Here we explore the consequences of this variation to the population's future dynamics -- i.e. do our predictions about the fate of the population change when we explicitly account for individual variation? To do so, we have developed a novel data augmentation approach whereby we quantify variation among past and present individuals using Bayesian hierarchical modelling, then apply random rates to hypothetical future individuals. Such inferences may be critical for populations of long-lived reptiles whose persistence many decades into the future may depend on current management interventions.

## Dalmatian oddities: functional divergence of head shape in lizards on two peculiar islands in the Adriatic

**Baeckens S<sup>1</sup>**

<sup>1</sup>*University of Antwerp*

Animals on islands are often morphologically divergent from their conspecifics on the mainland or on neighbouring islands as a result of selection favouring a specific 'optimal' phenotype for efficient resource acquisition in the unique insular environment they inhabit. To understand the adaptive significance of the observed phenotypic divergence on islands, it is crucial to have knowledge on the relationship between morphology and function.

The Dalmatian wall lizard (*Podarcis melisellensis*) is one of the most abundant members of the Adriatic insular herpetofauna. Although the general morphology of *P. melisellensis* lizards is relatively conservative, recent observations indicate that on two aberrant Adriatic islets (Brusnik and Mali-Barjak), lizards seem to deviate substantially in head shape from conspecifics on islands nearby. We predict that the observed divergence in head morphology is functionally significant, as a particular head shape allows lizards to feed more efficiently on the prey available. Indeed, diet analyses and estimates of invertebrate abundance showed that lizards on Mali-Barjak fed on smaller prey items than lizards on Brusnik as a consequence of differences in food availability. Moreover, geometric morphometric analyses of the head indicated that lizards on Brusnik had a short, wide head that enables them to bite hard and crush large prey, while Mali-Barjak lizards had a long, narrow head and low bite force. Lastly, behavioural assays of feeding performance showed that lizards from both islands fed most efficiently on prey that was available on their home islands, providing convincing evidence for functional divergence of head shape in *P. melisellensis*.

**The role of spatial structure on the social dynamics of estuarine crocodiles *Crocodylus porosus***

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How animals distribute themselves throughout a landscape is rarely random. Individual differences in resource requirements, physiological constraints, competitive abilities and perception of predation risk can cause patterns to emerge in space and time, and researchers often use these emerging patterns as indicators for the presence of spatial structure in wild animal populations. While significant research has been conducted examining the ecological factors that influence species occurrence and distribution patterns, little research has examined how this spatial structuring may influence a species' social dynamics. This is due to a historic focus in sociality research on the direct interactions between individuals. Determining where and when such interactions occur is often challenging, particularly for highly mobile and typically 'non-social' species, due to their cryptic nature and the rarity in which these individual interactions occur. By understanding how individuals use space and how they interact through time, we can gain insight into the underlying social structure of these typically difficult to observe species. Utilizing a long-term movement dataset containing 175 estuarine crocodiles *Crocodylus porosus* spanning 10-years of continuous monitoring, we investigated how individual crocodiles (Total length = 1 m - 4.7 m) structure themselves through space and time. We were particularly interested in whether individual home ranges overlap more or less than what might be expected by chance, if these patterns were stable through time and if emerging patterns were related to life history. Our findings help reveal novel insights into the social dynamics of this highly mobile and difficult to observe species.

## Rapid assessment of eradication success of an invasive lizard

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Attempts of eradicating invasive reptiles, particularly new incursions, are met with multiple challenges, and there are currently few records of success. Significant challenges include rapidly assessing what is required to eradicate, and declaring with confidence that eradication has been achieved. These issues are essential to the decision-making processes of managers and their stakeholders because resources (i.e. funding and labour) are often limited. Therefore, any attempt to eradicate must be achievable and affordable. Here, we apply a novel approach to rapidly assess the probability of eradication of an invasive lizard (*Lampropholis delicata*) in two experimental cell plots on Great Barrier Island, New Zealand. Originally developed for rat eradication, we built a spatial-survey model that predicts the requirements for eradication by using detection probabilities and home range data of the species from mark-recapture data collected before eradication efforts. We conducted model simulation trials to i) determine the required length and frequency of trapping sessions, and ii) identify influential parameters (e.g. spacing between traps, number of trapping nights) that will provide us with 95% confidence that eradication has been achieved. Results showed that more trapping nights per session and higher effectiveness of the trap to detect a single lizard would decrease the required length of time and increase confidence to declare eradication success. This study also identified gaps in biological knowledge of the species and a need for a more effective trapping device to improve eradication estimations.



**Social tactics and dominance relationships in a long-lived reptile, the eastern water dragon, *Intelegama lesueurii***

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<sup>1</sup>*University Of The Sunshine Coast*

The eastern water dragon is a highly gregarious, long lived agamid lizard native to Australia. During the breeding season, males have been observed to display a high degree of plasticity in their social tactics, with many engaging in fierce contests with one another to establish territories and gain access to females. Yet, despite this, relatively little is known about the variation in these tactics over time, and importantly, their associated fitness gains. Here, we use focal surveys to gather highly detailed information on agonistic behaviour use in relation to male dominance and score these behaviours by their intensity and outcome. We then use these scores to characterise interactions recorded as part of a longitudinal data set of behavioural surveys spanning ~8 years. This work gives a unique insight into how social dominance tactics varies across a free-living population and between multiple breeding seasons. Additionally, it allows us to examine the relationship between dominance status and affiliative social behaviour, as well as subsequent fitness benefits such as territory quality, body condition and reproductive output.

## Consequences of keystone plant decline for reptiles in fragmented agricultural landscapes

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Agriculture is the greatest threat to terrestrial reptiles worldwide, affecting 74% of species assessed for the IUCN Red List. Reptiles are particularly sensitive to agriculture and habitat fragmentation, but are routinely understudied. The consequences of ongoing habitat degradation for reptiles in the agricultural landscapes of southern Australia are poorly studied, but may be driving ongoing species decline. We aimed to investigate the effects of invasion of remnant vegetation by native grasses and exotic weeds and loss of native spinifex grass on reptile communities in fragmented agriculture landscapes, in south-eastern Australia. Reptile assemblages were surveyed through pitfall trapping at 20 sites spanning a gradient of vegetation degradation, including good condition sites dominated by spinifex grass through to sites dominated by native *Austrostipa* grass or exotic plants and with reduced, dead or no spinifex. A total of 857 reptiles from 18 species of squamates were recorded, including snakes (6), geckos (2), dragons (2), skinks (6) and goannas (2). Habitat variables were measured as percentage cover of spinifex, native and exotic grasses, canopy and leaf litter were measured adjacent to each trap. By relating reptile abundance to site attributes, we will identify whether loss of spinifex is a cause of reptile decline, which would make spinifex an important keystone structure for restoration focus. Analyses are currently underway and results will be presented at the conference.

## Re-regeneration to reduce negative effects associated with tail loss in lizards

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Many species of lizard use caudal autotomy, the ability to self-amputate a portion of their tail, as an effective anti-predation mechanism. The tail is regenerated over time, with a continual cartilage rod lacking autotomy planes in place of the original vertebrae. The importance of this tactic for survival varies based on the degree of predation risk, as there are both short- and long-term negative effects associated with caudal autotomy and subsequent regeneration. The general consensus is that, as the regenerated portion of the tail lacks autotomy planes, future autotomy events can only occur at a more proximal position on the original tail. This results in a larger portion of the tail lost, requiring more time and energy for regeneration of the tail. Anecdotal evidence suggests an uncoupling of autotomy and regeneration, that although the regenerated portion of the tail is unable to autotomise, it can re-regenerate following a physical shearing event. We assessed this concept of re-regeneration in a large scincid the King's skink, *Egernia kingii*, both using field data and micro CT. We show that re-regeneration is not an isolated event, occurring on an average of 17.2% across three populations and comprising up to 23.3% of their total tail length, with highest occurrence of re-regeneration observed in the population with the highest predation risk. We suggest that re-regeneration may assist in minimising the costs to an individual's fitness associated with tail loss, efficiently restoring ecological functions of the tail.

## Applied conservation research meets ecological restoration: Assessing early translocation success of captive-born Duvaucel's geckos

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Captive breeding is a recognised conservation-management tool. Yet, use of captive-born animals in reintroduction projects remains contentious due to variable outcomes. For lizard translocations in particular, conclusive data on establishment success of captive-born founders are sparse. Furthermore, adequate long-term post-release monitoring of species with 'slow' life-history traits (e.g. delayed maturity and low fecundity) is often lacking. However, comprehensive data from both failed and successful translocations of such species are needed to further improve translocation methodologies and species management strategies. We translocated captive-born and wild-sourced Duvaucel's geckos (*Hoplodactylus duvaucelii*) to two islands in the Hauraki Gulf as part of biodiversity restoration initiatives. A central objective of our five-year post-release population-monitoring program was to determine early establishment success in relation to rearing history and assess the suitability of captive-born founders for translocation. We radio-tracked adult captive-born and wild founders during the first 10 months after release and compared survival, habitat use and dispersal patterns between both groups. We conducted biannual mark-recapture surveys, recorded morphometric measurements and health parameters for all geckos, including reproductive state of females. We then investigated body condition, growth, health, and female reproductive performance of founders in relation to rearing status and time after release. Captive-born and wild founders performed similarly for most of our assessment criteria and we show that both groups reached milestones indicative for early translocation success. This study enhances our understanding of translocation responses, population biology and life history of *H. duvaucelii* and provides evidence that captive-born individuals are suitable candidates for biodiversity restoration.

### Impacts of translocation on a large urban-adapted venomous snake

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Translocation as a tool for management of nuisance or 'problem' snakes near urban areas is currently used worldwide with limited success. Translocated snakes experience modified behaviours, spatial use and survivorship, and few studies have investigated the impacts of translocation within a metropolitan area. We investigated the impacts of translocation on the most commonly encountered snake in Perth, Western Australia, the dugite (*Pseudonaja affinis*, Elapidae), by comparing the space use of resident and translocated snakes. We obtained GPS spatial-use data from dugites that were either released within 200m of their initial capture sites, or moved to new unconnected habitat at least 3km away. Translocation influenced space use of dugites and detrimentally affected their survivorship. Translocated snakes had larger activity ranges than did residents, and there was a trend towards travelling greater distances over time. Urban dugites face many threats, and snakes were negatively affected by translocation. Mortality for all snakes was high: 100% for translocated snakes, and 50% for residents.

## Johann Gottlob Schneider's *Historiae Amphibiorum* (1799-1801), a seminal contribution to herpetology

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The prominent naturalist and philologist J.G. Schneider (1750-1822) made numerous contributions to herpetology, most notably in his two volume (1799 and 1801) Latin language treatise *Historiae Amphibiorum*. In this work he reviewed both the ancient and modern literature on a diversity of snakes, lizards, frogs, salamanders and caecilians. He also contributed information from contemporary colleagues and presented his own observations from the 15 collections he had visited himself. The two volumes together contain the descriptions of more than 90 new species. Although many of Schneider's names remain in use today, few herpetologists can read the original Latin or are familiar with many of the sources cited by Schneider or with the numerous now defunct museums from which his material came. We provide an English translation of the *Historiae Amphibiorum* along with complete citations for the several hundred works cited therein and extensive annotations identifying all of the hundreds of persons and places mentioned in the books. We also clarify the identity and fate of all 37 museum collections referred to by Schneider and the current whereabouts of the type material for all of the names he coined. Some of the most important sources for Schneider were the personal collections of Marcus Elieser Bloch, Johann Bodo Lampe, and Johann Heinrich Linck, portions of which survive today in Berlin, Waldenburg and possibly Wrocław. Other types were housed in institutional collections, some of which have survived to the present, although others, most notably the Museum Barbyensis, did not survive through the centuries.

## **Bold New World: Urbanisation Promotes an Innate Behavioural Trait in a Lizard**

**Baxter-Gilbert J<sup>1,2</sup>**, Riley J<sup>1,2</sup>, Whiting M<sup>2</sup>

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Urban environments are novel landscapes that markedly alter animal behaviour. Divergence in behaviour in response to urbanisation may provide advantages in navigation, exploiting resources, and surviving under novel selective pressures. Relatively few studies have identified population-level behavioural changes in response to urbanisation that are not confounded by rearing environment and prior experience. To address this, we used the Australian Water Dragon (*Intellagama lesueurii*) to test whether populations under varying levels of urbanisation differ in their innate behavioral traits; acquired either heritably or due to population-specific maternal effects. Eggs were collected from wild mothers and hatched in the lab. Hatchlings were then reared in the lab using a common-garden study design, with individual behavioral traits (boldness, exploration, and neophilia) assayed five times over their first year. We compared behavioral traits, as well as their repeatability, between urban, semi-natural, and natural populations. Levels of neophilia and explorative behavior were similar among all populations. Dragons from semi-natural populations were significantly bolder than those from natural populations. Urban dragons were also bolder than dragons from natural populations, although this trend was not significant because of high variance in boldness. Yet, dragons from semi-natural and urban populations had near-identical boldness scores, suggesting a potentially biologically relevant difference in boldness between them and natural populations. Interestingly, boldness in individuals from urban environments was also the only repeatable trait across time. Overall, our study suggests that boldness is an innate, urban-derived divergent behavioral trait that likely contributes to the success of these lizards in anthropogenically altered environments.

## Linking host life history, landscape ecology, and climate to predict epizootics in Brazilian terrestrial-breeding amphibians

**Becker C<sup>1</sup>**

<sup>1</sup>*The University Of Alabama*

Accounting for how different amphibian species interact with the host-generalist pathogen *Batrachochytrium dendrobatidis* (*Bd*) is essential for resolving epidemiological dynamics. Scarce information on spatiotemporal pathogen dynamics in terrestrial-breeding amphibians -- a historically understudied, disease-sensitive group -- currently presents a major obstacle to estimating spillover events and amphibian declines. We studied how host life history (e.g., developmental mode, habitat use), community structure (e.g., population density, species richness), and environmental factors (e.g., climate, microclimate, and land cover) influence *Bd* outbreaks in Brazil. We found support for climatic anomalies explaining *Bd* spatiotemporal infection patterns and localized die-offs in populations of highly endemic terrestrial-breeding toadlets in the genus *Brachycephalus*. Die-offs were likely fueled by a drought-induced host aggregation near water bodies, enhancing the risk of *Bd* exposure in aquatic habitats or spillover from aquatic-breeding species. Through a manipulative laboratory experiment, we found that *Bd* spillover from even mildly infected aquatic-breeding hosts led to skin microbiome dysbiosis and mortality in terrestrial-breeding toadlets, underscoring the need to further investigate recent population declines of terrestrial-breeding amphibians in the tropics. In a study spanning multiple tropical landscapes, we found support for lower *Bd* prevalence, but higher average infection intensities in terrestrial-breeding frogs when compared to species undergoing aquatic larval development. Collectively, these results provide novel insights into community-level impacts of emerging diseases and the roles of environmental-climatic conditions and anthropogenic habitat disturbances in spillover events and *Bd*-induced die-offs.



## Habitat fragmentation reduces genetic diversity and increases susceptibility to infections in frogs of the Brazilian Atlantic Forest

**Belasen A<sup>1</sup>**, Smith D<sup>1</sup>

<sup>1</sup>*Conservation Research Postdoctoral Fellow, Cornell University*

Why are amphibians so susceptible to disease? Besides introductions of recently emerged pathogens such as the fungus *Batrachochytrium dendrobatidis* (*Bd*), amphibians are subject to anthropogenic (human-caused) modification of natural habitats. Amphibians are particularly sensitive to the negative consequences of habitat fragmentation, the process by which continuous habitats (such as forests) are divided into small isolated patches. Habitat fragmentation can result in loss of genetic diversity due to intense genetic drift (smaller population sizes) and inbreeding (isolation in habitat patches). We hypothesized that this renders fragmented amphibians more susceptible to infections, as inbred animals are known to exhibit higher disease susceptibility. We evaluated the impacts of intensive habitat fragmentation on frog genetic diversity and susceptibility to *Bd* in the Brazilian Atlantic Forest. We analyzed neutral genetic diversity and gene flow (a proxy for dispersal) using ddRAD-Seq, and detected *Bd* infections and quantified loads (intensity) using quantitative PCR (qPCR). We compared forest specialist frogs to “weedy” species that can exploit low-quality habitat to determine the range of effects experienced by diverse tropical amphibian faunas. We found that forest specialist frogs are definitively isolated in fragments, but that counterintuitively, genetic diversity loss has lagged behind high-dispersing weedy frogs. We hypothesize that this may be due to longer generation times in specialists. In addition, *Bd* prevalence was higher in fragmented areas and, and *Bd* loads were highest in fragmented forest specialists. Our results indicate a significant interaction between habitat fragmentation and infection susceptibility, which represent significant threats to the Brazilian Atlantic Forest biodiversity hotspot.

## **Archey's frog - an evolutionarily distinct and long-lived terrestrial frog at risk in New Zealand**

### **Bell B<sup>1</sup>**

<sup>1</sup>*Victoria University of Wellington*

The evolutionarily distinct Archey's frog *Leiopelma archeyi* (Anura: Leiopemmatidae) is a small (max 40mm SVL), mostly nocturnal, terrestrial, long-lived NZ endemic, inhabiting native forests and ridge tops. Remnant populations occur in the Coromandel Ranges and Whareorino forest. Marked declines occurred in Coromandel populations over 1996-2001 resulting in the species being ranked as Critically Endangered on the Red List, no. 1 on the Amphibian EDGE List and Nationally Critical on the NZ Threat Classification list (now At Risk/Declining). To Māori, endemic frogs are taonga or treasures. Handling or disturbance without approval is illegal under NZ legislation administered by the Department of Conservation (DOC). DOC is advised by the Native Frog Recovery Group comprising DOC and university expertise. Principal threats to Archey's frog are invasive organisms, particularly predatory mammals and disease. Climate change is an emerging challenge and there is concern over the potential impact of gold mining in Archey's frog habitat. Interventions include invasive amphibian and mammal control, long-term population monitoring, translocation, captive breeding and rearing, disease assessment and associated hygiene and veterinary investigation. Priorities and outcomes for Archey's frog are notified in DOC's periodic Recovery Plans and NZ Threat Status assessments. Some lessons learned are that: regular reviews of status are vital, research for effective management is important, on-going and new threats present continuing challenges, hygiene protocols are required for disease control, and community engagement has many benefits. Next steps include: increased predator control, clarification of distribution and habitat requirements, and review of potential translocation sites.

**Lure, Trap & Retreat! Development of a novel 'no-dig' trap for terrestrial herpetofauna.**

**Bell T<sup>1</sup>**, Upson Y<sup>2</sup>

<sup>1</sup>*Wildland Consultants Limited*, <sup>2</sup>*MechAdept*

For decades, pitfall traps have been a proven tool in the study of terrestrial amphibians and reptiles, however, there are some 'pitfalls' in the use of pitfall traps. These issues include the use of generic products made for other purposes (leading to ineffectiveness in design and function); the risk of interference by animals; escape or death of trapped individuals; and the inconvenience and labour cost involved in installation. Advanced statistical techniques, such as capture-mark-recapture methods, have been developed to estimate population parameters from ecological studies, partially as a consequence of data-poor datasets. We ask: could such datasets and outcomes from future herpetological studies be improved through significant advances in field tools? In other words, could we make better traps which operate more effectively and reduce escape rates? A novel 'no-dig' prototype trap will be revealed along with initial data from three field trials involving a range of different New Zealand lizard species and comparing results from several different methods including pitfall & g-minnow traps. At the end of this presentation, we invite the audience to examine the trap and contribute ideas towards the final trap design. We welcome collaboration with the international herpetological community in further testing and refining of this novel trap with a range of reptile and amphibian species.

## Responses of arboreal geckos to sanctuary management in pest-invaded environments of New Zealand

**Bell T<sup>1</sup>**, Herbert S<sup>2</sup>

<sup>1</sup>Wildland Consultants, <sup>2</sup>Victoria University of Wellington

In New Zealand, predatory pests such as rodents, mustelids, and cats have devastated the country's endemic, often evolutionary-naive fauna, many of which exhibit slow life histories. New Zealand lizards are among the affected, with 83% of the c. 110 species classified as threatened or at risk of extinction and two known extinctions. In a bid to combat this ecological devastation, wildlife sanctuaries have been created across the country, suppressing or eradicating these predatory pests in order to protect a range of fauna and restoring the functioning of native ecosystems. In our study, we looked at arboreal geckos, of the genera *Mokopirakau* and *Dactylocnemis*, present in a number of paired sanctuary and reference sites across the North Island. To monitor these geckos, we used repeated checks of closed-cell foam covers in transects throughout these multiple sites. We used a range of statistical techniques, including catch-per-unit-effort (CPUE), occupancy modelling and Bayesian analyses, to analyse the abundance of geckos in these sanctuaries relative to the reference sites. In our study, most of the sanctuaries had a higher CPUE of the following arboreal gecko species: *M. granulatus*, *M. 'southern North Island'*, and *D. pacificus*. It appears that sanctuaries benefit arboreal geckos even where the invasive rodent, the mouse (*Mus musculus*), were suppressed, not eradicated. Notably, several individuals of the arboreal striped skink *Oligosoma striatum* was also detected at one of our sites using closed-cell foam covers.

**Salvage, translocation and radio-telemetry of ngahere geckos (*Mokopirirakau* 'southern North Island') to Mana Island, NZ.**

**Bell T<sup>1</sup>**, Yee G<sup>2</sup>

<sup>1</sup>*Wildland Consultants*, <sup>2</sup>*University of Otago*

An arboreal gecko species, the ngahere gecko (*Mokopirirakau* 'southern North Island'), was relocated to Mana Island from a rock aggregate quarry undergoing expansion in the Hutt Valley, North Island, New Zealand. Forty-nine individual geckos were captured using closed-cell foam covers or spotlighting methods over the years 2015-2018. The majority of these geckos were translocated into a soft-release pen installed in seral forest on the island. The released geckos were monitored after release by members of Friends of Mana Island and iwi over time. Between January-March 2018, a radio-telemetry study was conducted comparing initial movements of penned geckos with hard-released geckos. This radio-telemetry study looked at behaviour, habitat use, dispersal and home ranges between the two release-type groups. The population is currently assessed to be still at an early stage in the four stages of translocation success framework that considers life-history and time since release, however, all indications insofar indicate that the translocation effort will eventually be successful. We found that hard-release geckos travelled further between fixes and from the initial release point, and were found at lower heights than penned geckos. Penned geckos had a smaller home range size than hard-release geckos using both minimum convex polygons and fixed kernel methods. However, there was significant variation within each group. We also found a high overlap in habitat use between penned and hard-release geckos, ranging from an overlap of 0.771–0.962. Soft-release methods, by the use of penning, is a recommended strategy for translocations of arboreal *Mokopirirakau* geckos.

## Highlights of long-term studies of endemic New Zealand frogs (Anura: Leiopelmatidae) over several decades

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Despite widespread global reports of declining amphibian populations, supporting long-term demographic data are few, limiting opportunities to study changes in numbers and survival over time. However, for the past 37 years (1982-2019) we have studied the evolutionarily distinct and globally threatened *Leiopelma* frogs of New Zealand (Anura:Leiopelmatiodae), particularly *L. archeyi* in the Coromandel Ranges and *L. hamiltoni* on Maud Island, revealing remarkable aspects of their life-histories and information of relevance to their conservation management. The study of *L. archeyi* in the Coromandel Ranges first identified a major decline of the species (88%) over 1994-96 but fears of continued decline did not eventuate, although population size estimates remain low. On Maud Island, recent *L. hamiltoni* declines have occurred on long-term study plots, but a population of 100 frogs translocated to a new site on Maud Island over 1984-85 continues to increase. We found remarkable longevity in the wild, estimates exceeding those generally reported for similar-sized species elsewhere. New Zealand frogs can take 4-7 years to reach maturity with estimated life-spans up to 46 years in *L. hamiltoni*, 38 years in *L. archeyi*, and 18 years in *L. hochstetteri*. Once settled, adult *L. archeyi* and *L. hamiltoni* were highly sedentary. For example, on Maud Island *L. hamiltoni* occupied a mean individual range area of only  $26.7 \pm 2.2$  m<sup>2</sup> (95% kernel density estimate), mean distances moved between successive range centroids being only 1.3 m per decade. Emerging demographic patterns are related to life-history traits and to conservation of these threatened species.

## Disentangling the mechanisms behind lizard-spinifex relationships

**Bell K<sup>1</sup>**

<sup>1</sup>*Deakin University*

The “unique spiky sclerophyllous structure” of spinifex grass (*Triodia* spp.) provides a keystone functional habitat for many reptiles, and is potentially a critical factor behind Australia’s remarkable arid zone lizard diversity. Spinifex may benefit numerous lizard species through the provision of refuge from predators, a repository for food resources, or the provision of thermoregulatory opportunities. However, despite 40 years since these initial hypotheses were proposed, there remains ambiguity over the relative importance and validity of these functions.

This project aims to tease apart the refuge, sustenance and thermoregulation hypotheses through a combination of field invertebrate sampling, temperature surveys, and manipulative behavioural and predation experiments. Seven lizard species from four families will be tested, each of which displays a close association with spinifex.

Understanding the mechanisms behind the wide-ranging impacts of a keystone structure would enable us to help protect and promote the specific properties underlying its 'keystone-ness', mitigate future habitat loss and degradation, and improve our understanding of wider ecosystem function and management.

**Shifting breeding phenology and the role of photoperiod as a developmental cue under climate change.**

**Benard M<sup>1</sup>**

<sup>1</sup>*Case Western Reserve University*

As climate change progresses, many amphibians are breeding earlier, which in turn exposes their offspring to different amounts of daily sunlight (i.e. photoperiod). If amphibian larvae use photoperiod as a biological cue to control development, as many other organisms do, then climate change is creating a potential ecological trap. As the average dates of optimal conditions shift with climate change, the annual patterns of photoperiod at a site will remain the same. Thus, the old relationships between seasonal temperature and precipitation versus photoperiod may no longer apply. In this study, I tested two hypotheses about how photoperiod may act as a cue to affect amphibian development rate. First, amphibian larvae will accelerate development rate in long photoperiods (simulating development later in the season) compared to short photoperiods. Second, amphibians from more northern populations, which face greater seasonal time constraints, will respond more strongly to photoperiod than those from southern populations. To test these hypotheses, wood frog (*Rana sylvatica*) larvae from populations spanning a latitudinal gradient from 36.2 ° N to 45.5 ° N were raised in the laboratory under early-season and late-season photoperiod treatments. Larval growth and development were measured through metamorphosis. There were population by photoperiod interaction effects, however they were not entirely in accordance with the predictions of the hypotheses. For example, longer photoperiods, simulating a later season, prolonged metamorphosis in some populations. These results demonstrate the importance of evaluating the effects of shifting breeding season mediated through developmental responses to novel photoperiod patterns.



## Urbanization modulates mating signals and antipredatory strategies in a Neotropical frog

**Bernal X<sup>1,2</sup>**, McMahon T<sup>3</sup>, Halfwerk W<sup>4</sup>, Anderson N<sup>1</sup>

<sup>1</sup>*Purdue University*, <sup>2</sup>*Smithsonian Tropical Research Institute*, <sup>3</sup>*University of Tampa*, <sup>4</sup>*Vrije Universiteit*

Humans have dramatically altered most habitats around the world. By transforming ecosystems to satisfy human demands, we change the selective pressures imposed on organisms that survive in such novel environments. Organisms living in cities are thus exposed to diverse selective pressures that may differentially affect their traits. Abundance and composition of diurnal and nocturnal predators, for example, may change the fitness landscape of urban prey. We synthesize three studies investigating how predation affects a Neotropical anuran, túngara frogs. These studies combined provide a novel perspective on the factors that modulate coloration pattern and calling behavior of frogs in cities. We find that urbanization differentially affects abundance of diurnal and nocturnal predators of túngara frogs. Using clay models of túngara frogs we detected a higher abundance of diurnal, visually-oriented predators (birds) in urban areas. Despite the increased frequency of attacks on city frogs, however, coloration pattern did not affect the number of attacks. In contrast, eavesdropping, nocturnal predators (frog-eating bats) and micro-predators (frog-biting midges) are rare in the city. Experimental manipulations revealed that both light and noise pollution negatively affect the ability of frog-biting midges to attack calling frogs. Similar effects are likely to occur in frog-eating bats but more studies that dissect the influence of different types of pollution are necessary. Reduced predation pressure from both bats and midges, combined with lower abundance of female frogs, results in calling males from the city producing calls of higher complexity. These studies provide a perspective of the complex effects of urbanization.

## The amphibian chytrid fungus in New Zealand

**Bishop P<sup>1</sup>**, Eda A<sup>1</sup>

<sup>1</sup>*University Of Otago*

Chytrids were first detected in introduced frogs in 1999 in a pond in Christchurch. The presence of *Bd* was first detected in native frogs (*Leiopelma archeyi*) on the Coromandel Peninsula in 2001 and it was suggested that *Bd* was responsible for a mass decline (88%) in that population between 1994 and 2002. Although *L. hochstetteri* is the only endemic frog that breeds and lives in small high-altitude streams all sampling for *Bd* was negative, and in an experimental study, *Bd* was suggested to pose a low risk to leiopelmatids. Swabs taken from the other two species of native frogs, which only occur on islands, were negative, although positive *Bd* results have been obtained from all three introduced species of treefrog. An incursion of Alpine newts (*Ichthyosaurus alpestris*) was recently detected at the southern end of the Coromandel Peninsula and they were thought to have escaped from an illegal importer between 1995 - 2005. Many of these newts tested positive for *Bd* and it has been suggested that this is the original source of *Bd* in New Zealand. Populations of introduced frogs on South Island started to decline in the mid-2000s, and by 2008 large populations of *R. raniformis* in Central Otago disappeared. Over the last 10 years populations of introduced frogs have been recovering, and in recent studies these frogs are impossible to infect with laboratory strains of *Bd*. Current research is underway comparing skin microbiomes of *Bd*-exposed frogs to *Bd*-naïve frogs.

## **The global effort to conserve amphibians under threat**

**Bishop P<sup>1</sup>**

<sup>1</sup>*University Of Otago*

Global amphibian declines and extinctions have been reported for many decades and it has become clear that amphibians are experiencing a global extinction crisis. The amphibian community has built a network to respond to this crisis, including: coordinating amphibian conservation scientists and practitioners through the Amphibian Specialist Group (ASG) which develops the scientific input to support conservation decision-making through recommendations set out in the Amphibian Conservation Action Plan (ACAP); the Amphibian Ark (AArk) which coordinates ex situ actions and how these can be effectively linked to conservation on-the-ground; and the Amphibian Survival Alliance (ASA) a partnership of global organisations that work to promote the conservation of amphibians and implement ACAP. This network needs to be continually strengthened by contributors both within and outside of the amphibian community.

ASG, AArk and ASA all share a common vision “Amphibians thriving in nature” and their new Strategic and Operational Plans include priority actions such as: mitigating the impact of other key threats such as emerging infectious diseases and trade; habitat protection (KBAs); communication and awareness-raising; assessments of the conservation status of amphibian diversity; rescue and capacity building; and developing applied science needed to inform effective conservation actions. While the future for amphibians is still precarious, global amphibian conservation is finally moving ahead in a coordinated and collaborative way.

## Leiopelma conservation: progress and key uncertainties

**Bishop P<sup>1</sup>**

<sup>1</sup>*University Of Otago*

New Zealand is home to only a few species of native frogs. However, these noteworthy highly endemic frogs comprise a small genus, *Leiopelma*, in the stem anuran family *Leiopemmatidae*, suborder *Archaeobatrachia* with divergence from modern frogs around the mid-Triassic. Humans first arrived in New Zealand in the mid-late 13th century and introduced the Polynesian rat (*Rattus exulans*), which probably had a major impact on the small vertebrates of forests. This was the first wave of invasive mammalian introductions and combined with the extensive clearing of native forests, three species of *Leiopelma* became extinct around the time of human settlement. The four surviving species (*L. archeyi*, *L. hamiltoni*, *L. hochstetteri*, *L. pakeka*) are all threatened, have undergone severe declines, are of urgent conservation concern and all strictly protected under the Wildlife Act 1954 and the Conservation Act 1987.

With the increasing human population in New Zealand and the concomitant increase in deforestation and prospecting for natural resources, such as gold and silver, habitat destruction and alteration continue to be the major threat to North Island *Leiopelma* species. New Zealand's endemic frogs depend upon ongoing conservation management tools, such as protection and restoration of the habitat, management of invasive vertebrate species, monitoring of populations, conservation translocations, hygiene protocols for the management of disease, and maintaining and breeding frogs in captivity.

**Confronting amphibian diseases: frontiers in effective mitigation**

**Bletz M**

Emerging infectious diseases have decimated amphibian communities and threaten naïve populations around the globe. Pathogens, such as *Batrachochytrium dendrobatidis*, *B. salamandrivorans*, Ranavirus, and severe perkinsea infections, present conservation challenges for researchers, managers and policy makers alike. Collaborative efforts to identify ways to prevent arrival and spread of these pathogens and to develop effective disease mitigation strategies can facilitate population persistence and species conservation. National and international wildlife disease policy and best practices for amphibian hobbyists can bolster our ability to minimize risk of pathogen entry and spread to new regions through amphibian trade. Effective mitigation strategies, ranging from skin probiotic therapy to vaccination, from environmental manipulations to selective breeding, can target (i) aspects of the hosts' skin mucosal environment to promote disease resistance or tolerance, (ii) components of the surrounding environment to reduce infection risk, or, (iii) the pathogen directly to reduce pathogen virulence. Integrative research across disciplines can spur new synergistic strategies to mitigate disease emergence, reduce spread, and promote population resilience through invasion, which is essential for confronting continued biodiversity loss of amphibians around the world.

## Proactive Conservation: Developing strategies to combat the salamander-eating fungus

**Bletz M<sup>1</sup>**, Nanjappa P<sup>2</sup>, Woodhams D<sup>1</sup>

<sup>1</sup>University Of Massachusetts Boston, <sup>2</sup>Conservation Science Partners

Arrival of the fungus, *Batrachochytrium salamandrivorans* (*Bsal*), looms over the United States – a salamander diversity hotspot rivaled by no other around the world. This ‘salamander-eating’ fungus is decimating fire salamanders in Europe and poses immense threat to global salamander diversity. Therefore, understanding risk of *Bsal* invasion in the US and preparing for both how to respond if it emerges, as well as how to support population resilience through invasion, is essential. Three key strategies include prevention, management, and mitigation.

Minimizing risk of pathogen entry through amphibian trade is critical; the 2016 USFWS listing of 201 salamanders as injurious under the Lacey Act was a first step in prevention. However, management of this pathogen will require additional regulatory measures, along with development of implementable science-based strategies to mitigate disease emergence and reduce spread. Disease response strategies in the wild can include altering water chemistry or temperature, modifying micropredator communities, applying fungicides, vaccination, and probiotic bioaugmentation. We characterized susceptibility across populations of eastern newts and two-lined salamanders, finding that populations differ in mucosal protection against *Bsal*. This crucial step identified geographic regions that are at greatest threat if *Bsal* invades, which in turn helps direct surveillance and management actions. Preliminary evidence from a controlled-laboratory experiment suggests that bacteria that produce volatile antifungal compounds (VOCs) may be able to limit infection buildup in hosts simply by being present in the environment. Continued development of integrative, multipronged approaches will be essential to combat significant biodiversity losses if *Bsal* emerges in the United States.

## **#Frogtraits and Swamp Trolleys: new art and creative strategies in amphibian conservation and public outreach**

**Blumstein C<sup>1</sup>**

<sup>1</sup>*Sussex Amphibian & Reptile Group (SxARG)*

Amphibians have suffered catastrophic declines around the world, including so-called common species. For example, the Common Toad (*Bufo bufo*) has declined by 68% in the UK over the past 30 years and more severely in some areas, such as the South East of England.

Since 2012, I have focussed my work on amphibians, in particular the Common Frog (*Rana temporaria*). Drawing on 30 years experience as a professional artist, I have applied my artistic approach to urban amphibian conservation, connecting non-science audiences with the global amphibian extinction crisis by developing new art, performance and creative strategies in public outreach.

My main aim is to inspire conservation support and action from the general public, by focussing on species that can be encountered within the urban environment, for example by creating ponds, frog-friendly habitats and installing toad ladders in drains.

Creative strategies developed thus far include:

Agent Amphibian, a performative character and #FrogCollaborator who gives frogs agency and a voice, in any context.

#Frogtraits - metal art prints of individually identified Common Frogs who have a name and story that connects their individual plight with that of frogs around the world.

TEDx talk by Agent Amphibian: introducing a broad, non-science audience of 1000+ to the amphibian extinction crisis.

Swamp Trolley art installation: Deployable exemplary habitat and interactive communication device.

In this presentation, we will discuss the effectiveness of these strategies so far in achieving the intended outcomes in public outreach.

Caution - this talk may contain croaks!

## **Adaptive radiation in the multidimensional phenotype**

**Bodensteiner B**<sup>1</sup>, Muñoz M<sup>1</sup>

<sup>1</sup>*Virginia Tech*

Adaptive radiations are considered a special case of evolutionary diversification in which a clade displays extraordinary ecological and phenotypic diversity. A common feature that unites studies of adaptive radiation is that the ecology-phenotype connection has been almost exclusively described in terms of morphology. For example, the adaptive radiation of Caribbean anoles is known for the evolution of distinct 'ecomorphs', which are so-named based on the tight association between structural habitat use and morphological traits in these lizards. Despite all the of the disproportionate attention that morphological traits have received, it has also been well-recognized that physiological evolution, along a thermal gradient, is also a key aspect of the adaptive radiation of anoles. Here we test how morphological and physiological disparity compare by examining the morphology and thermal physiology of Hispaniolan anoles. Elucidating that physiological patterns of evolution do not mirror morphological patterns of evolutionary divergence. We propose that thermoregulatory behavior may be guiding these macroevolutionary patterns revealed by this study.



## Functional diversity of predators drive variable trait- and density-mediated effects on prey life history

**Bohenek J<sup>1</sup>**, Resrtarits Jr. W<sup>1</sup>

<sup>1</sup>*The University Of Mississippi*

The consumptive effects of predators have been central to ecological theory, but non-consumptive predator effects have emerged as major components of predator-prey interactions. Consumptive and non-consumptive predator effects are expected to vary with predator functional traits (hunting mode, gape size). In a field mesocosm experiment, I exposed developing larval salamanders (*Ambystoma talpoideum*) to the consumptive (free-roaming) and non-consumptive (caged) effects of three predators with different hunting modes and gape sizes (*Lepomis cyanellus*, *Aphredoderus sayanus*, *Fundulus chrysotus*). I measured life history variables (body size, growth rates) along with plastic traits known to respond to predator presence: tail color and the expression of paedomorphic life history strategies. *Lepomis cyanellus* and *Aphredoderus sayanus* had equivalent consumptive effects on prey, but induced opposite effects on body size. Consumptive (but not non-consumptive) *L. cyanellus* evoked trait-mediated effects that suppressed growth, induced achromatic tails, and overrode density-mediated effects. Surviving larvae exposed to consumptive *A. sayanus* reached the largest body sizes, showing only density-mediated, and not trait-mediated, effects. *Fundulus chrysotus* imposed intermediate effects on prey survival and body size. Predator effects varied with functional traits with larger gaped predators having stronger consumptive effects, but active predators induced stronger trait-mediated effects. Paedomorphosis was mostly density-mediated, but appears suppressed in the presence of *F. chrysotus*. Non-consumptive predator cues had minimal effects on prey suggesting prey may have habituated to predator cues. Our results emphasize the role that species identity and functional traits play in determining density- and trait-mediated effects on prey and life history.

### **Diversification via paedomorphosis in salamanders**

**Bonett R**<sup>1</sup>, Ledbetter N<sup>1</sup>, Herrboldt M<sup>1</sup>, Hess A<sup>1</sup>

<sup>1</sup>*University of Tulsa*

Salamanders exhibit a continuum of life cycle patterns that involve shifts in the timing of metamorphosis within and among species. The biphasic (metamorphic) life cycle of salamanders includes an aquatic larval stage that metamorphoses into a terrestrial form before adulthood. An alternative life cycle is larval form paedomorphosis, where salamanders forgo metamorphosis and retain their aquatic larval morphology and ecology into adulthood. The transition between metamorphosis and paedomorphosis is a major shift in adaptive zones, which has implications for patterns of diversification at multiple levels of organization. Our talk will report on comparative studies of diversification in salamanders at micro and macroevolutionary scales.

### **The return to an aquatic life, propulsive thrust of swimming in terrestrial and marine snakes**

**Bonnet X<sup>1</sup>**, Ballouard J<sup>2</sup>, Michel C<sup>2</sup>

<sup>1</sup>*Cebc Cnrs*, <sup>2</sup>*SOPTOM*

Several lineages of snakes have returned to an aquatic life. Main evolutionary stages, terrestrial, amphibious and truly marine species, are represented by more than 3,700 living species. Thus, snakes offer a simple framework to explore a major, albeit poorly understood, evolutionary shift that shaped the diversity of extant and fossil vertebrates. The athletic performances of different species of snakes have been compared during swimming. Yet, the energetic efficiency of swimming has not been sufficiently investigated. We assessed the propulsive thrust of terrestrial and marine snakes (both in the field and in the laboratory). Surprisingly, marine snakes did not surpass the most vigorous terrestrial snake species; at least at the beginning of the experimental trials. Over time, the performances (e.g. of thrust) of the snakes decreased, more slowly in marine than in terrestrial snakes however. Therefore, a greater endurance is a possible major adaptation to an aquatic life; enabling snakes to undertake long foraging trips associated with the search of prey (e.g. days or weeks to be successful). In order to test this assumption, we need to quantify simultaneously performance and energetic expenditure of swimming using non-invasive methods; measuring the hydrodynamic drag coefficient while collecting precise kinematics of undulatory swimming for example is an appropriate albeit complex option.

### Variations of jaw size across genetically homogenous sea snake populations reveal phenotypic plasticity

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The lagoon of New Caledonia is the largest in the world, it covers more than 24,000 km<sup>2</sup> and hosts large colonies of amphibious sea kraits. One endemic species, the “tricot rayé jaune”, *Laticauda saintgironsi*, is particularly widespread and often locally abundant. High climbing ability enables this marine snake to occupy a great variety of sites, from precipitous rocky places to flat sandy islets scattered from the coastal zones to the remote barrier reefs, sometimes more than 50 km offshore. This diversity of terrestrial habitats is mirrored by the variability of associated marine landscapes, which in turn influences the type of fish consumed by the snakes. Indeed, the bottoms surrounding each snake colony are characterized by various depths, proportions of sandy versus hard substrate, coral reef assemblages and protection status. Sea krait's diet, represented by more than 30 species of moray eels and other benthic anguilliform fishes, depends on the nature of bottom habitats. We observed significant difference in relative jaw size among colonies, in relation to relative prey size. Because the peculiar breeding strategy of the tricot rayé jaune maintains a strong genetic homogeneity among populations, phenotypic plasticity likely explains the differences of relative jaw length.

## Comparison of call properties, morphometrics and genetics as clade delineating tools in North-Asian Hylid treefrogs

**Borzée A**<sup>1</sup>

<sup>1</sup>*Department of Biology and the Environment, Nanjing Forestry University*

Clade delimitation methods have transformed over time, starting from morphology and later primarily relying on tools such as genetic analyses. Recently, these methods have been combined with other variables such as call properties and ecological requirements to provide integrated results. However, each of the methods used may provide different results when used in isolation, raising questions on best practices. Here, we compare genetics, morphometrics and call properties for a the far-eastern Japanese treefrog, *Dryophytes japonicus*, from North East Asia. The species ranges from central Mongolia to Eastern Russia and the Japanese archipelago in the North, and to the two Koreas and Eastern China to the South West. We collected data from all six countries between 2013 and 2018 and conducted a taxonomic comparison comprising over 500 individuals, including two mtDNA and three nuclear gene fragments, call properties and morphological measurements. Our results support the described split of the clade in two species, but we also recover several sub-lineages. Most interestingly, while morphology only shows a trend towards segregation of sub-clades on the mainland and large islands, call properties and genetics agree on a divergent clade in the North East Asian mainland. Non-significant variations are also detected for isolated populations, such as smaller islands where the opposite pattern is observed with divergent morphologies. In conclusion, while any of the tools used is important on its own, we highlight the importance of combining all variables available for an integrated and geographically variable answer.

**World's largest tropical island may provide refuge from the amphibian chytrid fungus *Batrachochytrium dendrobatidis***

**Bower D**<sup>1</sup>, Jennings C<sup>2</sup>, Webb R<sup>3</sup>, Amepou Y<sup>4</sup>, Schwarzkopf L<sup>3</sup>, Berger L<sup>5</sup>, Georges A<sup>4</sup>, McKnight D<sup>3</sup>, Alford R<sup>3</sup>, Carr L<sup>3</sup>, Clulow S<sup>6</sup>

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Emerging infectious diseases threaten the persistence of biodiversity globally. The amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, is among the most widespread and damaging pathogen to biodiversity. New Guinea hosts 6% of the world's frogs and is the largest landmass climatically suitable for *Batrachochytrium dendrobatidis* that may be free of the pathogen. We surveyed for *Batrachochytrium dendrobatidis* in Papua New Guinea, by swabbing live frogs in the Gulf Province and Eastern Highlands Province and by examining museum specimens from a range of sites and elevations. In total, all 442 samples were negative for *Batrachochytrium dendrobatidis*. The entry of *Batrachochytrium dendrobatidis* may have been prevented by the remoteness of Papua New Guinea and the unsuitable climate of its lowlands, which surround the climatically suitable highlands. Alternatively, *Batrachochytrium dendrobatidis* may be present in isolated patches but remain undetected, to date. We recommend a national disease surveillance program for chytrid fungi and crucial pre-emptive actions, designed to reduce the risk of pathogen transmission. Measures should include improved biosecurity protocols for trade and travel and continued disease surveillance in areas of probable entry and spread.

### Fine-scale density modeling of an endangered sand-dune lizard with drone mapping

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Rapid consequences of global climate changes in habitats of threatened species urge accurate information in fine spatial and temporal scales to monitor and predict these effects. *Liolaemus arambarensis* (Iguania: Liolaemidae) is an endangered sand-dune lizard, endemic to Southern Brazil, that has not been seen in its type locality since 2009. This local extinction was probably caused by dune stabilization and depletion of tussock grasses associated with loose sands (keystone resource for *L. arambarensis*), a tendency of recent wetter and warmer climate. Here, we provide an example of how accurately predicting density of sand dune lizards in fine spatial scale using drone mapping and accounting for imperfect detection. Applying spatial capture-recapture (SCR) models, we modeled density in relation to tussock grasses coverage (from drone map) and baseline detection probability and area used per sex. For this, we used search-encounter SCR data collected in the reproductive season of *L. arambarensis* (Aug-Mar) from 2013 to 2017. Mean density ranged from 148 (123-198) lizards/ha in 2015-16 season to 282 (216-369) lizards/ha in 2014-15. We estimated densities of  $0.34 \pm 0.03$  ind/25m<sup>2</sup> in grid cells with no tussock, and  $1.35 \pm 0.31$  ind/25m<sup>2</sup> with 35% of tussock coverage. Males (that have territorial behavior) presented a smaller area used and a higher detection probability than females. Combination of SCR models and drone mapping provided a reliable framework to model density of sand-dune lizards in fine scales and thus to predict future impacts of global climate changes on this threatened species.

## **Recovering the white-bellied frog in south-west Western Australia: successes, challenges and the importance of collaborations**

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<sup>1</sup>Perth Zoo, <sup>2</sup>Parks and Wildlife Service

Critically Endangered white-bellied frogs (*Geocrinia alba*) are endemic to a small area of south-west Western Australia. As part of recovery efforts for this species, Perth Zoo runs a head-starting program where eggs / larvae are collected from the wild each spring and reared at the Zoo for 10-11 months prior to release back to the wild as juveniles the following spring. This increases survival during these vulnerable stages of the life-history from less than 20% to more than 90%.

Since 2010, approximately 800 head-started froglets have been released into six sites within their geographic range, both to establish new populations and augment existing ones. On-going monitoring indicates that populations have established and are persisting at the two oldest introduction sites, and that supplementation has increased numbers at an existing population. Releases to the remaining sites have occurred in more recent years and, while initial signs are positive, it is still too early to be certain of the outcomes at these sites.

The major challenge facing the program at present is the identification of additional suitable release and augmentation sites, particularly ones that are likely to remain suitable into the future given the warming, drying climate in south-west WA. Research to address this is currently occurring in collaboration with Perth Zoo and the WA Parks and Wildlife Service.



## Evaluating environmental-DNA as a tool for detecting an amphibian pathogen using an optimized extraction method

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<sup>1</sup>University of Melbourne, <sup>2</sup>University of Pittsburgh

Environmental DNA (eDNA) detection is a valuable conservation tool because it can be used to detect and monitor imperiled species, invasive species, and wildlife pathogens within the environment.

*Batrachochytrium* pathogens are of particular global conservation concern because they are a leading cause of amphibian decline. While there has been some research exploring the use of eDNA techniques to detect *Batrachochytrium* DNA in the environment, no single method appears to work in all situations. In this study, we first systematically compared eDNA extraction methods to find the most effective method for detecting *Batrachochytrium dendrobatidis* in water samples. Second, we used the extraction method we found to be most effective on field-collected samples of both water and sediment. Using a quantitative PCR assay, we found that the most effective method for extracting *Bd* DNA from water filters was a soil extraction kit (Qiagen PowerSoil). We found that water filters were equivalent to amphibian skin swab samples for detecting the presence or pathogen DNA at the level of habitat, but that seasonal patterns of pathogen quantity were not equivalent between swabs and water samples. We also found that detection probability increased with the volume of water filtered. Overall, our detection of *Bd* eDNA presence was less sensitive for sediment samples. Our results indicate that eDNA samples, particularly filtered water samples, can be accurate in detecting pathogen presence at the habitat scale and could be an important conservation tool, however, eDNA's utility in predicting infection risk and dynamics within a habitat is limited.

**A global phylogeny of monitor lizards, and the contribution of competition to extreme size disparity**

**Brennan I**<sup>1</sup>, Moriarty Lemmon E<sup>2</sup>, Lemmon A<sup>2</sup>, Welton L<sup>3</sup>, Portik D<sup>4</sup>, Weijola V<sup>5</sup>, Keogh S<sup>1</sup>, Donnellan S<sup>6,7</sup>  
<sup>1</sup>*Australian National University*, <sup>2</sup>*Florida State University*, <sup>3</sup>*University of Kansas Biodiversity Institute & Natural History Museum*, <sup>4</sup>*University of Arizona*, <sup>5</sup>*University of Turku*, <sup>6</sup>*University of Adelaide*, <sup>7</sup>*South Australian Museum*

Monitor lizards (*Varanus*) are an exceptional radiation of squamate reptiles which range from Africa, through the Middle East and Indian subcontinent, and across Southeast Asia into Australo-Papua. Among living vertebrates, monitors exhibit the greatest size disparity within a single genus, varying in orders of magnitude between the colossal Komodo Dragon *Varanus komodoensis* and the smallest Australian dwarf goannas. Using a coalescent species-tree approach, we first estimate the relationships among living and extinct varaniform lizards, incorporating exon-capture molecular and morphological datasets. We then trace the biogeographic history of the group from its origins in Paleocene Eurasia, across its broad current distribution, and focus on the Australian radiation of monitor lizards. Here we extend existing phylogenetic comparative methods which consider lineage interactions to test the evolution of extreme size disparity among Australian *Varanus*. Our results suggest that communities of Australian *Varanus* show high functional diversity as a result of continent-wide interspecific competition.

## **When chytrid fungus invades: Integrating theory and data to understand disease-induced amphibian declines**

**Briggs C<sup>1</sup>**

<sup>1</sup>*University Of California, Santa Barbara*

The emerging amphibian fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*) has led to severe amphibian declines around the globe. One of the challenges when attempting to mitigate the effects of *Bd* on amphibian populations is that different amphibian populations can show drastically divergent outcomes following *Bd* invasion. These include an increase in amphibian population density, no discernible change in population density, a decrease in density, and even population-level extinction. Here we integrate extensive data from amphibian-*Bd* systems and epidemiological theory to build a framework for predicting when and why amphibian populations might show different population-level trajectories upon *Bd* invasion. This framework allows us to place seemingly disparate population-level responses following *Bd* invasion in terms of known disease ecology theory to better understand and manage amphibian declines and recoveries.

**Identifying frog chorusing in environmental recordings using acoustic indices and false-colour spectrograms.**

**Brodie S**<sup>1</sup>, Towsey M<sup>2</sup>, Roe P<sup>2</sup>, Schwarzkopf L<sup>1</sup>

<sup>1</sup>*James Cook University*, <sup>2</sup>*Queensland University of Technology*

Acoustic monitoring has become a commonly used method in studies of frog populations with the development of affordable and portable sound recording technology. Advances in digital technology mean the amount of acoustic data that can be collected is now less constrained by battery power and data storage limitations. This allows bioacoustic studies of vocal animals to be scaled up both temporally and spatially. However, the utility of large bioacoustic data sets is limited by our capacity to analyse them. Automated call recognition software is ineffectual for noisy environments with high call overlap, such as in frog choruses. We demonstrate an approach to detecting frog choruses in acoustic data using acoustic indices and false-colour spectrograms. Acoustic indices are numeric summaries of the energy distribution in sound recordings. False-colour spectrograms are composed using multiple acoustic indices to give a graphical representation of the acoustic content in acoustic files, which allows for visual identification of sounds of interest in long-duration recordings. We used false-colour spectrograms in a study of the chorusing activity of frog communities in tropical savanna habitats in northern Australia and found the choruses of many species could be identified visually by their unique acoustic signatures. We also tested the use of acoustic indices as predictors in random forest classification models to automate the detection of several frog species in multi-species choruses with promising results. The analysis tools we demonstrate could be applied to long-term monitoring and study of frog species at the community and landscape scales.

### Evolution of dermal armour in monitor lizards (Squamata: Varanidae)

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<sup>4</sup>Free University of Brussels

Monitor lizards of the genus *Varanus* comprise a morphologically conservative, yet ecologically diverse clade that includes some of the largest known lizard species living today. Although monitor lizards have been the subject of a plethora of anatomical studies, little research has been devoted to exploring their expression of dermal armour – or osteoderms – a trait that is characteristic of the Anguimorpha. To date, osteoderms have only been reported in a handful of phylogenetically distant species of monitor lizards. The restriction of osteoderms to a minority of taxa is likely related to methodological difficulties in detecting osteoderms, because of their small size, anatomical positioning (i.e., embedded in the integument) and occurrence as isolated elements in this clade. In this study, we investigate the expression of osteoderms in a large number of monitor lizards (> 50 species) using micro-CT scans of skin tissues from various body regions. Based on the micro-CT data, we reconstruct the evolution of dermal armour in monitor lizards and address possible functional explanations by examining correlations between osteoderm expression and indices of climate, microhabitat use, as well as fighting ability. Ultimately, we aim to unravel the evolutionary and ecological relevance of these enigmatic structures in an even more enigmatic clade of lizards.

## **Mechanisms for coordinating expertise: New Zealand's Island Eradication Advisory Group**

### **Broome K<sup>1</sup>**

<sup>1</sup>*Doc*

The Island Eradication Advisory Group (IEAG) is made up of New Zealand Department of Conservation (DOC) staff with experience and knowledge in eradicating pests from islands. Set up in 1997 to provide technical advice to DOC projects, the role has diversified to include capability development and international networking to maintain DOC's knowledge base by participating in the resolution of island eradication issues worldwide. Key successes of the group are: a strong customer focus to meet project manager needs; clear separation between advice and decision making; a team approach to each project; and effective communication. Priorities are described in the terms of reference for the group. Meetings - about three times per year, involve discussion and problem solving with project staff followed up by written advice agreed at the meeting. The IEAG respond to requests for advice at any time to meet the needs of projects. Members can contribute to group discussion via email or conference call to provide a collective view. Many projects have the IEAG undertake pre-operational 'readiness checks' to identify outstanding issues that need to be addressed before implementation. Key elements in the success of IEAG advice are robust debate and review involving the IEAG and the project managers; making the most of collective knowledge; challenging assumptions and growing project managers' experience. We think this approach can be adapted to be useful in other parts of the world. We propose that this structure for rapid engagement and advice also has promising application for the EDRR to new incursions.

## Fluctuating toxins and the evolution of chemical defenses in a poisonous amphibian

**Bucciarelli G<sup>1</sup>**, Green D<sup>2</sup>, Shaffer B<sup>1</sup>, Kats L<sup>2</sup>

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Comparisons of range-wide phenotypic variation can reveal the evolutionary and ecological forces that have produced distinct trait differences between populations. When interacting species have well-matched phenotypes they may become tightly linked in an antagonistic relationship that leads to coevolution, if the traits are heritable and there is reciprocal selection. Observed wide-ranging phenotypic variation of chemical defenses in newts (genus *Taricha*) and resistance in predatory snakes (genus *Thamnophis*) has remained an exemplar of coevolution and an escalating arms-race. Under this coevolutionary model, traits evolved in lock-step, leading to greater and greater chemical defenses and resistance in populations. But, it is undetermined how *Taricha* possess their chemical defenses, a neurotoxin (tetrodotoxin, TTX) that may be either endogenously produced via a biosynthetic pathway or alternatively by endosymbionts. It is also not clear if newt chemical defenses are responsive to selection or are heritable. Moreover, TTX fluctuates within individuals, implying the trait is plastic. Thus, geographic phenotypic variation of newt defenses may not be the result of coevolutionary dynamics. In this study, we characterize spatial and temporal patterns of newt-TTX variation in nearly 50 populations found across California and test the potential role of bacteria in driving phenotypic variation and fluctuations. Ultimately, our results suggest that a coevolutionary model does not explain trait differences between *Taricha* populations.

**Chemically Mediated Self and Social Recognition in Juvenile Common Gartersnakes (*Thamnophis sirtalis*)**

**Burghardt G<sup>1</sup>**, Pepper H<sup>1</sup>, Jenkins M<sup>1</sup>, Rowland J<sup>1</sup>, Steele J<sup>1</sup>, Partin A<sup>1</sup>

<sup>1</sup>*University Of Tennessee*

Research on whether animals are self-aware and recognize themselves as individuals separate from conspecifics has relied upon the mirror recognition task. However, animals relying on different sensory modalities may use non-visual means of self-recognition. Chemical cues involving vomerolfaction are important components of behavior among squamate reptiles, especially snakes. Past studies have suggested that several species of snakes and lizards recognize siblings and others show they can discriminate chemicals deposited by themselves from conspecific or clean control stimuli. While suggestive, these studies lacked controls, such as for diet and genetics. We carried out a study on 24 juvenile common gartersnakes, *Thamnophis sirtalis* (12 of each sex), from a single litter. We manipulated substrate chemicals in test arenas using clean or previously occupied cage liners and measured several behaviors including frequency of tongue flicking and general activity. Each snake was tested under four conditions: one's own substrate, substrate of a same sex littermate fed the same or different diet (fish or worm), or a control clean substrate. Trials lasted 30 minutes, were video-recorded, divided into three 10-minute segments, and both tongue flick and activity counts tallied for each segment and inter-observer metrics calculated. We found that both tongue flick and movement rates were lower in the own chemical vs the control condition, replicating prior findings. We also found sex and diet differences in responses to own vs conspecific chemicals of littermates fed identical diets. These data appear to be the first showing pre-reproductive sex differences and further support the chemical self-recognition hypothesis.



## Behavioral Changes throughout the Span of *Batrachochytrium salamandrivorans* (*Bsal*) Infection in Various Salamander Species

**Burghardt G<sup>1</sup>**, Partin A<sup>1</sup>, Steele J<sup>1</sup>, Hartline K<sup>1</sup>, Wallace S<sup>1</sup>, Free A<sup>1</sup>, Carter E<sup>1</sup>, Cusaac J<sup>1</sup>, Miller D<sup>1</sup>, Gray M<sup>1</sup>

<sup>1</sup>University Of Tennessee

The chytrid fungus has decimated populations of amphibians around the world. A related fungus, *Batrachochytrium salamandrivorans* (*Bsal*), is also deadly to amphibians, especially salamanders. Originating in Asia, it has destroyed populations in Europe, but has not yet reached the Americas. In a proactive move, researchers are trying to determine which native amphibians are at risk. However, behavior changes accompanying the progression of the disease have not been studied. We developed a method to monitor behavior of individually housed control and infected animals ( $10^3$  to  $10^6$  fungal spores/10mL at 15C). Cameras were programmed to take time-lapse photos at 60-second intervals inside secured environmental chambers over the period during which susceptible animals will develop symptoms and die. Photos were viewed to assess number of behavioral changes and whether animals were or in or out of the provided cover object. Inter-rater reliabilities were high. Based on movement and exposure data gathered from the photos, we found that changes in movement and time undercover were detectable, often before animals exhibited physical signs of the disease. For example, *Eurycea wilderae* showed both increased time under cover and decreased locomotion and posture changes with increasing infection load. Behavior may be useful in future studies of this potentially devastating threat to American, and especially Appalachian, hotspots of salamander diversity.

**Indirect terrestrial transmission of chytrid fungus from reservoir to susceptible host species causes fatal chytridiomycosis**

**Burns T**<sup>1</sup>, Scheele B<sup>2</sup>, Brannelly L<sup>3</sup>, Clemann N<sup>4</sup>, Driscoll D<sup>1</sup>

<sup>1</sup>Deakin University, <sup>2</sup>Australian National University, <sup>3</sup>University of Melbourne, <sup>4</sup>Arthur Rylah Institute for Environmental Research

Amphibian chytrid fungus has had a devastating impact on biodiversity; causing the decline or extinction of over 500 species. Yet, understanding of chytrid transmission pathways remains incomplete, in particular for species with low aquatic associations and between reservoir and susceptible hosts. We examined chytrid transmission from a potential reservoir host to a potentially susceptible host; assessing directly the capacity of the former to transmit chytrid and the susceptibility of the latter as a chytrid host. Using cohousing vs sequential use of the same enclosure by the two species, we distinguished the effects of direct vs indirect chytrid transmission. We found that direct and indirect terrestrial transmission from a reservoir to susceptible host species can result in fatal chytridiomycosis and that mode of transmission had no effect on overall morbidity or disease progression. Our results demonstrate that reservoir and susceptible hosts need not be in the same place at the same time or within an aquatic environment for transmission to occur. Our work has implications for the management of species threatened by chytrid fungus, particularly those with low aquatic associations (28 % of the total species impacted by chytrid) or sharing a landscape with a chytrid reservoir host.

## The Contribution of Wetland Succession to Declines of Threatened Amphibians

**Burrow A<sup>1</sup>**, Maerz J<sup>1</sup>

<sup>1</sup>*University of Georgia*

Prescribed fire is an effective forest management tool for reinstating historic disturbance regimes. However, the necessary precautions precluding summer burning limit fire's restorative effects to terrestrial zones and remnant isolated seasonal wetlands are often overgrown and hydrologically altered due to fire exclusion or incompatible, cool season fire regimes. In the absence of warm season fires when wetlands are dry, shrubs and trees succeed herbaceous plants, which alters wetland productivity via effects on light and detritus quality. In turn, wetland productivity determines larval amphibian performance including survival and size at metamorphosis, which are large determinants of amphibian population growth. Therefore, the objectives of our work are to quantify the effects of hardwood encroachment on the growth, development, and survival of tadpoles of two threatened amphibians of the Southeastern United States, the gopher frog (*Lithobates capito*) and ornate chorus frog (*Pseudacris ornata*). Tadpoles were reared in outdoor aquatic mesocosms containing either leaf litter of an encroaching hardwood or a grass/sedge with and without a shading treatment. Results for *P. ornata* suggest that survival was minimally affected by light and litter treatments. However, larval periods were significantly increased in shade while mass decreased with shading and hardwood litters. Results for *L. capito* suggest that survival tends to decrease in shade and hardwood litters. Larval periods were significantly increased in hardwood litter and shading while mass decreased with shading. Our results suggest that prescribed fire regimes which exclude wetland zones may negatively impact amphibian populations through reduced survival, prolonged development and reduced mass.

**Amphibian “silent spring”: the catastrophic collapse of a mega-diverse community at La Planada Reserve, Colombia.**

**Burrowes P<sup>1</sup>**, De la Riva I<sup>2</sup>

<sup>1</sup>University Of Puerto Rico, <sup>2</sup>Museo Nacional de Ciencias Naturales

We re-visited La Planada in April 2019, a site where Burrowes conducted an extensive ecological and taxonomic study of the amphibian community in 1986, and where the presence of armed guerillas discouraged further herpetological studies. Our objective was to assess the status of the amphibians 33 years later, after modern stressors like climate warming, emergent infectious pathogens (e.g. *Batrachochytrium dendrobatidis* =*Bd*), and war may have affected this highly threatened class of vertebrates. We found that the forest is intact, but the decline of the amphibians has been drastic in diversity and abundance. Over 70 % (29/41) of the species have not been observed in recent surveys, and the relative abundance in the forest has declined by 98 %. Missing species include some in the families Bufonidae, Craugastoridae, Dendrobatidae and Hemiphractidae. Preliminary molecular screening revealed that *Bd* is currently present in the reserve, but thus far we have not found *Bd*-positive's among museum specimens collected between 1984–1986. While the average minimum temperature has increased significantly, the maximum temperature has decreased rendering a narrower daily range that would be more favorable for chytrid growth. Ongoing studies include continuous monitoring of amphibians, present and historical sampling of diseases, as well as, experiments on the thermal physiology of key extant species to determine their vulnerability to climate change. Past and present data from La Planada provide a framework to test theoretical models that predict the impact of climate warming and the effect of epidemics on an amphibian community during the Anthropocene.

**Predicting Future Range Shifts of a Stream Indicator Species, *Rhyacotriton variegatus*, Under Climate Change.**

**Bury G<sup>1</sup>**, Bury R<sup>2</sup>, Baggett C<sup>1</sup>

<sup>1</sup>Oregon State University, <sup>2</sup>Emeritus, USGS

Models that predict the potential range of indicator species under climate change could model the function of ecosystems. Before such models can be used with confidence, it is important to understand the mechanisms of physiological tolerance of species. We used field studies to find the thermal limits of the range of *Rhyacotriton variegatus*, laboratory experiments to determine the thermal tolerance of multiple life stages and sublethal heat effects, and input these data into the spatially explicit models. We used a multi-model approach with multiple representative concentration pathways (RCP). Our modeling included not only a MaxEnt model with 14 abiotic variables, but also a MaxEnt model with only the yearly maximum temperature change between scenarios, and a mechanistic model. The proportion of the modeled area that the models classed as excellent habitat declined with higher RCP and over time, with substantial differences between coupled climate models. Indicator species are important tools, but to be used well, we need to know specific physiological tolerances, range extent, environmental requirements, and then apply this knowledge to modeling efforts. Our data supports further research into indicator species in general, and *R. variegatus* as a cold water obligate in particular.

## Morphology, Performance, and Ecology of Three Sympatric Turtles in a Tropical Dry Forest

**Butterfield T<sup>1</sup>**, Olson M<sup>1</sup>, Beck D<sup>2</sup>, Macip R<sup>1</sup>

<sup>1</sup>*Universidad Nacional Autónoma De México*, <sup>2</sup>*Central Washington University*

Categorizing a turtle's ecology based on inferences from the literature has been fundamental in documenting general patterns of turtle form-function. Despite the utility of categorizations like "aquatic" or "omnivorous", it seems clear that classifications may not always capture that turtle lifestyles form a continuum. Therefore, it is important to develop a quantitative vocabulary that reflects biologically important diversity in turtle form-function. To do so, we focus on the three species (*Rhinoclemmys rubida*, *R. pulcherrima*, and *Kinosternon chimalhuaca*) that co-occur in the dry forests of western Mexico. We compare conclusions drawn regarding turtle lifestyles in this area based on traditional classifications versus those based on continuous measures of habitat, diet, morphology, and performance. Habitat was measured by documenting the frequency that each turtle species occurs in habitats that represent the elevational gradient in the forest, and diet using stable isotopes. We compared morphology and maximum swimming speed to see whether differences in habitat and diet were related to quantitative differences in morphology and performance. We found that continuous differences in habitat are associated to limb and shell morphology, and swimming performance. Relationships between morphology and diet were less clear, which might be the result of changes in behavior or habitat rather than morphology. In this study system, morphology could be used as a quantitative measure to represent habitat differences between turtle species. We conclude that a finer understanding of morphology and performance can be used to account for subtle differences observed in the habitat and diet of co-occurring turtle species.

## Genetic benefits of extreme sequential polyandry in a terrestrial-breeding frog

**Byrne P<sup>1</sup>**

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Sequential polyandry may evolve as an insurance mechanism to reduce the risk that females choose mates that are genetically inferior (intrinsic male quality hypothesis) or genetically incompatible (genetic incompatibility hypothesis). The prevalence of such indirect benefits remains controversial, however, because few studies have estimated the contributions of additive and non-additive sources of genetic variation to offspring fitness in species with sequential polyandry. Here, we use artificial fertilisation techniques combined with a cross-classified breeding design (North Carolina Type II) to simultaneously test the 'good genes hypothesis' and the 'genetic incompatibility hypothesis' in the brown toadlet (*Pseudophryne bibronii*); a terrestrial breeding species with extreme sequential polyandry. Our results revealed no significant additive or non-additive genetic effects on fertilisation success. Moreover, they revealed no significant additive genetic effects, but highly significant non-additive genetic effects (sire by dam interaction effects), on hatching success and larval survival to initial and complete metamorphosis. Taken together, these results indicate that offspring viability is significantly influenced by the combination of parental genotypes, and that negative interactions between parental genetic elements manifest during embryonic and larval development. More broadly, our findings provide novel quantitative genetic evidence that insurance against genetic incompatibility favours the evolution and maintenance of sequential polyandry.

**Utilizing do-it-yourself open-source technologies to make easier the life of the field herpetologist**

**Cain P<sup>1</sup>**

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Field herpetologists are commonly required to fix, slice, hack, and modify field equipment to meet the needs of their research question. Not only does much of a field herpetologist's training lack formal introduction to such methods, the cost of commercially available technology may also preclude certain unique or informal approaches to answering biological questions. Here, I present a primer on ways one can learn to become more familiar with and skilled at modifying common electronic field equipment. More specifically, I will introduce how open-source hardware and software (e.g. Arduino, Raspberry Pi, and Python) and the thriving do-it-yourself (DIY) community can help facilitate such skills. With just a cursory understanding of electrical engineering, soldering, circuits, and coding, field herpetologists can overcome many of the limitations of cost, and even develop customized field equipment themselves. I will briefly discuss few examples of open-source do-it-yourself equipment such as environmental loggers, GPS data loggers, and VHF systems (transmitters, receivers, and antennas).



## **Applying SNP-derived genotyping to the captive management of the endangered Mountain Yellow-legged frog.**

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<sup>1</sup>*San Diego Zoo Institute for Conservation Research*, <sup>2</sup>*Native Animal Researchers and Conservationists (NARC)*

Conservation breeding and reintroduction programs designed to reestablish wild populations are an important tool for recovering at-risk species. These programs rely on an understanding of genetics for managing population viability of both the captive population and the remaining wild populations. The mountain yellow-legged frog (*Rana muscosa*) program breeds and reintroduces captive-born frogs from three distinct populations, the San Gabriel, San Jacinto and San Bernardino Mountains. In the absence of genetic data, these populations have been managed separately, but extremely low numbers of frogs remaining in one population suggests that genetic rescue may be required to increase genetic diversity. Initially, data obtained from nine polymorphic microsatellite markers were used to evaluate population differentiation, genetic structure and heterozygosity of the captive populations. However, this method does not provide the genetic resolution necessary to estimate kinship, degree of relatedness or population differentiation. Assumptions that have driven MYLF management and reintroduction. were reassessed using restriction site associated DNA marker analysis. Founders and first-generation offspring from San Bernardino and San Jacinto populations were included, as well as wild samples from all three populations. Relationships from pedigree data were genetically validated and relatedness coefficients estimated among all captive frogs. We identified differences between the two captive populations in terms of genetic structure, population differentiation and heterozygosity. High levels of inbreeding in both captive frog populations, and low gene flow were identified between the San Bernardino and San Jacinto populations raising questions about appropriate strategies to keep these populations healthy and still contributing to wild augmentation.

**Spermiation and changes in serum hormone levels in the mountain yellow-legged frog, *Rana muscosa*.**

A B<sup>4</sup>, A M<sup>4</sup>, Calatayud N<sup>1,2</sup>, Curtis M<sup>1</sup>, Durrant B<sup>2</sup>, Shier D<sup>1,3</sup>, Swaisgood R<sup>1</sup>

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Sperm production in mountain yellow-legged frog (MYLF) males after exogenous hormone treatment is affected by season. A previous study established that spermiation is easily stimulated during the reproductive period but is followed by a period when the testes are unresponsive to hCG and GnRH stimulation. This study investigated the baseline and seasonal changes in reproductive hormone levels in young (1-4 yr) and adult (7-12 yr) males. Serum corticosterone, estriol, estrone, 17 $\alpha$  ethinylestradiol (17 $\alpha$ -EE), estradiol, progesterone and testosterone were measured by mass spectrometry. Preliminary findings indicated that total estrogens and testosterone were highest in young frogs. All males, regardless of age had seasonally associated changes in hormones. In adults, total estrogens were highest post-breeding when males are least likely to respond to artificial stimulation. In these mature frogs testosterone and corticosterone were highest during the reproductive period, consistent with the onset of reproductive behaviours. Additionally, we examined changes in hormone levels after inducing spermatogenesis by administration of different doses and combinations of hCG and GnRH. Monthly injections were administered from October-November 2016 and April-July 2017 and corresponding blood samples were collected at 0 h and 3.5 h post-injection. Increases in corticosterone and estrogen were detected within 3.5 h of injection while testosterone remained below detectable levels in males 3-12 years. These results support previous findings that, MYLF males have seasonal-associated changes in circulating sex hormone levels throughout the year. Although more research is required, preliminary findings indicate that induced spermiation is accompanied by changes in corticosterone and estrogen levels.

### **Outcome bias of auditory survey methods may negatively affect detectability of anurans**

**Callen A<sup>1</sup>**, Clulow J<sup>1</sup>, Clulow S<sup>2</sup>, Pizzatto L<sup>1</sup>, Stockwell M<sup>1</sup>, Mahony M<sup>1</sup>

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Traditional anuran survey methods such as visual encounter and capture-mark-recapture yield detailed data on population dynamics such as distribution, abundance and demography. By contrast, impact and biodiversity assessment guidelines often focus on auditory surveys using male calling to determine species' presence. Bioacoustic monitoring was used in this study to describe the seasonal and circadian calling phenology of a threatened Australian anuran, *Litoria aurea*, and compared with extant auditory survey guidelines. Calling data was combined with weather variables to develop a predictive model for seasonal and daily calling profiles under mean climatic and rainfall conditions. The probability of detecting males calling was greatest between midnight and 3am, several hours outside of the typical 'just after dark' recommended survey time. Mean daily air temperatures above 21.7°C were also shown to negatively correlate with the number of calling days across the breeding season. This was unexpected as anecdotally the species has been observed to increase attendance at wetlands in warmer months, which for many ecologists translates into an expectation of increased detection probability. Therefore, peak auditory detection for this species lies outside of current survey methodology recommendations, creating an outcome bias that may negatively affect detectability. As these methods are frequently used to underpin conservation studies or development/mitigation action, under-estimated detections caused by inappropriate sampling may pose a serious risk for anurans where calling phenology is poorly understood. These results confirm bioacoustic monitoring can refine predictions of the most suitable environmental conditions under which to survey, improving the overall conservation efforts for species.

### **When the world falls in love, case study of a small frog going global**

**Camacho Badani T<sup>1,2</sup>**, Jordan C<sup>3</sup>, Moore R<sup>3</sup>, Renick Mayer L<sup>3</sup>, Barrón Lavayen S<sup>1,2</sup>, Zurita Ugarte R<sup>1,2</sup>

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In 2008, after noticing a steep decline in wild populations, we collected a single male Sehuencas Water Frog (*Telmatobius yuracare*), dubbed Romeo, from a Bolivian cloud forest and introduced him into the captive breeding center of the Museum of Natural History Alcide d'Orbigny in Cochabamba, Bolivia. Despite significant effort, biologists were unable to find additional Sehuencas Water Frogs. Ten years later on Valentine's Day, 2018, Global Wildlife Conservation and the Museum teamed up with Match.com to launch a fundraising campaign to find a Juliet for Romeo, the world's loneliest frog, that included a dating profile for Romeo on the popular dating website. The campaign surpassed fundraising targets and resulted in significant global media attention. In December of 2018, a team from the Museum found Juliet and 4 additional individuals in a stream along the border of Bolivia's Carrasco and Amboró National Parks. Then the celebrity amphibian couple went viral. As of early February 2019 we had 1,244 media hits in at least 72 countries, for a reach of ~1.1 billion people and an ad value of US\$11 million. After being cleared of chytrid, Romeo and Juliet are now living together in our captive breeding center. With a strong communications platform from which to communicate about our advances, we are now embarking on a long-term holistic project to bring this species back from the brink from extinction. In this presentation we describe the strategies we used to make the world fall in love with a small water frog from Bolivia.

### **The critically endangered Titicaca water frog: Bolivia and Peru conservation efforts**

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The Andean genus *Telmatobius* is considered one of the most threatened in South America, among the most representative species is the Lake Titicaca water frog (*Telmatobius culeus*), considered the largest water frog in the world, is noted for its morphological adaptations as physiological to their particular lifestyle at the bottom of Lake Titicaca and surrounding lagoons at 3800 meters above sea level in the department of La Paz in Bolivia and Puno in Peru. It has been categorized as critically endangered, mainly due to over exploitation, contamination by pesticides, dumping of waste from mines and sewage, among other threats. In both Bolivia and Peru, several institutions have worked on the conservation of this species, including the Universidad Peruana Cayetano Heredia, which since 2007 is working together with other institutions in Peru, such as the Denver Zoological Foundation from USA, for conservation of the Titicaca water frog and in Bolivia the Natural History Museum Alcide d'Orbigny worked with several projects since 2008 and now with the K'ayra Center that depends on the Museum, it is the only amphibian rescue center in the country. However, this is the first time that an agreement has been reached between institutions of both countries to unify methodologies, share information, hold workshops and conserve the species as a single team and have an effective conservation, following the guidelines of binational commitments to which the governments of Bolivia and Peru have committed themselves to the conservation of the Titicaca water frog.

## **Limb reduction in skinks (Squamata: Scincidae): a comparative analysis based on morphological literature**

**Camaiti M<sup>1</sup>**, Aguilar R<sup>1,2,3</sup>, Evans A<sup>1</sup>, Hipsley C<sup>2,3</sup>, Chapple D<sup>1</sup>

<sup>1</sup>*Monash University*, <sup>2</sup>*University of Melbourne*, <sup>3</sup>*Museums Victoria*

One of the most dramatic examples of morphological change in animals is the transition from fully-limbed to limb-reduced or limbless body shapes in squamates. Among all squamates, skinks (Scincidae) have one of the highest recorded frequencies of independent occurrences of limb reduction and loss, also displaying all the possible degrees of limb reduction, sometimes even within the same genus. Here we provide a large-scale analysis of the morphology of limb-reduced and limbless representatives of the family Scincidae. We use the literature to create a database that organizes taxa according to their respective degrees of limb reduction. Subsequently, we set up a comparative analysis to identify the evolutionary trends and patterns of limb reduction and loss within the major lineages of skinks. One major takeaway from this work is the assessment of the existing historical gap between traditional morphological studies, such as external observations and measurements, and more modern approaches to studying morphology such as those involving geometric morphometrics and 3D computed tomographies. Moreover, since it provides for the first time a clear comparative analysis of all the known limb-reduced skink taxa and quantifies their different degrees and patterns of limb reduction, this study addresses some of the unanswered questions relevant to the field and the knowledge gaps that need to be filled for future research in reptile morphology.

**Winter is coming: Temperature dependent virulence of *Batrachochytrium salamandrivorans***

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*Batrachochytrium salamandrivorans* (*Bsal*) is a recently emergent fungal pathogen that has caused dramatic population declines of European fire salamanders (*Salamandra salamandra*). Preliminary risk analyses suggest temperature will be an important factor driving *Bsal* invasion in the United States. In order to understand how *Bsal* virulence and host susceptibility are affected by temperature we performed challenge experiments at three environmentally relevant temperatures. We exposed eastern newts (*Notophthalmus viridescens*) to a sham inoculation of autoclaved water (control) or one of four *Bsal* zoospore doses ( $5 \times 10^3$ - $6$ ) at one of three ambient temperatures, 6 C, 14 C or 22 C. We monitored animals twice daily for signs of disease. We swabbed each animal weekly to monitor infection intensity via qPCR. At 14 C, all newts became infected and died within one-month post-exposure. Median survival duration at 14 C was 26, 20, 14 and 7 days for  $5 \times 10^3$ ,  $5 \times 10^4$ ,  $5 \times 10^5$  and  $5 \times 10^6$ , respectively. No individuals became infected or died when exposed at 22 C. At 6 C, results were intermediate, with survival durations approximately two weeks longer than 14 C. Newts that died from chytridiomycosis at 6 C had lower zoospore loads than at 14 C, suggesting lower infection tolerance at lower temperature. Our results suggest that *Bsal* invasion in the United States may be geographically narrower than indicated in previous risk models, with greatest likelihood of emergence occurring at mid- to northern latitudes and higher elevations. Seasonal fluctuations in disease emergence also are expected, with autumn and winter months experiencing greatest mortality.

## **Breadth of the thermal response captures individual and geographic variation in temperature-dependent sex determination**

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Population-scale responses of key ecological traits to local environmental conditions provide insight into their adaptive potential. In reptiles with temperature-dependent sex determination (TSD), short-term, individual developmental responses to the incubation environment have long-term consequences for populations. We took a model-based approach to studying within and among-population variation in the physiological components of TSD in 14 populations of painted turtles (*Chrysemys picta*), a semi-aquatic turtle with a broad, endemic North American distribution. We used laboratory and field incubation data to quantify variation in thermal reaction norms at both population and clutch scales, focusing on the pivotal temperature ( $T_{piv}$ ) that produces a 1:1 sex ratio and the transitional range of incubation temperatures (TRT) that produces mixed sex ratios. Defying theoretical expectations, among-population variation in  $T_{piv}$  was not convincingly explained by geography or local thermal conditions. However, within some populations,  $T_{piv}$  varied by  $> 5^{\circ}\text{C}$  at the clutch scale, indicating that the temperature sensitivity of gonadal differentiation can vary substantially among individual nesting females. In addition, the TRT was wider at lower latitudes, suggesting responsiveness to local incubation conditions. Our results provide a potential explanation for discrepancies observed between constant-temperature experimental results and outcomes of fluctuating incubation conditions experienced in natural nests, exposing important knowledge gaps in our understanding of local adaptation in TSD and identifying shortcomings of traditional lab studies. Understanding individual variation and the timing of gonadal differentiation is likely to be far more useful in understanding local adaptation than previously acknowledged.



## Modeling incubation microclimates to predict impacts of climate change on embryonic development in tuatara

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A key aim of contemporary ecology is understanding how populations respond to environmental variation, especially rapid climate warming. While climate change is typically modelled on a continental or global scale, organismal responses can depend on complex, physiology-microenvironment interactions that manifest at both population and individual levels. In reptiles with temperature-dependent sex determination (TSD), fluctuating temperatures directly affect both embryonic development and gonadal differentiation. However, predictive models that rely on broad-scale climate data do not capture the microclimate-scale processes that drive these developmental responses. Here, we used a spatially-explicit model of embryonic development, driven by NicheMapR, a mechanistic microclimate model, to examine the fine-scale consequences climate warming for embryonic development in two wild populations of tuatara (*Sphenodon punctatus*), a New Zealand-endemic reptile with TSD. We found that a +4°C change in mean annual air temperatures - the magnitude of climate warming predicted by 2100 - would likely result in offspring sex ratios approaching 100% male, regardless of geographic location. In addition, warming could de-couple the currently-tight relationship between egg-laying date and sex ratio, creating an ecological trap. Changes in nesting phenology or in the location or depth of nests are unlikely to compensate for these effects. Our results not only provide insight into the potential impacts of climate change on an iconic New Zealand reptile but also highlight the necessity of quantifying those impacts at the spatial and temporal scales where they will actually affect organismal physiology.

## The undescribed frog diversity of the Australian Monsoonal Tropics

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The Australian monsoonal tropics represents one of the largest intact tropical savannahs in the world, and has a large number of endemic fauna. Many of these endemic taxa are described as single species that cross the entire ~2,300 kilometre (1,400 mile) region. Recent genetic studies of herpetofauna and other taxa have indicated few of these “wide-spread” species are actually a single species. Frogs from the tropics have been particularly poorly investigated. In part, this is because the vast floods of the wet season, combined with the sparse road network, make much of the region impassible while the frogs are above ground.

We have used a reduced representation sequencing approach to generate large-scale SNP datasets for hundreds of individuals from ~20 species of tropical frogs. Combined with morphological and acoustic data, our results indicate we are likely to at least double the frog diversity in our target species. In this talk, I will give an introduction to the Australian monsoonal tropics (including pictures of amazing places), and an overview of the diversity we are identifying in our tropical frogs. In particular, I will focus on common biogeographic breaks that are likely to be important to future conservation planning.

## Local adaptation to dietary toxins shapes poison frog chemical defenses

**Caty S<sup>1</sup>**, Coloma L<sup>2</sup>, O'Connell L<sup>1</sup>

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Poison frogs sequester alkaloids from their diet of arthropod prey for use as predator defense. These toxins vary across species and across geographically distinct populations of the same species. The extent to which these differences are driven by diet or by the genetically-determined expression of proteins for the uptake, modification and sequestration of specific alkaloids is unclear. We investigated how genetic and dietary influences impact toxin sequestration and modification in the diablito poison frog *Oophaga sylvatica* (Family Dendrobatidae). We sampled frogs from ten populations (nine caught in the field and one raised in the laboratory) across northwestern Ecuador. Using gas chromatography mass spectrometry, we found that toxins differ across populations of *O. sylvatica*, and the toxin profiles of the populations cluster by previously established genetic relationships. Using laboratory reared frogs from three populations, we demonstrate that those populations that are exposed to pumiliotoxin in their native diet are better able to metabolize pumiliotoxin to allopumiliotoxin. Since the liver is likely the site of toxin modification, we examined the liver proteome of each of the ten populations, as well as the three captive raised populations. Within the captive frogs, liver proteome clusters by genetic similarity, even when controlling diet. Across the wild caught populations, we find that liver proteome again clusters by population, however there are some proteins that correlate with the presence of specific toxins. Together, these results provide evidence for local adaptation, where frog populations have adapted to process and metabolize the toxins present in a local environment.

## **Intertwined evolution of swimming, morphology and microhabitat in tree frogs from the subfamily Hylinae**

**Caviedes-Solis I<sup>1</sup>**, Leache A<sup>1</sup>

<sup>1</sup>*University Of Washington*

Multidisciplinary studies including phylogenetic inference, functional morphology and phylogenetic comparative methods are imperative to understand how closely related species evolved. By combining those three approaches we aim to disentangle the evolution of morphological traits and their influence on swimming behavior and performance, both within multiple microhabitats for Neotropical tree frogs. The subfamily Hylinae includes 163 species; they have three main locomotion modes with a direct impact on species fitness: they jump, swim and climb. Convergences in morphotype and locomotion are primarily driven by microhabitat, but in some cases morphology is the result of long-term phylogenetic constraints. Swimming is a critical behavior during life history for predator avoidance, reproduction, and early developmental stages. However, it is still poorly understood how swimming evolved in closely related species with multiple locomotion modes. I included 225 individuals from 16 localities in Mexico, corresponding to 29 species and 14 genera. Our data set includes linear velocity, forelimb and hind limb behavior, and we categorized aquatic and arboreal microhabitats. I estimated the correlations among the evolution of traits along the phylogeny by using the threshold model. I found that Hylinae species use the two types of swimming reported for frogs, alternating gait (considered the primitive condition in frogs) and simultaneously gait (the derived mode). In addition there is a high rate of inter- and intra- specific variation in swimming behavior and performance. These results represent an important contribution to the study of tree frog evolution and habitat selection.

**Threat Assessment of *Taruga eques* (Amphibia: Rhacophoridae) Endemic in Tropical Island; Sri Lanka**

**Prabhath M<sup>2</sup>**, Silva G<sup>1</sup>, Mahaulpatha W<sup>1</sup>

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Parasites, pathogens and predators are major biotic threats for amphibian survival. According to the most of amphibian studies in the field, found that helminthes parasites act characterizing crucial role for vesting physical abnormalities. Even though, parasite- host relationship is a natural biotic interaction operating in the ecosystem, anthropogenic activities and aquatic invertebrates directly and indirectly effect to shift the community composition and lead to increase the prevalence of amphibian abnormalities.

Present study was conducted over one year period of time, including five lentic water bodies within and outside the Horton Plains National Park, assessed on *T. eques* abnormalities through gross visual inspections, utilizing six quadrates of 1m×2m in each sampling site with the purpose of providing precise information to support conservation and management of amphibians.

A total of 347 *T. eques* examined within the Park, thereby 6.34% amphibians were abnormal, including ectromelia (0.58%) and surficial abnormality (5.76%). Moreover, 100% abnormality index was recorded outside park and all of them appertained to cysts of *T. eques*. Most of the infections were assayed hind limb region of juvenile stage amphibians. However, 60% of mild and 77.55% of moderate infection severities were predominant consecutively within and outside the park.

The present study reveals that helminthes parasites are major threat for *T. eques* species and their affect is different on life stage and habitat conditions. Moreover, research findings toughly disclose that necessity of mandating urgent measures to carryout for conservation of *T. eques* species.

**Reproductive Habitat Use of Endangered *Minervarya greenii* (Amphibia:Dicroglossidae) in montane cloud forests of Sri Lanka**

**Chamara Prabhath M<sup>1</sup>**, Jayasekara E<sup>1</sup>, Mahaulpatha W<sup>1</sup>

<sup>1</sup>*Department Of Zoology, University Of Sri Jayewardenepura*

*Minervarya greenii* is an endemic endangered amphibian species restricted to montane areas of Sri Lanka and its' population is also declining. There is no available information on its reproduction. Current study addresses its reproductive habitat use which can be used effectively in selecting habitats to breed this species in captive and also in initiating conservation plans. Sampling was conducted in lentic and lotic water bodies in Horton Plains National Park, Sri Lanka from January to December in 2016. 1m\*1m quadrates were placed encircling calling males and couples in amplexus. In each quadrate habitat characteristics (temperatures, humidity, substrate covers etc) and reproductive behaviours (calling, courtship, amplexus) were recorded. Data were statistically analysed. Current study revealed that *M. greenii* is a reproductive habitat specialist and its reproduction is restricted to lentic water bodies in HPNP. It breeds throughout the year, hence it is a continuous breeder. Water and ambient temperatures, relative humidity and moisture, water depth and submerged plants collectively form reproductive habitats which are used to perform reproductive behaviours. Current study provides detailed description of the reproductive habitat of *M. greenii*, which can be used in captive breeding and this research also highlights the importance of lentic water bodies as target areas in conservation plans. Furthermore, this study indicates the potential threats of invasive Rainbow trouts and climate changes for this species.

## Phylogenetics and undiscovered diversity of the Mexican leopard frogs

**Chambers E<sup>1</sup>**

<sup>1</sup>*University Of Texas At Austin*

In Mexico alone, 58% of amphibians are threatened or have already been lost to extinction. Included in this estimate are six species of leopard frogs (subgenus *Pantherana*) that are classified as endangered, with three other species having already gone extinct. Currently, there are 28 recognized leopard frog species distributed widely from Canada to Panama, although the species boundaries in the group are poorly understood in these regions. Despite the conservation concern of Mesoamerican leopard frogs, little taxonomic work has been performed on the group in recent years. The last study with widespread sampling on the group was in the 1980s, where researchers noted that there appeared to be at least four undescribed species distributed across Mexico. These purported species have been left undescribed since this work was conducted. Here, we explore this undiscovered diversity in the group in Mexico using samples collected within the past several decades, with heavier sampling in contact zones between potentially undescribed species, to determine this undescribed diversity. Additionally, we explore two other instances of potentially undiscovered diversity: in the single widespread species that occupies much of the Pacific coastal lowlands, *Rana forreri*, as well as the central Mexico high elevation endemic, *Rana spectabilis*. To do so, we performed reduced representation genome sequencing across the complex using double digest restriction-site associated DNA sequencing (RADseq), and conducted additional analyses at contact zones to provide an updated examination of the phylogenetic relationships for the leopard frogs of Mexico.

## Lessons learned from 10 years of Eastern Indigo Snake (*Drymarchon couperi*) conservation

Chandler H<sup>1</sup>

<sup>1</sup>*The Orianna Society*

Eastern Indigo Snakes (*Drymarchon couperi*) are the longest snake species native to the United States and have been listed as Threatened under the U.S. Endangered Species Act since 1978. The Orianna Society was founded in 2008 with a mission to conserve indigo snake populations across their range, using a combination of science and active management. Over the past 10 years, research projects examining indigo snake home ranges, movements, habitat selection, population persistence, genetics, and diseases have been completed. These projects have dramatically increased our understanding of the species' biology, leading to better survey techniques that have been used to identify previously unknown populations. Refined survey methodologies have now been incorporated into a long-term monitoring project for indigo snake populations. This increased the detection probability for indigo snakes to over 50% in the northern portion of their range. In addition to research and monitoring projects, prescribed fires are now applied to tens of thousands of acres of Longleaf Pine forests each year. Indigo snakes are dependent on this ecosystem and have benefited from an increased focus on burning sites to promote habitat quality (i.e., growing season fires). Habitat management is now successful enough to pursue reintroductions of indigo snakes on sites where populations were historically extirpated. Over 150 snakes have been bred in captivity and released at two sites in the western portion of their range. Together, these efforts highlight how an integrated approach of research, land management, and captive breeding can promote the conservation of an imperiled snake species.



## History and geography resolve a problem in African Sand Frog taxonomy (*Tomopterna*)

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The Natural History Museum in Berlin appointed MHC Lichtenstein as its director in 1811. Lichtenstein approved funding for Ludwig Krebs to collect specimens in South Africa from 1821–1839, which were subsequently sent to Berlin and sold to generate funds for the museum. Krebs bought a farm in the Eastern Cape of South Africa, which he named "Lichtenstein" in 1832 in honour of his benefactor. Kurt Deckert, based at the Berlin museum, described *Arthroleptella ahli* from "Lichtenstein, South Africa" in 1938. The specimen is not extant, but the description refers to a Sand Frog, in the genus *Tomopterna*. As it was attributed to a locality in the Eastern Cape Province of South Africa, it was synonymised with *Tomopterna cryptotis* (Boulenger, 1907) or *T. delalandii* (Tschudi, 1838).

The specimen that Deckert described was collected by Fritz Kunze, and collected on the farm Lichtenstein. However, Kunze was a hunter who travelled extensively in Namibia (then German South West Africa), and who sent mammal, reptile and amphibian specimens to the Berlin Museum, many from the farm Lichtenstein situated just outside Windhoek, the capital of Namibia. Deckert had mistakenly attributed the material to Lichtenstein in South Africa.

The confusion caused by two farms named Lichtenstein, both with extensive links to the Berlin Natural History Museum, resulted in the misidentification of this specimen for nearly a century.

We demonstrate that *Tomopterna ahli* (Deckert, 1938) is a senior synonym of *Tomopterna damarensis* Dawood & Channing, 2002.

## **Conservation status of the world's skinks: geographic and taxonomic patterns in extinction risk**

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Skinks (Family Scincidae) are the world's most diverse terrestrial vertebrate family, and comprise ~26% of the global lizard fauna (~1,675 of 6,512 described species). Skinks have a global distribution on all continents except Antarctica, with Australia and tropical regions of the Old World being particularly diverse. With the recent establishment of the IUCN SSC Skink Specialist Group, we conducted the first global review and synthesis of the conservation status of skinks. To date, 1,199 species (71.6%) have been assessed against IUCN Red List criteria, with a further 206 species (12.3%) considered data deficient. Of those species assigned a Red List category, 9 are listed as Extinct, 1 as Extinct as the Wild, and 221 (18%) as threatened (65 CR, 78 EN, 78 VU). 60 species are listed as Near Threatened (5%), and 908 as Least Concern (76%). 476 species (28%, including DD species) are yet to be assessed against Red List criteria. We use this knowledge of the conservation status of the world's skinks to investigate the taxonomic and geographic patterns of extinction risk. Our analyses shed light on the species, regions, and threats in most urgent need of conservation intervention.

### Cryptic diversity and evolution of Asian horned toads (Megophryidae)

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Southeast Asia and southern China harbor a highly diverse and endemically important flora and fauna that is under increasing threat. An understanding of the property, history and drivers of this diversity is lacking, especially in some of the most diverse groups. Asian toads of the family Megophryidae Bonaparte, 1850 exhibit much diversity, making the family an ideal study group to test evolutionary hypotheses. However, the wide distribution and high species richness present a challenge when attempting a comprehensive assessment. Among the species, *Megophrys* and *Leptolalax* are the two most intractable groups. For the group *Megophrys*, our analysis showed that multiple mutually exclusive, geographically cohesive major clades exist in it. Analyses reveal statistically significant mito-nuclear discordance. The geographic separation among clades reflects several important biogeographic barriers. All analyses resolve paraphyly for horned toads as the genus *Ophryophryne*, *Brachytarsophrys* and *Borneophrys* are embedded in it and a revised classification is proposed. In addition, the analysis indicates the presence of 20 putative undescribed species. For the group *Leptolalax*, molecular analyses reveal 16 undescribed species. Our phylogeny shows that *Leptolalax* is not monophyletic with respect to *Leptobranchella* and a revised classification is proposed. The diversification pattern of the group is complex, involving a high degree of sympatry and prevalence of microendemic species. Northern Sundaland (Borneo) and eastern Indochina (Vietnam) appear to have played pivotal roles as geographical centers of diversification. Analyses fail to reject an “upstream” colonization hypothesis, and, thus, the genus appears to have originated in Sundaland and then colonized mainland Asia.

## Intraspecific differentiation and speciation in three viviparous *Phrynocephalus* lizards in the northeastern Qinghai-Tibet Plateau

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The processes and driving force of speciation and population differentiation continue to be an important topic in evolutionary biology. Being the world's highest and largest plateau, the Qinghai-Tibetan Plateau (QTP) is well suited for studying intraspecific differentiation and speciation due to its highly heterogeneous topography and complex paleoclimate history. Here, we sequenced one nuclear and three mitochondrial genes of 532 individuals from the entire range of three closely related viviparous *Phrynocephalus* species (*P. guinanensis*, *P. putjataia* and *P. vlangalii*) restricted to the northeastern QTP. Mitochondrial data indicated two major clades [PV (*P. vlangalii* only) and MIX (*P. guinanensis*, *P. putjataia* and *P. vlangalii*)], of which each had two subclades. The haplotypes from localities occupied only by *P. guinanensis* could be distinguished from the nearest population of *P. putjataia*, which indicates an early stage of speciation. Several mountains, geographical distance and environmental differentiation were found to serve as the barriers to genetic exchange among populations and species. Species distribution models predict that all the three species have experienced demographic expansion from the LGM to present and will undergo contraction from present to the period of 2061-2080 as the consequence of rapid climatic changes in the next decades. In conclusion, our study showed that the geological and climate change following the orogeny (uplift) of QTP may have promoted intraspecific differentiation and speciation of viviparous *Phrynocephalus* species, resulting in the current spatial pattern of species distribution on the plateau.

## Parasites of invasive amphibians and where to find them

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Alien species invasions pose major threats to biodiversity conservation. Among all the impacts, negative or positive, direct or indirect, caused by alien species invasions, parasites are often overlooked. When alien parasite invades along with their host, it may infect local naïve host and result in parasite spill-over, subsequently, threaten the local biota. On the other hand, alien host may acquire local parasites from invaded sites. This so-call parasite spill-back may jeopardize the anticipated long-term relationships between local parasites and their hosts. Herein, we examined the banded bullfrogs (*Kaloula pulchra*) which originated from southeastern Asia were first recognized in Taiwan two decades ago, to determine their parasitic infections. We compared the parasite communities of these invasive frogs with those from the most abundant and widespread native amphibian, the Asian toads (*Duttaphrynus melanostictus*). Based on these comparisons, we tested the hypothesized emery released, spill-over, spill-back as well as the potential assembling process for the parasite communities from these invasive frogs.

## Effects of polyandrous mating on fertilization, embryo survival and offspring development in the emerald treefrog

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Simultaneous polyandry, in which a female has more than one male mate at the same time, has been reported in many anuran species. However, an increasing number of mates may raise costs to the female. Several hypotheses predict that females gain benefits to compensate for the costs of polyandry. The adaptive explanations for females are that polyandry ensures the eggs' fertilization (fertilization insurance hypothesis) and provides genetic benefits to polyandrous females (genetic benefit hypothesis, e.g., genetic bet hedging). Polyandry and multiple paternity has been found in the emerald treefrog (*Rhacophorus prasinatus*). Females choose their mates (primary males) in a lek-chorus; then the mating pairs spawn into foam nests constructed by the females, while unpaired males gather around the mating pairs (peripheral male) and release sperm into the foam nests. In this study, we collected clutches after both monandrous and polyandrous mating from a wild population of the emerald tree frog, and compared fertilization and embryo survival between monandrous and polyandrous clutches. We further analyzed the paternity of polyandrous clutches; then compared the development of primary with peripheral male offspring. The results showed that polyandry did not increase clutches' fertilization and embryo survival, and no developmental difference was found between the offspring of primary males and peripheral males. These results do not support the fertilization insurance or genetic benefit hypotheses, suggesting that simultaneous polyandry is probably driven by alternative mating tactics for males to pursue reproductive success, and that females do not benefit from polyandry in *R. prasinatus*.

## Occupancy modeling as an approach to evaluate effectiveness of conservation translocations in a temperate-zone rattlesnake

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Conservation translocations are used as a tool to augment or reintroduce populations of herpetofauna; however, biologists lack a thorough understanding of how to conduct and evaluate effective translocations, especially with temperate-zone snakes. Standardized estimates of a species' distribution pre- and post-translocation can inform whether or not a project was effective at long-term population establishment. Occupancy modelling has grown in popularity as a means to estimate the distribution of cryptic animals, including snakes, as it incorporates imperfect detection. Rate of detection can be strongly influenced by factors including survey technique and timing, therefore, a comparison of different survey methods would assist in developing the most efficient monitoring regime. We estimated occupancy and detection probability in an endangered population of cryptic North American pit-vipers (Eastern Massasauga, *Sistrurus catenatus*), prior to experimenting with conservation translocations as a recovery tool. Over 6 years, we conducted repeated standardized surveys at 40 sites (~2.0 ha each; 20-30 total surveys in majority of sites) using two methods and in two seasons, and analyzed detection histories using the program Presence. Preliminary analyses suggest that detection probabilities were higher using visual encounter surveys compared to artificial cover objects, and during spring as opposed to summer. Massasauga occupancy was limited to only 10% of survey sites (n=4/40). This study will generate robust baseline distribution data which will be used to inform and evaluate effectiveness of planned conservation translocations to recover an endangered rattlesnake population. It will also provide direction to other conservation practitioners working with small populations of cryptic herpetofauna.

### Upper voluntary thermal limit and water loss in a temperate viviparous gecko: an interactive effect

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Hydric stress is expected to increase with increasing temperature as the climate warms further. Thus, interactions between upper thermal tolerance limits and water availability may aid in predicting responses of ectotherms to increasing temperatures within microhabitats. In this study, we first assessed in the laboratory the upper voluntary thermal limit (VT<sub>max</sub>), and total amount of water loss during heating to VT<sub>max</sub>, of *Woodworthia* "Otago/Southland" geckos. We compared different life-history stages (LHS) and sexes using a protocol developed for a retreat-dwelling species. Then, to establish whether geckos reach VT<sub>max</sub> in the wild, we measured field body temperatures (T<sub>b</sub>) concurrently with microhabitat thermal profiles. Overall, there was no effect of LHS or sex on VT<sub>max</sub>. The VT<sub>max</sub> value increased with body mass and with time to reach VT<sub>max</sub>. However, non-pregnant females lost more water than other LHS during heating to VT<sub>max</sub>. In the wild, geckos reached VT<sub>max</sub> and none were found under thin slabs on hot days. The rock thermal profile exceeded VT<sub>max</sub> in some microhabitats in summer. Our results suggest that geckos will abandon warm retreat sites more frequently if climate warming persists, implying a trade-off between benefits of thermoregulation and risk of predation. Interactions between upper thermal limits and water loss may have large effects on the future activity of ectotherms, especially in temperate climates.



**A Curly Tale: Stress and immunity across nonnative populations of differing ages in *Leiocephalus carinatus***

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The Enemy Release Hypothesis posits that species introduced to a new range are at an advantage because they are removed from their native predators, competitors, and pathogens. In the new range, however, continual exposure to new pathogens may induce a state of inflammation that could prevent individuals from dispersing or reproducing. Newly introduced individuals capable of partially suppressing the inflammatory response may prevent unnecessary reactions to innocuous substances or non-pathogenic bacteria. If the benefits to immune suppression outweigh the costs, and if reproduction can occur before species succumb to an infectious agent, suppressed inflammatory responses may be crucial to the establishment success of a non-native species. If heritable, anti-inflammatory phenotypes may reflect an early-stage invasion, as longer-established populations build an antibody repertoire for the new environment. This mechanism may also determine establishment success of a species via jump dispersal across a new range. Glucocorticoid hormones may also act as indicators of invasion history; they are implicated in other vertebrate range expansions and influence immune reactivity.

We examine the hypothesis that more recently-established populations will demonstrate suppressed inflammatory responses in the Northern curly-tailed lizard *Leiocephalus carinatus*, a species of lizard with multiple geographically-discrete populations in Florida with varying establishment ages. To explore potential physiological indicators of invasion history, we examine both circulating and acutely-stressed corticosterone levels as a mediator of anti-inflammatory phenotypes and bacterial killing ability of plasma as a proxy for inflammatory response. We found differential inflammatory capacity among populations as they relate to estimated and published establishment dates.

## Niche partitioning as a mechanism for the coexistence of pygmy bluetongue lizards and burrowing spiders

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Sheep grazed native grasslands of South Australia are home to burrow engineering spiders which play an important role in the persistence of the endangered pygmy bluetongue lizard (*Tiliqua adelaidensis*). These spiders dig burrows, providing refuges, basking sites and ambush points for the lizards. The lizards obligatorily occupy spider burrows, however, both spiders and lizards pose a potential threat to one another. In order for coexistence of the spiders and lizards in these grasslands to be maintained, there is likely differential use of the burrow niche. We aimed to investigate how spiders and lizards utilize burrows and to identify if niche partitioning could provide an explanation for coexistence of the spiders and lizards. We conducted our research in the Tiliqua Reserve, near Burra, South Australia. We monitored 12 30m X 30m plots over two lizard activity seasons (Sep-Mar 2012-2014), recording burrow dimensions and burrow occupancy. Our results show that lizards display temporal and fine-scale spatial niche partitioning. Lizards move into vacated burrows, reducing the risk of direct interaction with spiders. We also found that lizards more often moved into burrows constructed by trapdoor spiders, and had a preference for empty burrows. They selected deeper burrows as long-term residences, indicating that their preferences may be driven by burrow depth. These results indicate that lizards are unlikely to move into occupied burrows and that burrows are most likely to be abandoned by spiders prior to a lizard occupying a burrow. The lizard-spider relationship is complex with lizard conservation dependent on sustaining spider populations.

## Dietary specialisation in fang 3D shape and sharpness of venomous snakes

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Venomous snakes owe their success to their unique method of feeding, where venom is used to subdue and kill prey. The injection of the venom is ultimately dependent on the puncture effectiveness of the snake's fang. Puncturing a material relies on the tool's characteristics, such as its 3D shape, sharpness and mechanical properties. This study tests the hypothesis that fang shape and sharpness vary with a snake's diet. We suspect this since the shape of a tool is likely affected by the properties of the material it is trying to puncture.

We created high-resolution computer models from microCT scans of snake fangs. These were analysed using 3D geometric morphometrics to look at changes in the overall fang shape, with their cross-sectional sharpness measured in key functional regions. These measurements were then correlated with diet, using four categories based on the physical properties of known prey.

Our results demonstrate variation in both 3D shape, specifically fang robustness and curvature, and sharpness among venomous snakes. There is a clear relationship between fang robustness and food hardness where more robust fangs are found in snakes that specialise on hard-shelled prey, which might be an adaptation to resist breakage. Knowing the fang's relationship with diet can be of importance in both ecology and conservation, since it will help interpret the feeding range of lesser-known snakes. These results aid in demonstrating how fangs work, and provides insight into why snakes are such successful predators in ecosystems around the world.

## **Elevated salinity blocks amphibian chytrid transmission and improves host survival: Implications for translocations**

**Clulow S<sup>1</sup>**, Gould J<sup>2</sup>, James H<sup>2</sup>, Stockwell M<sup>2</sup>, Clulow J<sup>2</sup>, Mahony M<sup>2</sup>

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Emerging infectious diseases are one of the greatest threats to global biodiversity, with chytridiomycosis in amphibians perhaps the most extreme example of this phenomenon. Translocations are increasingly used to fight disease-induced extinctions. However, many fail because disease is still present or establishes in the translocation environment. Can environmental mismatch between host and pathogen, whereby environmental manipulation generates unfavourable environmental conditions for pathogens but remains favourable for the host, improve survival in populations? We tested the hypothesis, manipulating environmental salinity in outdoor mesocosms under near identical environmental conditions to a nearby translocation program for an endangered amphibian. 160 infected and 288 uninfected, captive-bred, juvenile frogs were released into 16 outdoor mesocosms in which salinity was controlled (high or low salinity treatment). The experiment ran for 25 weeks from the mid-austral winter to the mid-austral summer. Increasing salinity from ca. 0.5 to 3.5 - 4.5 ppt reduced pathogen transmission between infected and uninfected animals, resulting in significantly reduced mortality in elevated salt mesocosms (0.13, high salt versus 0.23, low salt survival at 23 weeks). Increasing water temperature associated with season eventually cleared all surviving animals of the pathogen. Thus, we identified a mechanism by which environmental salinity can protect amphibians from chytridiomycosis by reducing disease transmission rates and conclude that manipulating environmental salinity in landscapes where chytrid-affected amphibians are translocated could improve the probability of population persistence. More broadly, we provide support for the paradigm that environmental manipulation via environmental mismatch can be used to mitigate the impact of emerging infectious diseases.

## **Obtaining gametes by hormonal induction from Australian temperate frog species: differential phylogenetic and sex responses**

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Chytridiomycosis has been a strong driver of declines in the major Australian amphibian lineages, causing extinctions and placing many species under threat. Most Australian frogs occur in two deeply split lineages. For convenience, these are referred to as ground frogs (Myobatrachidae and Limnodynastidae) and tree frogs (Pelodyadidae). Species of both groups are at threat from the chytrid panzootic and captive breeding and assisted reproductive technologies (ARTs) are tools that are being used or proposed for use in conservation programs. Hormonal manipulation of gamete release is an important component of any application of ARTs requiring artificial fertilisation (IVF). Differences emerge between sexes and between groups of temperate Australian frogs with respect to responsiveness to the artificial induction of gamete release. Protocols for the non-invasive induction of sperm release, relying on single doses of gonadotropin-releasing hormone (GnRH) or human chorionic gonadotropin are very effective in both ground and tree frog species. However, while protocols utilising GnRH, and GnRH and dopamine antagonists are moderately efficient at inducing ovulation or oviposition in ground frogs, such protocols have been not been successful in tree frogs. The reasons for the lack of response in female pelodyadids are not clear, especially since there is no reason to expect fundamental differences in the neuro-endocrine pathways leading to ovulation between pelodyadids and other groups of temperate Australian anurans. Further research and tools are needed to address this problem, including a source of purified or recombinant pelodyadid follicle-stimulating and luteinising hormones.

**Putting the ARTs into practice: emerging opportunities and priorities for the application of amphibian ARTs**

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The unprecedented decline of hundreds of amphibian species worldwide in mere decades from disease, habitat loss and climate change has resulted in a significant and urgent need for biobanking and assisted reproductive technologies (ARTs) to assist the conservation and genetic management of populations globally. Yet, scientists and conservationists have typically been slow to uptake these technologies for the conservation of threatened amphibians (in part hampered by a lack of technologies in non-model vertebrates) and attempts have typically been reactive, long after severe declines have taken place. Here, we identify and discuss a major opportunity for the use of biobanking and ARTs as a proactive conservation approach ahead of predictable and significant amphibian declines on the island of New Guinea – the world’s largest tropical island containing > 6% of the world’s frog species on < 1 % of the world’s land surface. Importantly, New Guinea is the last major climatically suitable landmass for the amphibian chytrid fungus that remains free of the disease. We have conducted a risk analysis for the frogs of New Guinea, identified opportunities and limitations for biobanking and ARTs based upon today’s technologies and will discuss a new ex situ conservation program involving the use of biobanking and ARTs being initiated in Papua New Guinea ahead of time to prevent the dramatic declines and extinctions seen elsewhere around the globe.

## **A global synthesis of phenological responses to climate change in amphibians and other animal taxa**

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Shifts in phenology are already resulting in disruptions to the timing of breeding in amphibians and asynchronies between amphibians and their predators and prey. Recent syntheses have concluded that trophic level, latitude, and how phenological responses are measured are key to determining the strength of phenological responses to climate change. However, researchers still lack a comprehensive framework that can predict responses to climate change globally and across diverse taxa. Here, we synthesize hundreds of published time series of animal phenology from across the planet to show that in amphibians and other taxa, temperature primarily drives phenological responses at mid-latitudes, with precipitation becoming important at subtropical latitudes, likely reflecting factors that drive seasonality in each region. Phylogeny and body size are associated with the strength of phenological shifts, suggesting emerging asynchronies between amphibians and their prey, which may peak in abundance earlier than amphibians. Amphibians are advancing their phenology at a faster pace than most migrating birds, which could benefit higher-latitude amphibians by freeing them from predation during the beginning of breeding season. Although there are many compelling biological explanations for spring phenological delays, some examples of delays are associated with short annual records prone to sampling error. Finally, our analysis highlights a near-complete absence of available phenological data from tropical latitudes. Our findings arm biologists with predictions concerning which climatic variables and organismal traits drive phenological shifts.

## **An interaction between climate change and infectious disease drove widespread amphibian declines**

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Climate change might drive species declines by altering species interactions, such as host–parasite interactions. However, few studies have combined experiments, field data, and historical climate records to provide evidence that an interaction between climate change and disease caused any host declines. A recently proposed hypothesis, the thermal mismatch hypothesis, could identify host species that are vulnerable to disease under climate change because it predicts that cool- and warm-adapted hosts should be vulnerable to disease at unusually warm and cool temperatures, respectively. We conducted experiments on *Atelopus zeteki*, a critically endangered, captive bred frog that prefers relatively cool temperatures, and show that it has high mortality rates only when exposed to a combination of the chytrid fungus (*Batrachochytrium dendrobatidis*) and high temperatures, as predicted by the thermal mismatch hypothesis. Further, we tested various hypotheses to explain recent declines in the amphibian genus *Atelopus* and reveal that these declines are best explained by the thermal mismatch hypothesis. Only the combination of rapid increases in temperature and chytridiomycosis could account for the patterns of declines. After combining experiments on declining hosts with spatiotemporal patterns in the field, our findings are consistent with the hypothesis that widespread amphibian declines have been driven by an interaction between increasing temperatures and infectious disease. Finally, we provide data that suggest that the thermal mismatch hypothesis can explain variation in infection prevalence across multiple host-pathogen systems, providing a framework for predicting how variation in weather and climate might affect disease risk more generally.



### **Rebuilding the endemic reptile community of Gunner's Quoin, Mauritius**

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The colonisation of Mauritius from the 16th Century resulted in extensive habitat degradation and species invasion, which caused multiple reptile extinctions and extirpations. Several endemic reptile species became restricted to single islet populations to the north of Mauritius, but were threatened by the presence of introduced mammalian herbivores and predators. The introduced mammals were eradicated between 1979 and 1998 with the aim to prevent further reptile extinctions and permit the rebuilding of the reptile communities on the islets through reintroductions to reduce future extinction risk. Gunner's Quoin (0.70 km<sup>2</sup>) with only four small surviving reptile species; having suitable habitat structure; being free from invasive terrestrial predators and competitors and closed to public access was selected as a suitable recipient islet to rebuild its lost reptile community. The saurivorous keel-scaled boa and Telfair's skink were present on Gunner's Quoin until rats invaded in the mid-1800s, but survived on the rat-free Round Island (2.19 km<sup>2</sup>). Smaller reptile species, such as the orange-tailed skink that became restricted to Flat Island (2.53 km<sup>2</sup>), were also thought to have been part of the reptile community on Gunner's Quoin. We report the process over the last 12-years of reintroducing the keel-scaled boa and Telfair's skink, and the emergency translocation of orange-tailed skinks to Gunner's Quoin that prevented their extinction. Frequent monitoring of the reptile populations has shown the resident species have remained abundant and healthy, that the translocated skinks are established and the boa population is on track to becoming established.

## Contribution to the herpetofauna of south-eastern Angola

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The herpetofauna of Angola has been neglected for many years, but recent surveys have revealed previously unknown diversity and a consequent increase in the number of species recorded for the country. The majority of these historical surveys focused on the north-eastern and south-western parts of the country, with the south-east, comprising the provinces of Bié, Moxico, and Cuando-Cubango, being neglected. To address this gap, a series of rapid biodiversity surveys of the upper Okavango, Cuando, Zambezi and Kwanza River basins were conducted by the National Geographic Okavango Wilderness Project between 2015 and 2018. Here we present the results of these surveys, together with a checklist of current and historical herpetofaunal records from the region. In summary, ~142 species are known from the region, comprising 48 snakes, 45 lizards, 6 chelonians, a single crocodile and 42 amphibians. The surveys further added 7 new country records and some novel species. These herpetological surveys are the most comprehensive ever undertaken in Angola. As a result of these surveys the region is now scientifically relatively well known, and our understanding of its biogeographic importance and complex biodiversity more fully appreciated. Our increased understanding of such aspects can now be used to underpin sound and integrated ecological management of these sensitive and unique habitats.

**Impacts of locust control pesticides on the physiology and behaviour of the central bearded dragon.**

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The application of pesticides as a locust control measure is a regular practice in the Australian arid zone. Risk assessments associated with pesticide application are critical for the protection of non-target fauna, but are largely based on laboratory studies of non-native species. Reptiles represent a crucial part of arid ecosystems, yet are underrepresented in toxicology research. We tested the impact of two pesticides, Fenitrothion (organophosphate pesticide) and Fipronil (phenyl pyrazole insecticide), via aerial spray, on the physiology and behaviour of the central bearded dragon (*Pogona vitticeps*). The experiment was conducted at Fowlers Gap Arid Zone Research Station, using a BACI experimental design at two replicate sites per treatment (control, fipronil, fenitrothion). Over a two-month period, we monitored for changes in physical body measurements (mass, SVL, tail length, tail width, jaw width), haematological measures (haemoglobin and haematocrit), and activity. Activity was measured using accelerometer tags to describe the movement patterns of dragons before and after exposure. Results showed a non-significant negative trend in haemoglobin levels and body mass at treatment sites. Blood residue assays describing the degree of pesticide exposure experienced by each dragon in conjunction with physiological and behavioural data suggested a high level of individual variation in the results. It is imperative that we understand the impact locust control pesticides have on native species, where new risk assessments and action can be taken appropriately.

### Variation on the Atlas- Axis Complex in Gekkota

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We scored characters of the atlas-axis complex for members of Gekkota, with the intention to find diagnostic features. The atlas is responsible for holding the weight of the skull. The axis is responsible for supporting movement of the first vertebra and skull, and in geckos, the dens interacts also with the brain case. Atlas and axis elements are important for defining the range of skull motion. CT scans of the atlas-axis complex of multiple species were analyzed. Eighteen characters were assembled and scored using visual observation. These characters were then mapped onto phylogenetic trees. Through this comparison, we found some traits that are consistent with some groups and might be informative. In general, pygopodoideans have an atlas with a poorly defined posterolateral process, while in gekkonoideans this process is more defined. Pygopodoideans tend to have a fused atlas intercentrum, while in gekkonoideans it is sutured. The number of intercentrum processes of the axis fluctuates from 0 to 4 among geckos, and only *Pseudogonatodes* and *Chatogekko* having the highest number. The dens length vs width was sub equal for the vast majority of geckos, but *Aprasia* has a dens more than 2 times longer than the width. Pygopodoideans have a unique dense shape that was occurred in 6 of the 8 genera observed. Variations in the atlas- axis complex may be influenced by different ecological niches, and further analysis could be done to determine this relationship more precisely.

## Bad Neighbours? Amphibian Chytrid Fungus Infection Dynamics in Three Frog Species of Sydney

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Wildlife disease is a major cause of global biodiversity loss. Amongst the most devastating is Chytridiomycosis, caused by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), contributing to declines and extinctions in hundreds of amphibian species. In Australia, our understanding of *Bd* is derived from studies that are highly geographically biased, focusing on the rainforests of tropical north Queensland and alpine regions of south-eastern Australia. Our ability to extrapolate lessons learnt in these unique environments to other regions is unknown. To gain a greater understanding of the dynamics of *Bd* in the Sydney region, we examined the prevalence and intensity of *Bd* infection in three frog species; *Litoria citropa*, a poorly-known species that has experienced population declines in parts of its range was predicted to be susceptible to *Bd* infection, and *Crinia signifera* and *Litoria lesueuri*, two known reservoir species in other regions. *Litoria citropa* and *L. lesueuri* were infected with *Bd* at a high prevalence and intensity, while the reverse was true for *C. signifera*. The results indicate that *L. lesueuri* may be a reservoir species for *Bd* in this habitat, but that *C. signifera* may not- conflicting with previous studies in other regions. Further research is needed to understand the impact of *Bd* on *L. citropa*. Our findings highlight the importance of region-specific studies to inform the conservation management of frog species.

## Enhancing conservation management of New Zealand's lizards – the relevance of laboratory-based studies

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To date, conservation management of New Zealand's lizards has mainly focussed on removing and/or limiting threats from introduced species, restoring habitat, and translocating populations to safe environments. However, captive management involving "breed for release" is increasingly used. Here we outline how laboratory studies can contribute to management programmes, providing the opportunity to remove variables that are impossible to control in the field, and thus enabling researchers to determine environmental limitations that may affect species and individuals. Using three well-studied lizard species (the oviparous skink *Oligosoma suteri*, the viviparous skink *O. maccanni* and the viviparous gecko *Woodworthia "Otago/Southland"*) we summarise the effects of thermal conditions on embryonic development. Together, these studies confirm effects on developmental success, time of birth/hatching and offspring phenotypes; furthermore, thermal requirements for successful development can differ among species, even for sympatric species, and may have long-term implications for offspring years after their release to the wild. Recent increases in emergency salvage operations and captive breeding of lizards highlight the need to understand thermal requirements for development, preferably before the need for urgent management arises. We outline information on thermal ecology that may be especially helpful to captive management, and new tools that make it easier to gather. We invite discussion on ways to facilitate information-sharing among those with interests in this area.

## **Diversion mini-hydropower plants on the Balkans and impact on local amphibian (and reptile) populations**

**Crnobrnja-isailović J<sup>1</sup>**

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Contemporary biodiversity crisis speeds up. The knowledge on amphibian important roles in the ecosystems and their survival requirements has been significantly increasing, but nevertheless we witness the ignorance to scholars'-conservationists' warning messages. One of the new threats on the Balkan peninsula is intensive building of diversion-mini hydro power plants (DMHPP) for "producing renewable electric energy". Private investors get monthly profit from the state once DMHPP is established. DMHPP are redirecting water flow from the riverbed into the tube in the length of several kilometers and leave the part of riverbed literally dry. Affected are the countries of ex-Yugoslavia plus Albania, Bulgaria and Greece. As an example, here is the current situation in Serbia: this country harbors populations of 21 amphibian species, only one is viviparous and others need aquatic habitats for breeding and development of early life stages. *Rana graeca* exclusively breeds in running waters, but *Salamandra salamandra*, *Ichthyosaura alpestris*, *Bufo bufo* and *Bombina variegata* also do it in many local ecosystems. Recently, even species usually breeding in stagnant waters (*R. dalmatina*, *R. temporaria*) were observed spawning in the mountainous water flows, probably due to the loss of their common breeding sites by climate changes, or by intentional drying out and introducing fish species in those water bodies. Local reptile populations would be also (indirectly) affected. There is about 800 DMHPP planned to be established in Serbia - on almost every single water flow - and they would have devastating effect on local biodiversity.

**Citizens capturing cryptic and charismatic chelonians for conservation: photographic capture-recapture and mobile app development**

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<sup>1</sup>*The Toledo Zoo and Aquarium*

Blanding's turtles (*Emydoidea blandingii*) are considered threatened or endangered throughout most of their range. A critical step in determining appropriate conservation actions for this species is assessing the status of remaining populations. However, the long-term surveys required to adequately document population trends are lacking as they are generally labor-intensive and time-consuming. We used citizen science-collected data and free pattern-recognition software to conduct a mark-recapture study on female Blanding's turtles in a northwest Ohio wetland. Over a five-year period, citizen scientists gathered 115 images of 51 individual female Blanding's turtles. The results of this work led to development a mobile app that will allow users to submit observations of turtle species with distinguishing carapace and plastron patterns and perform photographic pattern-recognition on-the-fly. The app also allows users to enter morphometric and location data, and provides information on turtle biology and conservation, local wildlife laws, and biosecurity guidelines. With the help of citizen scientists, we are working to fill in data gaps that will meet state agency assessment goals and aid in conservation planning.



## Using monitors to monitor restoration: how does Australia's largest lizard respond to mine site restoration?

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Globally increasing rates of mine site discontinuations are resulting in the need for immediate implementation of effective biodiversity and conservation management strategies. Over 60 000 mines across Australia have been identified as discontinued, yet despite restoration being a legislative requirement, the number of these sites confirmed as restored and officially closed is extremely low. Monitoring vegetation structure and condition is a common method of assessing restoration success, however monitoring animal responses is relatively uncommon. Animals are generally assumed to return to pre-disturbance abundances following the return of vegetation (Field of Dreams hypothesis; 'build it and they will come'). In practice, recovering animal biodiversity and community structure can be some of the most difficult components to achieve and assess following the restoration of degraded sites. Using VHF and GPS tracking, and the T-LoCoH method of home range construction, we assessed the behavioural responses of a sub-adult female perentie (*Varanus giganteus*) to habitat change and differing thermal environments presented in reference and restoration vegetation at a Mid West Western Australian mine site. We highlight a reduction of vegetation cover and spatial heterogeneity as a major constraint to the movements and behavioural ecology of the perentie, and hence although restoration may be facilitating return, behavioural use of restoration vegetation differs from that in the reference vegetation. Understanding the complex interactions between animals, and their behavioural responses to their environment, is fundamental to their conservation in the face of ever-increasing rates of human induced habitat change and degradation.

**Recent advances on the taxonomy, systematics and biology of cophyline frogs of the genus *Plethodontohyla***

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Frogs of the genus *Plethodontohyla* form a morphologically diverse group of species comprising a wide range of ecomorphologies (terrestrial, fossorial and semi-arboreal) and sizes (25-100mm). Most species are only occasionally encountered in the field, due to their secretive habits, seasonal breeding, reduced detectability outside of the reproductive period and, sometimes, by their restricted distributions. Scansorial species are known to breed in water-filled holes in trees, while terrestrial and fossorial taxa probably breed in cavities in the leaf litter. While semi-arboreal and predominantly terrestrial taxa possess expanded terminal discs, all other terrestrial and fossorial taxa possess knob-shaped terminal phalanges, but the monophyly of these phenetic groups (arboreal and terrestrial/fossorial) still needed to be established.

Species of the genus *Plethodontohyla* have an exceptionally convoluted taxonomic history, and only after the recent inspection of all type material it has been possible to reveal the names that are probably misapplied, to solve a taxonomic and nomenclatural conundrum, and finally set the basis to advance with their taxonomy and systematics.

We performed a molecular taxonomic identification of almost 200 individuals collected over the last 30 years, and assembled a multilocus dataset of all identified lineages to explore the phylogenetic relationships of the analysed taxa. Integrating data from external and internal morphology, natural history, phylogenetic relationships and the comparative inspection of museum material we present an update of their systematics.

## Digging into the diversification of the mantellid frogs of Madagascar (Anura: Mantellidae)

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Frogs of the family Mantellidae (including the Laliostominae, Boophinae and Mantellinae) underwent an exceptional diversification, which resulted in the evolution of a plethora of morphological and ecological traits. Among them, the mantellins is the most species-rich group. Mantellins have a derived reproductive behaviour (no amplexus) and males have femoral glands, whose position, sex-specific occurrence and species-specific secretion, suggests that they probably played a crucial role in mantellid frogs diversification.

We sequenced 24 tissue-specific (femoral glands, skin and liver) transcriptomes of two sympatric sister species of the genus *Gephyromantis* to identify the candidate genes involved in the expression of the femoral glands. We assembled and annotated the full transcriptomes of each species and used whole species transcript and differential gene expression analyses to identify the genes that are differentially-expressed in each tissue. We used the genome of *Nanorana parkeri* to identify the orthologous genes and found almost 500 genes differentially expressed in both species' reference transcriptomes. Of these around 200 genes are differentially expressed in the femoral glands. We used functional network analyses to identify functionally enriched gene association hubs that are differentially expressed in the femoral glands and identify the major biological processes and biochemical pathways in which the femoral glands are involved.

## The invasion of the Asian Toad in Madagascar

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In 2014 the Asian common toad (*Duttaphrynus melanostictus*) has been reported in Toamasina (eastern Madagascar), the major seaport town of Madagascar. This alien species was accidentally introduced from South-East Asia around 2010, and since then has established a rapidly-growing invasive population. This species is poisonous, generalist, highly fecund, and it is spreading across the lowland habitats of north-eastern Madagascar. Updates of its distribution range revealed a fivefold increase of the invaded area over 3 years (2014-2016), a doubling of the rate of spread, the capacity to invade forested areas and heterogeneous densities across different invaded areas.

Here we present an overview of the Asian toad invasion in Madagascar, tracing back the activities undertaken within the framework of the invasion risk assessment in the past five years, and outlining what should be investigated in the future.

### Experimentally testing the evolutionary drivers of a colorful signal in *Anolis* lizards

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Colorful signals among animals present a compelling example of phenotypic diversity found in nature. In some systems, the evolution of these traits is driven by environmental heterogeneity, with signals being most adaptive in the ecological contexts in which they are transmitted most efficiently. We investigated this phenomenon by leveraging a unique system to test selection on a colorful signal using experimental manipulation in natural settings. Our study species was the slender anole (*Anolis apletophallus*), a small lizard that possesses a colorful throat fan (dewlap) used in signaling. To test the hypothesis that selection acting on dewlap color would differ among environments that differ in light levels, we established and studied experimental populations of these slender anoles on islands in Lake Gatún, Panama. Slender anoles were captured from the mainland (Soberanía National Park), measured and tagged in the lab, and then released onto each experimental island. We characterized the light environment experienced by each population using hemispherical photography and a light meter. We then visited each island every six months and recaptured all individuals, allowing us to generate survival and reproductive data. These data were used to generate selection gradients and fitness surfaces for each island, which were then compared across light levels across all islands and on the mainland. Our results elucidate the role of environmental variation in driving signal evolution, which can in turn lend insight into the evolution and maintenance of phenotypic diversity across the tree of life.

## Surveying frogs from the bellies of their parasites

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Effective conservation of species and their habitats depends on reliable occurrence data. However, the collection of sufficient species records is often made difficult by factors that reduce species' detectability. Recently, iDNA (invertebrate-derived DNA) has emerged as a potentially powerful tool for increased detection of terrestrial vertebrates. Invertebrate parasites are effective samplers of some vertebrate diversity, and by trapping them and sequencing DNA from their meals, they can be used to identify and detect vertebrate hosts. Most vertebrate surveys incorporating iDNA have been general or targeted mammals, but frogs are an ideal candidate for iDNA surveys. Frogs are highly threatened, often have low detectability during surveys, and can attract impressive amounts of blood-feeding parasites. To determine whether frogs can be surveyed via their hematophagous parasites, and whether some frog species could be detected more effectively in this way than by using traditional survey techniques, we carried out 'traditional' and iDNA surveys at six stream sites in eastern Australia. iDNA survey results were comparable to traditional surveys in terms of total species diversity, and successfully detected a threatened frog species that traditional surveys did not. We demonstrate for the first time the potential for iDNA in improving the detectability of elusive frog species.

## Antipredator defenses promote morphological evolution in Uropeltid snakes

Cyriac V<sup>1</sup>

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Many species possess warning signals such as conspicuous colours that signal their unprofitability to predators. Apart from reducing predation risk, warning signals can also promote niche expansion by providing prey with a predator-free space to utilize other resources in its environment. However, how escaping predation by evolving such warning signals can release a species from environmental constraints and facilitate niche expansion is not clearly understood.

Fossoriality in reptiles imposes several morphological modifications associated with burrowing into the soil. Studies indicate that fossorial reptiles tend to have small, narrow heads with thin and long bodies for better burrowing performance. However, species occurring near the surface can exhibit more robust morphologies as constraints imposed by fossoriality reduces. Evolving antipredator defences that reduce predation pressure when fossorial species move to the surface could potentially reduce these morphological constraints as well as promote morphological diversification. In previous experiments, I show that conspicuous colouration in uropeltid snakes reduces avian predation when active on the surface, allowing them to occupy above ground habitats. Thus, I predicted that species with more colouration would exhibit more robust body forms and show faster rates of morphological diversification. Using a comparative phylogenetic approach, I confirm that Uropeltis species with more conspicuous colouration tend to have greater girth and larger heads. I also show that the rates of head shape evolution are much faster in more conspicuous species. Overall, I show that the evolution of warning colourations in uropeltid snakes can facilitate niche expansion by influencing rates of morphological diversification.

## **Benchmarking tissue preservation methods for ultra-high molecular weight DNA and long-read genome sequencing technologies**

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Difficulty in retrieving high-quality DNA from species that exist in remote regions, such as numerous reptiles and amphibians, has impeded the application of recent advancements in long-read sequencing to these groups. Long-read sequencing technologies have redefined the requirements for DNA quality. Yet methods for addressing limitations in sample collection, protocols for DNA extraction, and DNA quality have not been sufficiently expanded in parallel. Long-read genomic technologies require significant amounts of ultra-High Molecular Weight (uHMW) DNA >150kb in fragment length. Many species reside in geographical locations where liquid nitrogen or dry ice are not readily available, and tissue reagents may not survive at atmospheric temperatures well. To support the mission of the Vertebrate Genomes Project (VGP) in generating high-quality chromosome-level reference genome assemblies of all ~70,000 extant vertebrate species, we present a comparative study of preservation methods across different environmental conditions and sample types (muscle, soft tissue, and blood) for the major vertebrate groups (mammals, birds, reptiles, amphibians, and fishes). We identified methods adapted to field collection that yield sufficient DNA quantity and quality, and discuss ways in which other upstream factors, such as extraction methods, affect the quality of sequencing data. The guidelines presented here provide methods for optimally collecting samples in laboratory conditions or in remote field sites for downstream uHMW DNA extraction and long-read technologies.



**Insights from evolution and biomechanics reveal form-function relationships in reptile eggshells.**

**D'Alba L<sup>1</sup>**, Nallapaneni A<sup>2</sup>, De Clerck K<sup>1</sup>, Daelemans L<sup>1</sup>

<sup>1</sup>*Universiteit Gent*, <sup>2</sup>*The University of Akron*

Eggs are multifunctional structures that enabled early tetrapods to colonize diverse terrestrial habitats millions of years ago, and are now the reproductive mode of over 70% of land vertebrates. Egg morphology is essential for animal survival, for example, eggshells mediate the interactions between embryos and their environment, and a result have evolved into an enormous diversity of forms and functions in modern reptiles. Eggshell functions have had profound effects on vertebrate evolution and may serve as models for new antimicrobial and/or breathable materials.

However, we lack basic knowledge on most properties of eggshells, including their chemical composition, ultrastructure, and material properties. This is particularly true of reptiles. These data are critically needed if we are to understand their effects on vertebrate evolution and diversification. Here the goal was to determine the relative contributions of structural constituents and chemical composition of eggshells to their biomechanical performance using.

First, we characterized eggshell structure using X-ray micro Computed Tomography ( $\mu$ CT) coupled with scanning electron microscopy, then we analysed eggshell chemical composition using FTIR and conducted experiments to determine eggshell functional properties (optical, mechanical, interaction with water and microbes). We show that reptile eggs display larger diversity in egg phenotypes than previously thought and that this diversity is coupled with a large range of functional properties, some of which might serve as inspiration for biomimetic materials. The inferences generated in this project are of great relevance to evolutionary biologists, palaeontologists as well as bioengineers interested in biomimicry and bio-inspired design.

## How the "World's rarest snake" was saved, and its implications for other endangered Caribbean species

**Daltry J**<sup>1</sup>

<sup>1</sup>*Fauna & Flora International*

Caribbean islands cover less than 0.15% of the earth's land surface but account for over 65% of the world's reptile extinctions since 1500. Almost half of all remaining species in this region are threatened with extinction due to invasive alien species, hunting, habitat loss and climate change. Among them is the Critically Endangered Antigua racer *Alsophis antiguae*, which was abundant across Antigua and Barbuda (441 km<sup>2</sup>) until Asian mongooses and other alien mammals were introduced. Their range shrank to only 8.4 hectares (Great Bird Island), but they continued to be killed by people, fearful of all snakes, and preyed on by invasive rats. A mark-recapture survey in 1995 found only 51±7 Antigua racers remained. To prevent extinction, a consortium of national and international organizations launched an ambitious recovery programme to eradicate rats and mongooses, transform public perceptions of these non-venomous snakes, and reintroduce racers to parts their former range in Antigua. In 1999, 10 wild racers were successfully translocated from Great Bird Island to Rabbit Island. Further reintroductions followed to Green Island (from 2002) and York Island (from 2008), increasing the snakes' distribution range to 63 ha. The Antigua racer meta-population has increased to >1,100 individuals in the wild and the snakes have won a legion of supporters in Antigua. Methods and lessons learned from this project to restore habitats, change public attitudes towards reptiles, and reintroduce endangered reptiles are being applied to other threatened Caribbean reptiles, including the Lesser Antillean iguana *Iguana delicatissima*, and Saint Lucia racer *Erythrolamprus ornatus*.

**Biomechanical modeling of turtle jaws: linking brevirostry and edentulism in Testudines.**

**D'Amore D<sup>1</sup>**

<sup>1</sup>*Daemen College*

Major modifications to the turtle body plan include the development of a shell, remodeling of the skull, and the replacement of teeth with a rhamphotheca or beak. Through biomechanical modeling, I propose the loss of teeth in turtles may be a direct consequence rostral shortening. Hypothetical vertebrates were modeled through parabolic equations outlining the rostral margin at variable lengths. I used a torque formula to calculate force, determined the degree of rotation for a standardized tooth height, and calculated the tangent to determine the orientation of labial-lingual compressed crowns. Data were collected along this margin at 10% intervals of rostral-to-caudal length, representing hypothetical tooth positions. Results show the amount of force and degree of rotation increased at an exponential rate as the rostrum shortened, with the most pronounced change influencing positions furthest from the hinge. The tangents became more parallel to the axis of jaw rotation in those with the shortest rostrum. This indicates brevirostry may compromise the integrity of typical reptile teeth. As the snout shortens, teeth must endure greater bite forces, rotate more to open the mouth, and cope with the weaker, flattened section rotating about the axis of jaw rotation. Turtles would therefore require tooth crowns to be much shorter and/or thicker to compensate, or replace said teeth with a uniform cutting edge such as a beak. Future work will directly test this model by measuring distances between the hinges and beak margins of turtles, as well as applying in vivo bite forces collected from live specimens.

**Electric blue: novel toxins in long-glanded *Calliophis* sidestep neurotoxic arms race**

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The genus *Calliophis*, sometimes called Asian coral snakes, are the most basal branch of the elapid radiation. Several species of the genus are known to possess extremely enlarged venom glands that run to 1/3 the length of the snake's body. Beyond this highly unusual morphology, these species are known for their striking colors and diet that consists largely of other snakes. This includes other ophiophagous specialists like *Bungarus* (kraits) and *Ophiophagus* (king cobras). The venom of *C. bivirgatus* has previously been shown to contain toxins that activate voltage-gated sodium channels, the first such toxins known from a vertebrate venom. Using a combination of transcriptomics, proteomics, and in vitro testing of toxic activity, we further characterized the venom of this iconic snake species as well as that of the sister species *C. intestinalis*. We found that *C. intestinalis* venom has the opposite effect on sodium channels as that of *C. bivirgatus*, but that closely related toxins are responsible for these disparate activities. These findings shed light on the forces that have shaped the evolution of the peculiar venom system of this genus and our transcriptomic dataset also increases our understanding of the origin of elapid venoms overall.

## Research to conservation practice through Durrell's SAFE Strategy 2014-2020

**Dawson J**<sup>1</sup>

<sup>1</sup>*Durrell Wildlife Conservation Trust*

In the face of the global amphibian crisis Durrell Wildlife Conservation Trust decided to launch a dedicated amphibian conservation programme, Saving Amphibians From Extinction – SAFE Programme in 2013. To guide this a SAFE Programme strategy was developed focussing on four priority regions – Madagascar, Western Caribbean, Tropical Andes, Sri Lanka – for the period of 2014-2020. Within this work is broadly undertaken through four themes: understanding the situation of species in the wild; captive management development; developing in-country capacity and promoting amphibian conservation at a global level. Research is a key element of the strategy that cuts across all these themes. As our strategy approaches it is timely to review what has been a success and what has not, to understand reasons for that so that future strategies can be more effective. This presentation will look at some of the varied amphibian research undertaken over the last six years through the SAFE Programme and importantly how this has been translated into conservation practice and challenges in doing so. Research undertaken includes developing knowledge of amphibian communities and species populations; social based surveys to understand threats and research to stimulate debate and guide amphibian conservation practice at a global level.

### **Restoring the Critically Endangered mountain chicken in the face of chytridiomycosis**

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The Critically Endangered mountain chicken (*Leptodactylus fallax*), found on the Caribbean islands of Dominica and Montserrat, underwent one of the fastest declines observed in any vertebrate species due to the chytrid fungus *Batrachochytrium dendrobatidis*. A remnant population of c.130 individuals survives on Dominica but the Montserrat population is near extinct with just 2 known individuals. Research is ongoing into the Dominica population and reasons for its survival but it remains highly vulnerable to extinction from multiple non-disease threats as well as chytridiomycosis. The long-term goal of the multi-partner Mountain Chicken Recovery Programme is to restore populations across the species native range. This means identifying ways to restore populations to areas where the irreversible persistence of chytrid fungus and associated chytridiomycosis risk persists. A decade of research efforts which have included trialling field based anti-fungal treatments and trial reintroductions to understand the disease dynamics in the wild have led us to the current stage of trialling environmental manipulation. This strategy is well supported in the literature for the conservation of chytrid threatened amphibians but has not been trialled in the field. As of July 2019 on Montserrat, we will be undertaking trials to make the habitat unsuitable for the chytrid fungus and enable the persistent survival of released captive-bred mountain chickens. This project highlights the need of a multi-faceted and multi-partner approach when attempting to recover a Critically Endangered species in the light of an irreversible threat.

## Skull anatomy of a rare mid-Cretaceous tetrapod using HRCT data

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Albanerpetontids are enigmatic fossil tetrapods recorded from the Middle Jurassic to the Pliocene, when they apparently became extinct. Although they are known from fragmentary material, there are some complete specimens that provide details about their morphology and phylogenetic position. The most complete albanerpetontid skull is represented by disarticulated material of *Shirerpeton isajii* from the Early Cretaceous of Japan. Here we present the skull of a closely related form from the amber mines of Hukawng Valley, Northern Myanmar, a deposit of mid-Cretaceous age. The new specimen shares many similarities with *S. isajii*, and is fully articulated, preserving details of the soft anatomy. The skull has a large temporal opening that is confluent with the orbit. The jugal is directed posteriorly rather than dorsally (no postorbital bar) but does not reach the quadrate. Based on the size of the orbits, the eyes may have been very large. The supraoccipital and the parietals define two large dorsal post-temporal fenestrae, confirming the morphology reconstructed for *S. isajii*. The occipital condyle is double implying amphibian affinities, as previously interpreted from atlas morphology. This new material helps resolve the identity of some mysterious bones previously described in related forms, yields the first details of the palate, and provides new information on the convergent evolution of the ballistic tongue foraging mode, together with salamanders and chameleons.

## How does incubation temperature affect behaviour, physiology and growth in delicate skinks?

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Species across the globe face a rapidly changing climate. Reptiles are one group that will be especially vulnerable to rising average temperatures, particularly as many species are egg-laying. Conditions experienced during embryonic development have significant and potentially long-lasting impacts on phenotype and survival. Understanding how a range of incubation temperatures influence reptiles is therefore critical in assessing climate change impacts. While research in this area is growing, we still lack information on a greater diversity of traits, as well as a detailed understanding of the sensitivity of different traits to developmental temperature. Here we examined the effect of incubation temperature on ecologically relevant traits using delicate skinks, *Lampropholis delicata*, as a model species. Eggs were incubated at one of three temperature regimes, averaging 22 °C, 26 °C and 30 °C and fluctuating 3 °C above and below these averages, in a split-clutch design. We recorded incubation duration, egg mortality and body size on hatching. At four to six weeks of age we measured body size, sprint speed, resting metabolic rate, thermal preferences, habitat preferences, and response to a simulated predator attack. We took a multivariate approach to assessing what impact differences in incubation temperature have for this species, and also compared the magnitude and direction of change in these traits to assess potential differences in their capacity for plasticity. The results of this study will demonstrate the impact of a changing thermal landscape on lizards.



## Synopsis of the amphibians from a poorly surveyed African country: Equatorial Guinea

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Equatorial Guinea is a small central African tropical country situated in the Gulf of Guinea. It has a main insular area composed of the volcanic Bioko island, and a continental part known as Río Muni, which borders on Cameroon to the north and Gabon to the east and south, and accounts for most of the country's surface. Only a few punctual and mostly old publications have dealt specifically with the amphibians of Equatorial Guinea, and an accurate and updated catalogue is lacking. Based on fieldwork, a compilation of literature, and the examination of two important Spanish scientific collections, we present a comprehensive catalogue of the amphibian fauna for Equatorial Guinea. We report 77 species belonging to 30 genera, 13 families and two orders. Twelve species are present in Bioko only, 36 are known exclusively from Río Muni, and 29 occur in both regions. There is a very low level of endemism, with only one species endemic to Bioko. This may be due to the country's small size, to the relatively uniform landscape (lowland rainforest) of Río Muni, and to the recent connections between Bioko and the continent. Our work revealed several new species and unveiled problems in the taxonomic status of many amphibian populations that need to be addressed. As further field and taxonomic work is carried out, we expect new species records for the country that will also contribute to enrich this catalog.

## Population success of common skinks (*Oligosoma polychroma*) following mitigation translocation

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Of the 106 recognized lizard species native to New Zealand today, approximately 83% are listed as threatened or declining by the country's Department of Conservation. Major factors contributing to native species decline include urbanization, habitat loss, and introduced predators. In an effort to "save" lizards from destruction at development sites, mitigation translocations are often implemented. For this study lizards were collected, toe clipped for individual identification purposes, and relocated to "suitable" sites, which have varying short-term population monitoring and predator control strategies in place. Today, only 41% of herpetofauna translocation mitigations can be considered successful long-term. Here we investigated the outcomes of a mitigation translocation of 389 common skink individuals (*Oligosoma polychroma*) to two release sites to understand factors that determine success of translocated populations. We evaluated the habitat available at the release sites as well as individual survival, dispersal from the release points, and signs of recruitment. To date, pitfall monitoring at both release sites has recorded the presence of translocated individuals. These included juveniles and adults of both sex, with one possibly pregnant female. Additionally, there were adults and juveniles recorded who were not identified as translocated lizards at one location. We found that individuals were recaptured in greater frequency at pitfalls placed near or in native bush outcroppings at a distance from the public access areas. While it is encouraging that skinks were recaptured at both locations, further monitoring is necessary to investigate long-term establishment of a population via habitat use, recruitment, and individual survival.

## **Scales, tails, and trails: A review of recreational effects on reptiles and amphibians**

**Dean S<sup>1</sup>**

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Recreation is the primary way people regularly interact with wildlife and wild spaces. Recreational impacts to mammals and birds have been extensively documented, while reptiles and amphibians – taxa including globally imperiled vertebrates – have received little attention in the recreational-impact wildlife ecology literature. Recreational activities present unique challenges to herpetofauna, where negative effects of trail systems (which act similarly to road systems), edge effects, and anthropogenic disturbance compound to affect dispersal, survival, reproduction, health, and habitat quality. In urban recreational areas, these issues can be further complicated by the negative effects of urbanization. Here, I summarize the complex and often interrelated effects of recreational activities (including hiking, biking, off-road vehicles, horseback riding, dog walking, wildlife viewing, and boating) and recreational infrastructure on reptiles and amphibians, discuss how these impacts may differ based on landscape context and taxa, identify future research topics, and suggest best practices for land managers to minimize recreational impacts on herpetofauna.

## Ecological and genetic variation of the distribution of various species of amphibians at the southern border of their distribution

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The current mini-review describes the distribution of amphibian species in terms of their adaptation from Mediterranean to desert climates. According to the data collected in this mini-review and from some unpublished data, it was found that the adaptation of amphibian species from arid to Mediterranean climates was highest for *Bufo variabilis*, followed by *Triturus vittatus*, *Hyla savignyi*, *Pelobates syriacus*, *Rana bedriagae* and *Latonia nigriventris*. Many parameters affecting adaptation to different habitats have been described, including aquatic and terrestrial habitats. Among them, the most important parameters affecting semi-arid and arid habitats are the large number of tadpoles, the short growth and complete metamorphosis period, finding hiding places to prevent dehydration, and physiological adaptation to accumulate urea in the body fluid. A quality model is suggested to show the adaptation of various amphibian species to habitats at the southern border of its distribution

**Species Diversity, Distribution and Microhabitat Preferences of Anurans in Mount Kalatungan, Bukidnon, Central Mindanao Philippines**

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<sup>1</sup>*Department of Biological Sciences, Mindanao State University- Iligan Institute Of Technology*

Anurans are considered good indicators of habitat quality due to their dual life mode, limited dispersal capabilities, and sensitivity to environmental changes brought about by habitat fragmentation. Their important function as ecological indicator species, along with continued threats to Mount Kalatungan provides the basis for this study. Fieldwork was conducted from an elevation of 1,000 to 1,600 meters above sea level to assess the distribution of anuran species, employing cruising technique. Eight species of anurans were recorded in all sampling sites. The lower montane forest has higher diversity index ( $H' = 0.744$ ) than the upper montane forest ( $H' = 0.534$ ). Even with the low diversity indices, the level of endemism of anurans was 75% where five of the recorded species are Mindanao faunal region endemic and one species is Philippine endemic. One species is of near-threatened status and five endemic species have decreasing population trend of mature individuals. Most of the species prefer terrestrial and aquatic microhabitats specifically in the shrubs, rocks, and bank substrates along the rivers and streams. An increasing trend of endemic species along with the increasing elevational gradient of the forest that is from submontane to montane forests was observed. Field observations showed that the major threat to the anurans is habitat destruction, particularly the conversion of the forest to agricultural farms by the local people. Despite habitat loss in Mt. Kalatungan, considering the high endemism of anurans, the conservation of this mountain range is important.

## **Development and Application of Assisted Reproductive Technologies in Several Endangered Panamanian Amphibian Species**

**Della Togna G<sup>1</sup>**

<sup>1</sup>*Universidad Interamericana De Panama*

Knowledge of basic reproductive biology is critical to better protect and propagate endangered amphibian species. The objectives of the study were to develop optimal hormonal stimulations to characterize the sperm quality of five endangered species, as well as to improve the breeding success of non-breeding pairs in captivity. Spermic urine samples were collected following intraperitoneal injection of gonadotropin-releasing hormone agonist, human chorionic gonadotropin, or Amphiplex™. We assessed sperm quality parameters such as percentage motility, percentage forward progressive movement, concentration, morphology, pH and osmolality and identified the timepoints where treatments were most effective in terms of recovery of samples with higher sperm concentrations and quality. We will also present preliminary results on hormonal stimulation for sperm collection on other species (*Atelopus certus*, *A. varius*, *A. glyphus*), of females for egg collection, and breeding pairs for the successful stimulation of reproductive behaviors, as well as sperm cryopreservation. This is the first comprehensive characterization of the optimized application of Assisted Reproductive Technologies in several endangered Panamanian amphibian species for sperm collection, characterization and storage, as well as successful stimulation of breeding pairs.

**Social tolerance behaviour in a reptile, the Eastern water dragon (*Intellagama lesueurii*): a new perspective**

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<sup>1</sup>*University of the Sunshine Coast*

All animal species, regardless of their levels of gregariousness, constantly negotiate an ecological landscape containing other individuals. However, a significant bias in the study of social behaviour has persisted in the literature, where most work has focussed on describing complex social systems in group-living animals. Yet, both group and non-group living animals engage in social interactions, ranging from avoidance, tolerance, aggression and cooperation. Social tolerance represents an important factor explaining the likelihood and success of cooperation, and may therefore, represent the first requirement to allow the evolution of social behaviour. In this paper, we studied social tolerance behaviour in a social reptile, the Eastern water dragon (*Intellagama lesueurii*). First, we assessed social tolerance by recording all approaches without displacements between focal individuals and their neighbours, as well as the time spent in proximity after the approach. Secondly, we investigated the consistency of social tolerance behaviour across time. We recorded 228 approaches between focals and neighbours. Among these, 89% involved no displacements of the individual approached, indicating that eastern water dragons tolerate one another. Additionally, our results showed that both males and females were consistent in their social tolerance behaviour across time. Consistent social behaviour may be advantageous, by allowing individuals to predict the behaviour of others, therefore reducing uncertainties about their social environment. Overall, our results highlight the importance of studying social behaviour in lineages differing in key aspects of their biology, as this will help improve our knowledge of the factors driving the origin and persistence of social behaviour.

## Conservation ecology of facultative paedomorphosis in newts in a world of introduced alien species

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Facultative paedomorphosis is a fascinating example of heterochrony that implies the development of two alternative adult phenotypes in newts and salamanders: the metamorphs that lose their gills at metamorphosis and the paedomorphs, which retain them. Although this is a widespread process across taxa, it is much rarer than metamorphosis and therefore may be threatened by environmental change. In this study, we analyzed distribution data over several decades in southern Europe to find out the extent and consequences of fish introductions. Moreover, we explored how fish could be particularly detrimental for paedomorphs. Fish was the main driver of the extirpation of paedomorphosis in a large variety of habitats including ponds and lakes in all studied taxa and regions and this, even in wild environments. The highest declines occurred in some Balkan countries where more than 99% of aquatic area of occupancy of paedomorphs were lost, including disappearance of endemic taxa. Metamorphic phenotypes declined later than paedomorphs but followed the same trend. Beyond a direct predatory pressure, fish affected paedomorphs more than metamorphs through inhibition of vital activities such as reproduction and feeding. Conservation management was effective in allowing the resilience of paedomorphs in pond networks but this is less likely in isolated lakes. These results highlight that despite some hope for the persistence of paedomorphosis in ponds, the main populations of paedomorphs are critically endangered or already extinct. If fish introductions are not urgently stopped, what is a remarkable example of intraspecific heterochronic diversity may soon belong to the past.



### **The Brisbane Python Project: how mitigated translocations potentially affect urban pythons in Brisbane**

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<sup>1</sup>*University Of Queensland*

Translocation of nuisance wildlife is commonly used in urban areas to mitigate actual and perceived human-wildlife conflicts. This process often results in large numbers of animals being moved from their resident locations to 'suitable' novel environments. Areas classed as suitable, are at the discretion of the snake relocater and over a season, can involve many snakes being released at the same site, which may also be utilised by multiple relocators. Most reptile translocation studies focus on survivorship, stress responses, time until movement is stabilised, homing behaviour or high movement rates, but often there is no investigation on how these translocations effect resident conspecifics. In this particular study, we are investigating the ecology of urban carpet pythons through radio-tracking resident snakes with implanted radio-transmitters, examining thermal preferences within their known environment and testing for baseline stress factors. In following years, translocated pythons with implanted radio-transmitters will be introduced into the same environment, monitored for movement, thermal preference and stress factors. Resident snakes will continue to be radio-tracked to monitor changes in behaviour and stress responses for comparison to baseline data.

## The New Caledonian Leopard Skink *Lacertoides pardalis*: Ecology and Conservation in a Mining Context

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New Caledonia is famous worldwide for its spectacular and coveted giant geckos, yet it still remains a poorly known herpetological hotspot. To date only a few studies have dealt with the ecology of New Caledonian lizards, most of them focusing on geckos.

The New Caledonian Leopard skink is an elusive species whose ecology and distribution are only partially understood. This monotypic genus endemic to the southern ultramafic region stands out by several original features such as large size, viviparity and frugivory. Its dependency to rocky outcrops within low shrubland maquis puts half of the known populations at risk from the intense mining activities in the region.

The first step of our research project is focused on field evaluation of monitoring techniques seldom used in New Caledonia such as funnel traps, artificial retreats and camera traps, as an alternative to the more widely used sticky traps. We here present our first results which shed light on some biological features of this species, including population density. We also have the opportunity to explore the frugivory and related seed dispersal with ex situ specimens. These first methodological steps will help to shape further assessments of habitat use through radio-telemetry. These elements, in addition to a proper population monitoring based on CMR, will help future conservation measures through a better understanding of home ranges and intraspecific interactions of the Leopard skink.

Our project appears a unique opportunity to develop technical innovations and positively influence the way lizards are studied and protected in New Caledonia.

### **Functional morphology and evolution of dorsal shields in horned frogs (Anura: Ceratophryidae)**

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Horned frogs (Ceratophryidae) comprise a relatively small family of species endemic to South America. They occupy a diversity of habitats ranging from humid tropical forests to the semi-arid planes of Chaco and Caatinga. Several horned frog species are characterised by the presence of a large dorsal shield - a trait shared only with brachycephalid frogs. Recent studies have suggested that horned frogs diversified in semi-arid environments with multiple independent transitions to more humid habitats. In the present study, we test the hypothesis that the evolution of dorsal shield formation coincided with habitat transitions. Our approach is threefold. Firstly, we use micro-CT scanning to quantify the expression of dorsal shields both within and among horned frog species. Next, we test whether the observed variation in dorsal shield expression is related to habitat use using a phylogenetic comparative approach. Lastly, we examine the functional role of the dorsal shield in water balance and thermoregulation using state-of-the-art evaporimetry and thermal imaging. By using a holistic approach, we aim to shed light on the evolution of osteoderms, particularly their role in organismal physiology.

### **Will the World be too hot for Lepidosauria to survive?**

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In recent decades, many species have shifted their geographic ranges, seasonal activities, physiological issues, migration patterns, abundances and interactions in response to ongoing changes in the World's climatic system. Projections of future climate change are uncertain, but the Earth's warming is likely to exceed 4.8°C by the end of 21st century. Predicting the potential future risks to biodiversity caused by climate change has become a crucial and extremely active field of research, and several studies in the last two decades had focused on determining possible impacts of climate change on Lepidosaurians, at global, regional and local level. Here we aim to describe the main factors acting to increase the vulnerability of lizards, snakes, amphisbaenians and tuatara to climate changes and the most common methods used to evaluate that. We also identified potential biogeographical and phylogenetic patterns regarding climate change effects on this group. We highlight the importance of an integrative approach including biological, bioclimatic and environmental variables to buffer the climate change effects on these reptiles through the application of consistent and truly effective strategies for species conservation.

## Alpine temperatures and sex reversal of alpine Eastern three-lined skinks in South Eastern Australia

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Sex determination in reptiles is under the influence of genetic factors (genotypic-sex-determination / GSD) or environmental factors which, if incubation temperature is the environmental cue, is referred to as temperature sex determination. These two mechanisms of sex determinations are mainly observed across species as alternatives, however recent findings of sex reversal of two GSD species under extreme environmental conditions, confirmed in wild populations, has revealed that intermediate states are possible. The *B. duperreyi* has XX:XY sex chromosomes, but in cool nests this genetic mechanism is overridden by temperature. Eggs incubated at 20°C produce predominately male hatchlings which includes a proportion of individuals with XX chromosomes reversed to a male phenotype. The degree to which sex reversal occurs in natural nests and the success of sex reversed males reaching adulthood is poorly known. We developed seven Y chromosome specific markers using novel techniques of genome subtraction to replace the less reliable marker developed using AFLPs. We converted these markers to a PCR sex test and identified sex reversed adult males and hatchlings across the range of the species along an altitudinal gradient in south eastern Australia. Sex reversal occurs across a range of alpine populations (725 to 1640 m elevational range) evident in both hatchlings and adults, but not in lowland populations. Our results contrast with those of earlier studies in demonstrating that sex reversal in the wild persists into adulthood, and so admits a range of demographic and evolutionary implications including the possibility of rapid evolutionary responses climate change.

## Breaking the law: Sexual mate choice behavior of female European common frogs

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Mate choice in amphibians is often considered to be male based. It is assumed that in explosive breeding anuran species scramble competition precludes female choice and males are the choosy sex. In general, all males should prefer to mate with a large female, because these usually produce more eggs and therefore the fitness of the successful male would increase. In a former publication we have shown that smaller *Rana temporaria* males are faster grabbing a female which could be a sign of indiscriminate mate choice behavior. This could be due to large males having advantages during scramble competition. In the current study we studied pair formation in the European common frog without competition. We conducted mate choice experiments placing a male and two differently sized female in a box and recorded their mating behavior. We found that mate choice by large males was random. There was no preference for larger over smaller females (or vice versa). In contrast small males on the other hand preferred larger females. More important, females apparently could choose their mates alike. We recorded two different release calls emitted by females when grasped by an unwanted male. Females could terminate amplexus by applying different strategies. They either turned their bodies to free themselves or they literally played dead until released. So far, death feigning as a strategy to avoid or finish mating has only been described in invertebrates and one newt species.

## **Community engagement in amphibian and reptile research as a path for the new century**

**Donnelly M<sup>1</sup>**

<sup>1</sup>*Florida International University*

The majority of amphibian species are found in the developing countries of the tropics. These species-rich regions of the world are home to increasing human populations and I propose that we can engage communities that live adjacent to wild lands that are home to amphibians and reptiles using Citizen Science approaches and by selecting target species that are easy to identify but can inform us of critical aspects of ecosystem function. I will draw on two examples of Integrated Conservation and Development programs that stand as models for the future: Wildlife Clubs and Ranger Training Programs in central Guyana and Parabiologists working in Wara Sera, Papua New Guinea. In the Guyana case, Wildlife clubs were formed to expose young people to aspects of their natural heritage so they would grow up to care for the ecosystem. In the Paupa case, a series of researchers trained residents from two towns near a remote field station. The amphibian researcher in PNG paired young team members (good at numeracy and writing) with mature team members (good in the field at locating organisms) so that everyone had an opportunity to contribute to the project. Citizen science efforts could flourish in the tropics and I suggest these approaches as a way forward to monitor species of tropical amphibians and reptiles as well as providing capacity building in the developing world.

## The Role of Education in Amphibian Conservation (via video)

**Dos Santos M<sup>1</sup>**

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We are living in the 'Anthropocene Era' with irreversible biodiversity loss and support system of planet Earth is compromised. Although extinctions are natural events, it is the current rate of species extinction that is particularly alarming. I assessed two aspects of the conservation efforts towards minimising species declines and extinction (environmental education amphibian focus at zoo and schools), using amphibians as a model, with a view to improving species conservation tools to further minimise the rate of biodiversity loss. My findings suggest that amphibian focussed conservation tools need further development in order to be used effectively. Furthermore, conservation messages about global amphibian declines are largely failing to reach the public through zoos. Additionally, this finding suggests that conservation of endangered classes, such as amphibians should be emphasised throughout the school years for all science curricula explicitly, including the importance and benefits of amphibians for our ecosystem and for humans both directly and indirectly. There are several focus points that need to be addressed, for example, it is critical for the development of future amphibian conservation strategies to work with communities, enabling a multidisciplinary approach, at global, regional and local levels. The models in my recommendations fall into three categories; effectively using the conservation tools available, increasing collaboration, and, improving species focused environmental education, highlighting some clear directions to improve the conservation of amphibians globally and prevent further species and population-level declines.



## **Behavioral and physiological responses of desert lizards to climate change**

**Du W<sup>1</sup>**

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The global decline of reptiles is already evident due to environmental change. The lizard in arid regions is one of the reptilian lineages that need additional attention and conservation actions. We carried out experiments both in the field and laboratory to demonstrate behavioral and physiological responses of lizards to climate change in the desert steppe of Inner Mongolia, China. Here are our results: (1) Despite seasonal variation in ambient temperatures, females selected warm and moist nests that improve the growth and survival of offspring; (2) Early eggs produced high-quality offspring, but late eggs only produced high-quality offspring at falling incubation temperatures, a case of temporal adaptation. (3) The vulnerability of lizards to climate warming differed between sympatric species.

## **Adaptive responses of the embryos of oviparous reptiles to spatial and temporal variations in nest temperatures**

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Natural nests of egg-laying reptiles exhibit substantial thermal variation, at a range of spatial and temporal scales. Rates and trajectories of embryonic development are highly sensitive to temperature, favoring an ability of embryos to respond adaptively (i.e., match their developmental biology to local thermal regimes). Some thermal variation is unpredictable (e.g., driven by day-to-day variation in weather) and can be accommodated via phenotypic plasticity. In contrast, thermal patterns that are consistent through time and space might generate selective forces that result in adaptations of embryonic development. Spatially, thermal variation can be significant within a single nest (top to bottom), among adjacent nests (as function of shading, nest depth etc.), across populations that inhabit areas with different weather conditions, and across species that differ in climates occupied and/or nest characteristics. Thermal regimes also vary temporally, in ways that generate differences among nests within a single population (e.g., due to seasonal timing of laying), and among populations, and across species. Anthropogenic activities (e.g., habitat clearing, climate change) add to this spatial and temporal diversity in thermal regimes. We review published literature on embryonic adaptations to spatiotemporal heterogeneity in nest temperatures. Our review identifies many cases in which natural selection appears to have fine-tuned embryogenesis to match local thermal regimes. For example, developmental rates have been reported to differ between uppermost versus lower eggs within a single nest, between eggs laid early versus late in the season, and between populations from cooler versus warmer climates. Aspects of embryogenesis also appear to be evolving to deal with recent anthropogenically-derived shifts in incubation conditions. We identify gaps in our understanding of thermal adaptations of early (embryonic) phases of the life history, and suggest fruitful opportunities for future research.

**Phylogenetic relationships in the slug-eater snakes, *Duberria* (Lamprophiidae)**

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Slug-eater snakes, of the genus *Duberria*, are a fairly unique group as their specialised diet of molluscs is one that is rarely found in snakes. The genus, comprising four species, is distributed across the eastern regions of Africa stretching from Ethiopia down into South Africa, with each of the five subspecies of *Duberria lutrix* occupying isolated distributional ranges. We investigated the taxonomic level of the four currently described species (*D. lutrix*, *D. rhodesiana*, *D. shirana*, and *D. variegata*) and subspecies of *D. lutrix* (*D. l. abyssinica*, *D. l. atriventris*, *D. l. basilewskyi*, *D. l. currylindahli*, and *D. l. lutrix*). We produced phylogenetic trees from a concatenated dataset of two mitochondrial genes and one nuclear gene, and used Maximum Likelihood and Bayesian Inference to construct two trees. We assessed species level delineations using sequence divergence values, bGMYC methods and a program called 'Species Identifier'. From the analyses, we investigate whether currently described *D. lutrix* subspecies should be raised to species level.

## **A comparison of eDNA field sampling methods to characterise amphibian communities in turbid aquatic environments**

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Metabarcoding of environmental DNA has emerged as a powerful tool for species detection and has the potential to overcome many of the limitations of traditional amphibian surveys. Turbid water can harbour a large diversity of amphibian species, but sampling of such systems is very challenging, in some cases prohibitively so. We compared three eDNA field sampling methodologies (precipitation, standard 47mm filters and high-capacity filtering capsules) in detecting amphibian species and characterising amphibian communities in ponds in Portugal with varying turbidity levels. On average, capsules filtered c. 7 times more water than standard filters (mean = 7.89 L and 1.1 L respectively; precipitation was always limited to 0.015 L). Capsules also captured more total DNA (mean = 367 ng) than either standard filters (mean = 194 ng) or precipitation (mean = 70 ng). However, there was no significant difference between capsules and standard filters either for the detection of single target species or for overall amphibian community characterisation. Capsules and standard filters both outperformed precipitation, but none of the eDNA methods consistently outperformed traditional sampling, in these relatively small ponds. Site-occupancy modelling indicated that species detection was positively related to pH and negatively related to conductivity. This is the first time that high-capacity filtering capsules have been compared with standard filters for eDNA sampling. The study highlights a number of causes of substantial variation in eDNA sampling and provides recommendations for optimising metabarcoding of amphibian communities.

## Social Networking of the Otago Skink (*Oligosoma otagense*) at Ōrokonui Ecosanctuary

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The social behaviour of New Zealand's native lizards is poorly understood. We explored the social behaviour of the Otago skink (*Oligosoma otagense*), one of New Zealand's largest-bodied and rarest species of lizards. This endangered species has previously been observed in pairs and small groups (up to 4 individuals), but little else is known about its social behaviour. We were particularly interested in the stability of social interactions over time. We studied a translocated population of 33 Otago skinks in an outdoor enclosure at Ōrokonui Ecosanctuary and asked whether social interactions between skinks remained stable over time. Regular photo surveys were carried out from April to October 2018. Skinks were identified using unique pattern markings identifiable from photographs, and patterns of association were recorded (in particular, when skinks were aggregated). Individuals within 10 cm of each other were considered to be interacting, and each interaction was recorded as being either passive or aggressive. In order to understand whether interactions were stable over time, we compared association patterns in autumn/winter to those in winter/spring using social network analysis. Among adults and subadults, there was a positive correlation between the interactions in autumn/winter and winter/spring, thus indicating stable interactions among adults and subadults. However, juveniles showed no stability in their interaction patterns between these two periods. Our study provides an insight into the social networking of Otago skinks, as well as their movement and habitat. These results may assist successful future translocations, and other conservation management strategies for this species.

## High-country herpetology: insights from a 20-year ecological study of alpine lizards

Elphick M<sup>1</sup>

<sup>1</sup>*University Of Sydney*

My association with Professor Shine began way back in 1995 when I embarked on an Honours degree in his lab. My project involved an investigation into the effects of incubation temperature on hatchling 3-lined alpine skinks (*Bassiana duperreyi*), from the Brindabella Range near Canberra. Since graduating with First Class Honours, I have been employed for the past 24 years as Rick's research assistant. For two decades I assisted Rick with his continuing research on the incubation biology of *Bassiana duperreyi*. A typical season involved monitoring natural nest temperatures using miniature data loggers, collecting eggs from the field, and then incubating them in the laboratory at a variety of temperatures. Once the hatchlings emerged, we assessed their morphology, and various other behavioural characteristics, to investigate the ecological and evolutionary consequences of a female's choice of egg-laying site. At the end of every season, after the data was entered and analysed, more questions arose to be explored in the next field season. This research taught me about the importance of long-term projects and the valuable datasets they provide. In my talk I will highlight just a few of the exciting results arising from our long-term research on an alpine skink.

### Preventing the extinction of two of Australia's most threatened lizards

**Emery J<sup>1,5</sup>**, Mitchell N<sup>1,5</sup>, Tiernan B<sup>2</sup>, Retallick K<sup>2</sup>, Flakus S<sup>2</sup>, Valentine L<sup>1,5</sup>, Cogger H<sup>3</sup>, Woinarski J<sup>4,5</sup>

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The blue-tailed skink (*Cryptoblepharus egeriae*) and Lister's gecko (*Lepidodactylus listeri*) are endemic to Christmas Island and were considered widespread, common and secure in 1979. By 2010 both species vanished from their last known locations, and are now listed as Extinct in the Wild. The cause of their demise is unresolved, but strong temporal and spatial evidence implicates the introduced Asian wolf snake (*Lycodon capucinus*) as a primary suspect. However, other introduced species including yellow crazy ants (*Anoplolepis gracilipes*) and giant centipedes (*Scoleopendra subspinipes*) are also possible contributors. Fortuitously, a captive breeding program was initiated in late 2009 by Parks Australia, and 67 blue-tailed skinks and 43 Lister's geckos were captured. Captive breeding has been extremely successful, and as of May 2019, approximately 1500 captive individuals of both species exist on Christmas Island and at Taronga Zoo. Managing introduced predators at a landscape scale is unlikely, but soft release reintroductions into predator managed enclosures have been trialled as potential options for the blue-tailed skink. The first attempt in April 2017 was unsuccessful due to unsuitable habitat and predation by giant centipedes. These two factors were rectified in a second trial undertaken in August 2018. This trial has been viewed as successful due to reaching short-term objectives of (1) having 60% of the released animals' alive 1-month post release and (2) successful breeding within 6 months of release. Future trial soft release reintroductions are planned in late 2019 and a trial assisted colonisation to the Cocos (Keeling) islands in June 2019.

### **The structural characteristics of anuran advertisement calls in different habitats**

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Acoustic communication is an important part of the anuran life history and reproduction. Especially the advertisement calls are usually species specific. Stemming from subjective impressions that calls from different species from around the globe, living in similar habitats, seemed to sound similar, we asked if call characteristics were predominantly shaped by phylogeny or environment. We therefore performed a global analysis of the advertisement calls of 1158 anuran species, representing 208 genera from 40 families. Calls were collected from freely available databases, literature and own call records. The respective species occur in various habitats from arid to humid and temperate to tropical climates. Our analyses revealed a mix, whereas some characteristics, e.g. dominant frequency, could be best explained by phylogeny, others, e.g. call duration, seem to be predominately influenced by habitat variables. We further applied a random forest approach to investigate which combinations of morphology, calling behaviour and preferred habitat could have been the original state of respective call characteristics.



## Phylogenomics of the South American *Liolaemus* lizards demonstrates rapid radiation and rampant reticulation

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Heterogeneity in gene trees can hinder accurate inference of species trees. Discordant signals across the genome are commonly produced by incomplete lineage sorting (ILS) and introgression, which in turn can result in reticulate evolution. Modern species tree inference programs based on the multispecies coalescent are designed to deal with ILS and are robust to low levels of introgression, but extensive introgression violates the fundamental assumption of phylogenetic inference methods based on strictly bifurcating relationships. In this study we explore the phylogenomics of the charismatic *Liolaemus* subgenus of lizards from South America, a group of over 100 species mostly distributed in or around the Andes mountains. Using mitochondrial genes (mtDNA) and genome-wide restriction-site associated sequencing (nDNA), we inferred time calibrated mtDNA gene trees, and nDNA species trees and phylogenetic networks. We find very high levels of discordance between mtDNA and nDNA, which we attribute in part to extensive ILS coupled with very rapid diversification. Our data also reveal extensive and deep introgression. We discuss these findings in the context of Andean orogeny and glacial cycles that fragmented, expanded and contracted species distributions. Finally, we shed light for many long-standing taxonomic issues in one of the most studied lizard groups in the New World.

## The evolution of direct development in Southeast Asian Rhacophorid frogs (*Philautus*, Rhacophoridae, Anura)

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<sup>1</sup>Natural History Museum Bern, Institute of Ecology and Evolution, University of Bern

The more than 300 extant species of rhacophorid frogs exhibit a broad diversity of ecological adaptations and modes of reproduction. The genus *Philautus* was formerly used as a collective genus for all small-sized species suspected of direct development with the lack of a free-swimming larval stage. It is assumed that direct development is a successful strategy of reproduction in habitats without permanent waterbodies. As a consequence of phylogenetic studies the species that exhibit direct development have recently been assigned to three genera: The sister groups *Pseudophilautus* and *Raorchestes* from Asian mainland and Sri Lanka, and *Philautus*, from the Sunda Shelf. Direct development evolved independently within *Philautus*, because this genus is not closely related to the West Asian clade and basal species of this genus breed via endotrophic, free-swimming tadpoles. Recently, a further species that uses the pitchers of *Nepenthes mollis* for breeding was discovered on Borneo. Eggs and tadpoles with yolk-filled guts and reduced mouthparts were found and genetically matched to the adults. Phylogenetic analyses (RAxML, MrBayes) show that this species is nested amongst basal species of *Philautus* with lecithotrophic tadpoles (*P. hosii*, *P. macroscelis*). Endotrophic tadpoles, which completely rely on the egg yolk as their sole nutrient source, are thought to be a precursor stage of directly developing larval forms. This discovery of a further basal species with endotrophic larvae supports the hypothesis that direct development within *Philautus* evolved from ancestors with endotrophic larvae rather than exotrophic tadpoles.

## Incubation temperature affects the color expression and embryonic development of a terrestrial salamander

**Evans A<sup>1</sup>**, Urban M<sup>1</sup>, Jockusch E<sup>1</sup>

<sup>1</sup>*University of Connecticut*

Phenotypic plasticity can be a crucial adaptive response to climate change, particularly for dispersal-limited species living in fragmented habitats. In some amphibians, factors such as developmental temperature can induce plasticity in the expression of color and patterns, suggesting that plasticity may explain some of the spatial variation in relative frequency of alternative color morphs in natural populations. Temperature-induced plasticity also occurs in amphibian life history traits. For example, warmer developmental temperatures typically increase development rate and thus shorten the time to reach metamorphosis but might also decrease size at metamorphosis. In the forests of northeastern North America, populations of red-backed salamanders (*Plethodon cinereus*) show differing proportions of two main color morphs, striped and unstriped. Although the color polymorphism has a genetic basis, plasticity may also contribute to the observed variation. We used a split-clutch common garden experiment to test the effects of developmental temperature on the phenotype (color), growth, and development of *P. cinereus* from six different populations across two states. Our results provide the first evidence for temperature-induced plasticity in coloration of *P. cinereus* hatchlings. We did not detect any morph-specific reproductive differences and surprisingly found that neither temperature nor initial egg size affected the size of hatchlings. The evidence for plasticity shown in our study adds a new dimension to the ecological and evolutionary puzzles about the long-term persistence of this polymorphism. Additionally, our reproductive biology results provide insights into how reproductive traits of this ecologically important species may be impacted by climate changes across the species' range.

## Population trends of European amphibians and reptiles are jointly determined by multiple drivers

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The continuous decline of biodiversity is determined by the complex and joint effects of multiple environmental drivers. Still, a large part of past global change studies reporting and explaining biodiversity trends have focused on a single driver. In most cases, we are unable to attribute biodiversity changes to different drivers, since a multi-factorial design is required to disentangle joint effects and interactions. In this work, we used meta-regression within a Bayesian framework to analyze 843 time-series of population abundance from seventeen European amphibian and reptile species over the last 45 years. We investigated the relative effects of climate change, alien species, habitat availability, and habitat change in driving trends of population abundance over time, and evaluated how the importance of these factors differs across species. A large number of populations declined but differences between species were strong, with some species showing positive trends. Populations declined more often in areas with a high number of alien species, and in areas where climate change has caused loss of suitability. Moreover, a strong interaction between habitat availability and the richness of alien species indicated that the negative impact of alien species was weaker for populations living in landscapes with more suitable habitat. Furthermore, when excluding the two commonest species, habitat loss was the main correlate of negative population trends for the remaining species. By analyzing trends for multiple species across a broad spatial scale, we identify alien species, climate change, and landscape changes as the major drivers of European amphibian and reptile decline.

**Variation of the skin bacterial community and *Batrachochytrium dendrobatidis* infection in the frog *Phyllorhina loveridgei***

**Familia López M<sup>1</sup>**, Rebollar E<sup>2</sup>, Harris R<sup>3</sup>, Vredenburg V<sup>4</sup>, Hero J<sup>5</sup>

<sup>1</sup>*School Of Environment And Science, Griffith University*, <sup>2</sup>*Centre for Genomic Sciences*, <sup>3</sup>*Department of Biology, James Madison University*, <sup>4</sup>*Department of Biology, San Francisco State University*, <sup>5</sup>*School of Science and Engineering, University of the Sunshine Coast*

Globally, amphibian population decline and extinction has largely been driven by chytridiomycosis (*Batrachochytrium dendrobatidis*- *Bd*). Amphibian skin bacteria are increasingly recognized as important symbiont communities with a relevant role in the defense against pathogens, inhibiting the growth of *Bd*. We aimed to describe the *Bd* infection status of wild populations of a terrestrial cryptic frog (*Phyllorhina loveridgei*), and to determine whether infection status was correlated with changes in the skin microbial communities. Skin samples of *P. loveridgei* were collected along an altitudinal range in subtropical rainforests of southeast Australia. Sampling was conducted in two years during two breeding seasons with the first classified as a “La Niña” year. We used Taqman real-time PCR to determine *Bd* infection status and 16S amplicon sequencing techniques to describe the skin community structure. We found *Bd*-positive frogs only in the second sampling year with low infection intensities, and no correlation between *Bd* infection status and altitude, frog sex or size. Skin bacterial diversity was significantly higher in *P. loveridgei* frogs sampled in the 1st year than in the 2nd year. In addition, 7.4% of the total OTUs were significantly more abundant in the 1st year compared to the 2nd year. We identified 67 bacterial OTUs with a significant positive correlation between infection intensity and an OTU’s relative abundance. Forty-five percent of these OTUs belonged to the family Enterobacteriaceae. Overall, temporal variation was strongly associated with changes in *Bd* infection status and bacterial community structure of wild populations of *P. loveridgei*.

**What determines the range margins of the Delicate Skink (*Lampropholis delicata*)?**

**Farquhar J**

<sup>1</sup>*Monash University*

Phylogeographic studies have furthered our understanding of species distributions and divergence patterns in the mesic biota of southeast Australia. Such studies emphasise the profound impact Pleistocene climate has had on current patterns of distribution, implicating abiotic factors as fundamental determinants of species' range limits. However, the underlying eco-physiological processes driving these distributional patterns are poorly studied. I therefore use empirically determined physiological data from the delicate skink (*Lampropholis delicata*) to predict its geographical range limits as a function of spatial climatic data. This research focuses on an isolated peripheral population of *L. delicata* occurring on the Coolah Tops – a high elevation inland plateau in New South Wales. The plateau is surrounded by dry lowland habitat, of Pleistocene origin, which may act as a barrier to dispersal. I use mechanistic niche modelling to elucidate whether such environments are too physiologically demanding, and beyond the limits of the species' capacity to adapt. This research supplements our phylogeographic literature with a species-specific understanding of the consequences physiological adaptation to climate has on distributional limits.

## Timing is critical when inducing oviposition in turtles

Feldman M<sup>1</sup>

<sup>1</sup>TSA

Oxytocin has been used for decades to induce turtles to lay their eggs. Unfortunately, we found it had a success rate of only 37% with a variety of *Trachemys*. It often yields partial clutches or fails to work at all. Oxytocin also causes delays in producing the next clutch and false nesting.

After trying a wide variety of alternative agents on thousands of captive turtles at the Concordia Turtle Farm we found that Lutalyse (prostaglandin F2 alpha) is far more effective (94% success rate) and free of side effects in a variety of North American *Trachemys*, *Chrysemys* and *Graptemys*.

However, when we tried Lutalyse alone on a variety of North American *Trionyx* the success rate decreased to 48%. If we administered the alpha agonist Sedivet (romifidine) 20 minutes before the Lutalyse the success rate only rose to 80%.

Thinking that the reduced success rate was because some of the *Trionyx* were on a “false crawl” we took a series of time lapse videos in July, 2018 of the softshell nesting area. We found that many turtles did engage in false crawls but the period of filming was at the very end of the nesting season. This may have prejudiced our results.

In June, 2019 we will repeat the process, but at the height of the nesting season, to see if false crawls are still a frequent event that may account for our reduced success rate. The resulting time lapse videos will form part of the presentation.

**Using environmental DNA to map winter hibernacula in temperate freshwater turtles.**

Feng W<sup>1</sup>, Loughheed S<sup>1</sup>

<sup>1</sup>*Queen's University*

To disentangle the factors that limit species distribution or facilitate dispersal, accurate occurrence data for the species of interest is key. Using detailed presence or abundance data, we can build models that predict a species distribution across landscapes and help us to discern the factors that constrain their geographic range. A common hurdle in this is assembling accurate occurrence data where the task is often time-consuming and expensive, especially for species that are cryptic or have life histories where the species may be directly observed for only short periods of its annual cycle. Here we use environmental DNA (eDNA) to map the distribution of freshwater turtle hibernacula within a single temperate lake in Eastern Ontario. We detected eDNA concentration gradients for two target species, musk turtles and northern map turtles. For the latter, the signal is consistent with known hibernacula, suggesting that eDNA holds promise as a way of mapping



## Investigating patterns of ecomorphological diversification and convergence in savannas from Australia and Brazil

**Fenker J<sup>1</sup>**, Esquerré D<sup>1</sup>, Rabosky D<sup>2</sup>, Colli G<sup>3</sup>, Moritz C<sup>1</sup>

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Convergent evolution of species' ecological and morphological traits, is often produced by similar selective pressures across biomes. There are many recognized and quantified examples of convergence between distantly related species or even entire clades, like Caribbean anoles and pythons and boas. However, convergent evolution between entire animal communities has remained comparatively poorly studied. If environmental features strongly influence community structure and species' adaptations, we would expect convergence between geographically distant biomes that share similar climate, vegetation and other ecological factors. Using the entire squamate community present in the savannas of South America and Australia (~400 species in total), similar environments on two distant continents, we investigate the presence of phenotypic and ecological convergence. Using a variety of methods, we assess the frequency and strength of convergence across the squamate tree. Additionally, using species distribution data, we look for the overlap of beta diversity traits compared to neutral expectations. We expect strong convergence between independent radiations occupying the equivalent ecological niches in different communities.

## Combining micro- and microhabitat analyses to understand niche evolution in European cave salamanders

**Ficetola G<sup>1</sup>**, Lunghi E<sup>2</sup>, Manenti R<sup>1</sup>

<sup>1</sup>University of Milan, <sup>2</sup>Institute of Zoology Chinese Academy of Sciences

The analysis of ecological niches is a powerful approach to understand the drivers of species distribution. Bioclimatic niches are often analysed by combining distribution records with broad-scale climatic variables, but interactions between species and their environment often occur at fine scales. We combined broad- and fine-scale (microhabitat) approaches to analyse the niches of European plethodontid salamanders, and assessed the factors determining range limits and size. First, we show that microhabitat selection provides accurate information on species tolerance. Second, we analyzed populations living along a hybrid zone (*H. ambrosii*/*H. italicus*) to assess whether hybrids have intermediate or transgressive niches compared to parental species. Introgressed populations showed a transgressive niche, occupying sectors with warmer temperature, lower humidity and more light, compared to the non-introgressed ones. Introgressed salamanders exploited the stressful conditions that exist nearby cave entrance, where food availability is highest, suggesting that transgressive niche is important for the evolution of hybrid zones. Finally, we tested whether a broad ecological niche allows species to occupy large ranges. The relationship between niche breadth and range size was positive at both the broad- and at the microhabitat levels. At the macroclimatic scale, strong autocorrelation inflated the possibility to observe such a positive relationship. Spatial autocorrelation was weaker for microhabitat; spatial null-models showed that microhabitat data provide more direct measure of conditions selected by ectotherms, and provide less biased measures of niche breadth. The direct links between Plethodontids and their microhabitat makes them excellent models to understand how evolution and ecology shape the species distribution.

## Assessing avian predation on New Zealand's lizard fauna using 3D-printed replicas

**Florence-Bennett B<sup>1</sup>**, Nelson N<sup>1</sup>

<sup>1</sup>*Victoria University Of Wellington*

Predation is an important factor influencing the viability and evolution of prey species. Prey populations are generally more robust to the effects of selection pressures exerted by predators with which they co-evolved. This is particularly true within island ecosystems where isolated evolutionary histories have resulted in complex co-evolved linkages among endemic species. Prior to the introduction of mammalian predators, birds and reptiles were considered the top predators within New Zealand ecosystems, however, we know little about the impacts that these predators have on native species. Globally, birds are considered key predators of herpetofauna but within New Zealand the impact of avian predation on lizards is largely unknown. We used clay-coated skink (Scincidae) replicas to quantify the impacts of avian predation on lizard populations around the Wellington region, New Zealand. We used impressions left by birds on the soft clay as a proxy for predation pressure experienced. Frequency of predator interactions largely depended on the foraging strategy of different bird species, with a large proportion of opportunistic ground foragers interacting with replicas. Bird predation rates were lower than expected in mammal-free areas, however, even episodic or opportunistic foraging by birds can result in cumulative impacts on lizard species that are already threatened. Avian-reptile interactions are largely understudied, in the presence of invasive mammals, and following their eradication. Understanding these interactions will become increasingly important for informing management, such as by guiding translocations, to aid the recovery of endemic species following mammal eradications or the creation of exclusion zones.

### **Modeling strategies and evaluating success during repatriations of elusive and endangered species**

**Folt B<sup>1</sup>**, McGowan C<sup>2</sup>, Steen D<sup>3</sup>, Piccolomini S<sup>1</sup>, Hoffman M<sup>4</sup>, Godwin J<sup>1</sup>, Guyer C<sup>1</sup>

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Wildlife repatriation has become an important tool to decrease extinction risk for imperiled species, but successful repatriations require significant time, resources, and planning. Because repatriations can be long and expensive processes, clear release strategies and monitoring programs are essential to efficiently use resources and evaluate success. However, monitoring can be challenging and surrounded by significant uncertainty, particularly for secretive species with slow population growth. Here, we simulated how alternative repatriation strategies influence repatriation success for the eastern indigo snake (*Drymarchon couperi*), an endangered species that is being repatriated in extirpated areas of its range in southeastern North America. We used a stochastic stage-based population model to predict population growth and extinction risk under different release strategies, and then applied data from ongoing repatriations to predict success and guide future releases in an adaptive-management framework. Because *D. couperi* is difficult to monitor, we modeled how detection probability influenced perceptions of abundance and population growth by monitoring programs. Simulated repatriation strategies releasing older, head-started snakes in greater abundance and frequency created wild populations with decreased extinction risk relative to scenarios releasing fewer and younger snakes less frequently. Simulated abundances observed after imperfect detection suggested that monitoring programs during repatriations of secretive species may indicate repatriation failure when populations are in fact growing. Overall, our modeling framework informs release strategies to maximize repatriation success while demonstrating the need to consider how detection processes influence assessment of success during conservation interventions, particularly in an adaptive-management framework.

## **A novel approach to addressing development impacts on *Triturus cristatus*, a strictly protected UK amphibian**

**Foster J<sup>1</sup>**, Buxton A<sup>1,2,3</sup>, Griffiths R<sup>2</sup>

<sup>1</sup>*Amphibian & Reptile Conservation Trust*, <sup>2</sup>*Durrell Institute of Conservation, University of Kent*, <sup>3</sup>*South Midlands Newt Conservation Partnership*

The great crested newt *Triturus cristatus* (Urodela: Salamandridae) is the UK's most strictly protected amphibian, yet its widespread distribution means that the species is frequently in conflict with land use change. Until recently construction impacts were addressed on a site-by-site basis that typically involves survey, capture and local translocation of newts, alongside habitat enhancement. However, evidence demonstrates that poor outcomes for *T. cristatus* often arise, apparently driven by structural issues associated with the site-by-site approach. In addition, stakeholders frequently express concern over the risk, cost and delays associated with extensive newt capture programmes. Government recently introduced policies allowing "District Licensing", which aims to improve outcomes for *T. cristatus* and relieve burdens on developers and regulators. Here we describe the first full-scale implementation of District Licensing, covering seven contiguous land use planning authorities. Components that distinguish it significantly from conventional licensing include: proactive landscape-wide assessment of newt status; definition of a clear conservation status goal; generation of risk maps based on Species Distribution Modelling; creation of newt habitat linked to a long-term, landscape-wide conservation strategy; guarantee of long-term monitoring and habitat management; focus on habitat provision rather than individual newt protection; funding via developer contributions proportionate to impact. The scheme cost c.£1M (NZD1.9M, USD1.3M) to set up. Now approaching two years of operation, the scheme has attracted uptake from developers and has delivered substantial habitat provision. Based on early findings, wider adoption of District Licensing could be beneficial to *T. cristatus*, so long as there are suitable standards and co-ordination nationally.

## Learning from UK conservation translocations of *Pelophylax lessonae*

**Foster J<sup>1</sup>**

<sup>1</sup>*Amphibian & Reptile Conservation Trust*

The pool frog *Pelophylax lessonae* (Anura: Ranidae) went extinct in the UK in the 1990s. There have been two phases of conservation translocation aimed at restoring this species: firstly, a wild-to-wild translocation using founders from Sweden released in Eastern England, and a subsequent within-country translocation. This has resulted in two UK populations, and this presentation assesses lessons learned from the two translocation approaches. Although still in the early stages by the conventions of amphibian conservation, there are encouraging indicators from both translocations, including regular evidence of overwintering survival, breeding, recruitment, dispersal and apparently normal behaviours. The initial translocation of frogs from Sweden was undertaken over four consecutive years. It was considered inappropriate to source animals for the second population from Sweden, chiefly to limit impacts on the donor populations. Instead, we decided to head-start using spawn from the first UK population. Captive breeding has been rejected, on the grounds of cost, logistics, programme management and disease risk, although this position will be reviewed for future reintroductions. The approach to head-starting has evolved from a “low tech” approach using small containers located in a private residence, to a dedicated facility involving larger aquaria and more complex water management and husbandry. This has essentially allowed us to increase the quality and quantity of founders for the translocation. Challenges have included resourcing of head-starting, monitoring and scaling up for further candidate release sites. The benefits of intensive post-release monitoring and partnership working cannot be over-stated.

### Phenotypic and life-history diversification in *Anomaloglossus* despite past introgressions

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The advent of genomics strengthened the perception that conflicts among gene trees are frequent and often due to introgressions. However, hybridization occurs mostly among species that exhibit little phenotypic differentiation. A recent study delineating species in *Anomaloglossus*, a frog genus endemic to the Guiana Shield, identified an intriguing pattern in the *A. baeobatrachus* species complex. This complex comprises two sympatric phenotypes contrasting not only in body size, habitat, and advertisement call, but also in larval development mode (endotrophic vs exotrophic tadpoles). However, molecular and phenotypic divergences are, in some cases, incongruent, i.e. specimens sharing mtDNA haplotypes are phenotypically distinct, suggesting a complex evolutionary history. Therefore, we genotyped 106 *Anomaloglossus* individuals using ddRADseq to test whether this phenotype/genotype incongruence was a product of phenotypic plasticity, incomplete lineage sorting, multiple speciation events, or admixture. Based on more than 16,000 SNPs, phylogenetic and population genetic approaches demonstrated that exotrophic populations are paraphyletic. Species tree and admixture analyses revealed a strikingly reticulate pattern, suggesting multiple historical introgression events. The evolutionary history of one exotrophic population in northern French Guiana is particularly compelling given that it received genetic material from exotrophic ancestors but shows very strong genetic affinity with the nearby endotrophic populations. This suggests strong selection on larval development and mating call after secondary contact and hybridization. The case of *A. baeobatrachus* represents a striking example of introgression among lineages that are phenotypically distinct, even in their larval development mode, and highlights how high-resolution genomic data can unravel unexpectedly complex evolutionary scenarios.

## **The Asian Toad in Madagascar, when EDRR fails**

**Freeman K<sup>1</sup>**, Porton I<sup>1</sup>, Reardon J<sup>2</sup>

*<sup>1</sup>Madagascar Fauna and Flora Group, <sup>2</sup>Department of Conservation*

It is as important to take lessons from examples of failed Early Detection Rapid Response (EDRR) reptile and amphibian cases as it is from successful ones. Here we examine the multiple and diverse factors that contributed to the failure to take rapid action following the recent invasion of Asian toads in Madagascar's eastern port city of Toamasina. Factors ranged from the lack of 1) in-country capacity to assess and address invasive species issues, 2) a legislative framework to manage biosecurity issues within Madagascar, 3) a recognized reporting network for newly arriving exotic species, and 4) clear guidelines from the IUCN or other authoritative bodies on how to react to emerging invasions. We found that the inability of coordinating NGOs to exert influence over local government decisions hindered progress, the lack of available, accessible funding prevented the ability to react quickly and the absence of assigned, full-time personnel made it very difficult to coordinate multiple national and international actors. Here we present positive steps being taken in Madagascar to improve biosecurity and the in-country capacity to respond more quickly to new invasion scenarios.



## Comparative phylogeography of the Brazilian Atlantic Forest leaf litter lizards (genus *Enyalius*)

**French C<sup>1</sup>**

<sup>1</sup>City University of New York

The composition of communities is shaped by historical dynamics. Past climate change, shifts in ecological interactions, and differences in dispersal potential lead to the current snapshot of biodiversity. The Brazilian Atlantic Forest (BAF) has distinct local dynamics, most notably northern (NAF), central (CAF), and southern (SAF) regions of unique phylogeographic and environmental composition. While broad-scale diversity patterns are evident among these regions, the historical processes that have led to them and the conditions maintaining them remain an open question. Using six species of leaf-litter lizards (genus *Enyalius*) distributed across the Atlantic Forest as a focal assemblage, I assess the influence of past climate change, ecological interactions, and dispersal potential on their current distribution patterns. I project ENMs to past climate to generate hypotheses about their historical distributions and population dynamics through time. I use physiological data to estimate their thermal tolerance and dispersal potential for use in these models. I then use genomic data from 172 individuals to assess the demographic history of the species. I use comparative landscape genetics as an integrative approach to link historical and contemporary dynamics. I find that the biogeographic history of *Enyalius* lizards is consistent with patterns found in other taxa, most notably long-term stability in the CAF and disequilibrium in the SAF. In addition, species from the same region occupy distinct environmental niches. In most species, ecological variables explain a significant amount of variation in genetic diversity, suggesting that ecological interactions and differences in dispersal potential play a role in their biogeographic history

**Sex differences in kidney gene expression: Reproductive costs of the renal sexual segment?**

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The male North American red-sided garter snake produces a large, gelatinous copulatory plug that functions to reduce the probability that a female will remate, and also prevents sperm leakage or ejection. Thus, this copulatory plug is critical to male reproductive fitness. The copulatory plug is produced in the renal sexual segments (RSS) of the male kidney, which is hypertrophied during the breeding season. Using RNA-Seq we compare the gene expression in female, unmated and mated male kidneys. We interpret these new transcriptomic data in light of our past work on the male costs of reproduction in this species as well as more recent work on blood chemistry reflecting possible sex differences in renal function during an especially dry breeding season.

**It came from the land: Venom variation in aquatic snakes relative to terrestrial ancestors**

**Fry B**<sup>1</sup>

<sup>1</sup>*University Of Queensland*

The aquatic environment has been invaded on multiple independent occasions by terrestrial snakes and therefore represents an excellent opportunity to study convergent selection pressures. Aquatic snakes are under unusual pressures in regards to prey escape relative to their terrestrial ancestors, in addition to predated upon a novel prey item. A comparison of 3 aquatic lineages of elapid snakes was undertaken - sea kraits, sea snakes, and water cobras. All venoms were streamlined relative to the more complex venoms of their terrestrial ancestors, reflective of their streamlined diets consisting of a single prey lineage (fish). Studies were also undertaken to determine if there has been specialisation of their venoms for fish relative to that of terrestrial relatives which have a broader diet. Antivenom studies were also undertaken to determine if the convergent selection pressures resulted in similar proteomes that were well neutralised despite the evolutionary distance between these independent aquatic lineages. These results not only inform about evolutionary theory, but also present information vital for the treatment of the envenomed patient.

## Transcriptional comparison of MHC II $\beta$ 1 in amphibians that are susceptible and resistant to chytrid fungus

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Globally, except in Asia, amphibian populations are threatened by the chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). The resilience of Asian amphibians resulted from evolved resistance of Asian amphibian hosts. Differential susceptibilities of hosts have been shown to reflect genetic variation in MHC class II genes. However, genotyping has been based mostly on single PCR derived traditional cloning, which often yields limited genotypes with relatively low statistical confidence. We applied next generation sequencing derived from three independent amplicons to compare MHC II $\beta$ 1 genetic variation in *Bd*-susceptible (*Litoria caerulea*) and *Bd*-resistant (*Bufo gargarizans*) species. High copy number variation of MHC II loci was detected in both species, and allele frequencies differed significantly among individuals. We found differential MHC II allele expression in both species, but no clear relationship between susceptibility and genotype in *Bd*-infection experiments. We found evidence of balancing selection in the MHC II in both species. WuKabat variation was significantly higher in *L. caerulea* than in *B. gargarizans*. Certain codons of MHC II were under strong positive and negative selection in *L. caerulea*. By contrast, only negatively selected codons were identified in *B. gargarizans*. Moreover, among the positively selected sites in *L. caerulea*, five were conserved in *B. gargarizans*. The results suggest that long-term interaction between Asian amphibians and endemic *Bd* lineages drove evolution for resistance to pathogens as they, in turn, became increasingly virulent. Immunogenetic comparison between hosts with different susceptibilities provides novel insights into host-pathogen evolutionary dynamics and selection on the MHC in response to pathogens.

## Toads or fireflies? Which do Asian natricine snakes prefer as a source of steroidal toxins?

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<sup>1</sup>Kyoto University, <sup>2</sup>Chengdu Institute of Biology

Several animals sequester toxins from their prey to use for their own defense. This phenomenon is well studied in some terrestrial vertebrates including Asian natricine snakes, genus *Rhabdophis*. Several *Rhabdophis* species mainly feed on amphibians and sequester cardiac steroidal toxins, bufadienolides (BDs), from toads. Based on our stomach content examination and chemical analysis, three Chinese earthworm-eating species, *R. pentasupralabialis*, *R. nuchalis*, and *R. leonardi*, are considered to sequester BDs from firefly larvae of Lamprinae. However, it was unknown how strongly these snakes depend on fireflies as a toxin resource. In this study, we conducted two experiments to answer this question. First, we conducted feeding experiments to examine dietary preference to toads and fireflies. We found that most snakes only fed on fireflies. Secondly, we conducted chemical preference experiments. We presented a cotton swab bearing chemical stimuli of toads and fireflies and BD itself to the snakes. We counted the number of tongue flicks emitted toward the cotton swabs. We found that most snakes showed higher number of tongue flicks toward the fireflies than toads and showed lower number of tongue flicks toward BD than fireflies. Our findings strongly suggest that the three Chinese *Rhabdophis* species prefer fireflies to toads and that they use chemical cues to discriminate toxic fireflies but the source of the cue is not BD itself.

## Sex chromosome evolution in snakes and lizards

**Gamble T<sup>1</sup>**

<sup>1</sup>*Marquette University*

Evolutionary studies of sex determination and sex chromosomes have been hindered by a lack of information on the types of sex-determining mechanisms that occur among different species. This is particularly problematic in groups where most species lack visually heteromorphic sex chromosomes, such as amphibians, reptiles, and fish, because conventional cytogenetic analyses fail to identify the sex chromosomes in these species. I describe the use of several DNA sequencing tools, including RADseq, RNAseq, and whole genome sequencing, to determine whether a species has male or female heterogamety (XX/XY or ZZ/ZW sex chromosomes) and subsequently identify which chromosomes are the sex chromosomes. Combining newly generated data with data from the literature, I reinterpret the evolution of sex-determining systems in lizards and snakes (squamates) to discover numerous transitions, even in clades that were presumed to have stable systems. The large number of transitions observed in squamates provides an excellent set of replicate evolutionary “experiments” to examine the origin and evolution of sex chromosomes and sex-determining systems. Identifying multiple examples of independently derived XY and ZW taxa is important to distinguish factors common to all XY (or ZW) clades from factors unique to each individual group and can help answer the many outstanding questions about the origins and evolution of sex chromosomes. These methods will undoubtedly prove useful in evaluating other species for male or female heterogamety, particularly the vertebrate clades that lack visibly heteromorphic sex chromosomes, and will significantly accelerate the pace of biological discovery.

## Co-evolutionary History Matters: Competition Strength Between Introduced Frogs and Native Toads on Haida Gwaii, Canada

Gamlen-Greene R<sup>1</sup>, Richardson J<sup>1</sup>

<sup>1</sup>*University of British Columbia*

As climate change pushes species' distributions poleward, we expect to see novel interactions between previously isolated species. The intentional introduction of the Northern red-legged frog (*Rana aurora*) to an island (Haida Gwaii, BC, Canada) circa 2002, provided a chance to test whether the only native amphibian, the Western toad (*Anaxyrus boreas*) was adapted to deal with a newcomer. Frogs are expanding on Haida Gwaii (HG) while native toads appear to be declining, but with little evidence of causality. The two species co-occur naturally on the mainland, so we experimentally tested whether competition from frogs had a larger impact on toads from HG than from the mainland. We ran an outdoor mesocosm experiment with tadpoles in 72 cattle tanks, with three toad populations from each region (HG vs. mainland) paired with frogs or in high and low intraspecific competition treatments. While the effect of intraspecific competition on toad growth and biomass was similar in both regions, on the mainland, intraspecific competition decreased toad growth and biomass two times more than interspecific competition from frogs, whereas on HG interspecific and intraspecific competition were the same strength. Interspecific competition being on par with intraspecific competition on HG may suggest there is more resource overlap between frogs and toads from HG compared to the mainland. This suggests that if toads do not have a recent co-evolutionary history with another amphibian, they may lack adaptations to partition resources as effectively as species with a longer co-evolutionary history.

## Metabolic heat in sea turtle nests: does it matter?

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Incubation temperatures around 28-32°C are critical to the successful development and survival of sea turtle embryos. Much work has gone into predicting how global warming will increase sand temperature at nesting beaches, and the resulting effects on hatchling sex ratios and survival. The incubation temperature of a nest is not defined by abiotic drivers alone, and many factors, including decomposition of organic material and metabolic heat generated by older embryos also contribute to incubation temperature, and ultimately to the viability of sea turtle populations.

As sand temperatures are increasingly breaching the critical thermal limits of sea turtle embryos, the additional input of heat from metabolic processes should be incorporated into models that predict incubation temperatures as that may dramatically reduce survival. This study presents the results of a systematic literature review of the metabolic heat produced by all extant species of sea turtle. We explore empirical and mechanistic methods for predicting metabolic heat, its impact on development and survival, and how researchers have incorporated metabolic heat into predictions of incubation temperature.

Finally, we present a modeling framework that can account for both abiotic and biotic drivers of incubation temperature, and that could lead to greater accuracy when predicting hatching success under current and future climates.



## Two tests of the Hierarchical Mechanisms of Thermal Limitation hypothesis in lizards

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Describing the mechanisms underlying performance is critical for understanding and predicting how species respond to changes in the thermal environment. We present results from two experiments testing predictions of the recent Hierarchical Mechanisms of Thermal Limitation (HMTL) hypothesis. This hypothesis states that both oxygen limitation and the functionality of subcellular structures affect thermal performance and tolerance in a hierarchical manner. We tested two novel predictions of this hypothesis in lizards: First, we tested whether animals cool their bodies in low-oxygen environments (i.e. behavioral anapyrexia) more rapidly when metabolic demand is elevated. To do this, we experimentally increased the metabolic rate of western fence lizards (*Sceloporus occidentalis*) and measured their thermal preference along a gradient within varied oxygen environments. Second, we tested the prediction that optimum temperatures and peak performance for aerobic scope are reduced in naturally hypoxic environments (i.e. high elevation) in transplanted common wall lizards (*Podarcis muralis*), even when critical limits are unaffected. Our results generally support the HMTL framework, but additional work is needed to identify conditions in which the interaction between oxygen and temperature on performance are most ecologically relevant.

## Genomic and transcriptomic investigations of the evolutionary transition from oviparity to viviparity

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Viviparous (live-bearing) vertebrates have evolved repeatedly within otherwise oviparous (egg-laying) clades. Over two-thirds of these changes in vertebrate reproductive parity mode happened in squamate reptiles, which makes them suitable to study the evolutionary transition from oviparity to viviparity in vertebrates. The transition from oviparity to viviparity requires numerous physiological, morphological, and immunological changes to the female reproductive tract, including eggshell reduction, delayed oviposition, placental development for supply of water and nutrition to the embryo by the mother, and suppression of maternal immune rejection of the embryo. We studied the genetic bases of this transition by comparing genomic and transcriptomic data of a closely related oviparous–viviparous pair of lizards (*Phrynocephalus przewalskii* and *P. vlangalii*). Expression patterns of maternal oviduct through reproductive development of the egg and embryo differ markedly between the two species. We identified genes whose temporal and spatial changes in expression account for the major aspects of the oviparity–viviparity transition. In addition, we discovered low levels of convergence in both amino acid replacement and evolutionary rate shift by comparing the gene sequences in transcriptomes of four oviparous–viviparous pairs of lizards in different genera (*Phrynocephalus*, *Eremias*, *Scincella*, and *Sphenomorphus*). This suggests that most of the changes that produce the oviparity–viviparity transition are changes in gene expression, so the occasional evolutionary reversal from viviparity to oviparity may not be as difficult to achieve as has been thought to date.

### **Empowering communities and new generations in Lake Patzcuaro: the case of Achoque (*Ambystoma dumerilii*)**

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Lake Pátzcuaro (Michoacán, Mexico) has been subject to a dramatic decline of native species and a decrease in environmental functionality due to a variety of anthropogenic pressures. The local economy depends on the success of the fishing industry, tourism activities, and craft manufacturing. Most of these activities are undertaken by native Mexican people belonging to the Purépecha community, a pre-Hispanic culture whose livelihoods and worldview are closely tied to the Lake.

One species that is particularly important in purépecha culture is the Achoque (*Ambystoma dumerilii*), a neotenic salamander endemic to Lake Pátzcuaro, classified as critically endangered by the IUCN. Over the last decade conservationists, scientists, and local breeders of this species have united to conserve the Achoque and restore its traditional cultural importance. Making up this group are scientists from Michoacán University, Center for Aquaculture and Fisheries Research, Sisters of the Monastery of the Dominican Order, local Achoque breeders, and international Zoos. Together this team generated a project which aims to use ecological, biological, and social science techniques to manage the Achoque. Preliminary results suggest that the population of Achoque is as low as 50-70 mature individuals, with a peak of reproduction in winter in the deepest area of the lake (north central area). As part of this project local communities have participated in breeding this species with semi-technical implementations as a pilot project for potential future conservation translocations. Through the education program local children (age 5-18 years) are now aware of the existence of Achoques where they weren't before.

**Behavioural observations of the Bermuda Skink *Plestiodon longirostris* in captivity: informing in-situ and ex-situ conservation**

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The Bermuda skink (*Plestiodon longirostris*), the only extant endemic terrestrial vertebrate of Bermuda, has been listed as Critically Endangered by the IUCN Red List for over 20 years. However, because of its secretive behaviour in the wild, making field observations is difficult. Behavioural observations of pairs of Bermuda skinks were undertaken using cameras fitted to replicated, climatically controlled enclosures at Chester Zoo, with a focus on daily activity patterns, basking, and feeding behaviour. This revealed the first ever observations of courtship, mating and egg guarding in the species. We also report the first nocturnal behaviour for this species and examine their feeding and food preferences. Based on recent observations of wild Bermuda skinks, there may be a link between the skinks' nocturnal activities and their relationship with nesting seabirds. Recommendations are made as to how the husbandry can be improved to aid the captive breeding of this highly threatened skink. Equally, the activity profiles observed in captivity can be used to refine the design of ongoing monitoring programmes of the remaining wild populations.

### **LIFE Tritó Montseny: The conservation toolbox for an endemic newt (*Calotriton arnoldi*)**

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The Montseny brook newt (*Calotriton arnoldi*) is an endemic amphibian of Montseny Natural Park and Biosphere Reserve, Spain. It was acknowledged as a new taxon in 2005 and, based on its small range and population size, is listed as critically endangered by the IUCN. Research has confirmed a strong geographic structure within *C. arnoldi* populations, with the species split into two distinct populations. At the end of 2016 the project "Life Tritó Montseny" was established to conserve *Calotriton arnoldi*, focusing on two main goals: 1) Reduce the threats to *C. arnoldi* by improving the riverside habitat. 2) Increase the range of *C. arnoldi* and raise the number of newts in the wild by implementing reintroductions.

In 2007 a captive breeding program was initiated for *Calotriton arnoldi* at four breeding centres (Wildlife Management of Torreferrussa and Pont de Suert, and Barcelona and Chester Zoos) to safeguard the species from extinction and facilitate reintroductions. Three years later an experimental trial release of captive bred newts started with 700 individuals in four different populations. One of the new populations achieved success showing an average annual survivorship of 2.4-8.9%. Currently, from 20 wild founders over 2,500 newts have been bred at an average rate of 300 newts/year.

A recent habitat suitability study has been completed to identify new release sites for the two distinct populations and improve the success of future conservation translocations. Here we discuss the progress of the captive breeding and reintroduction program and plans for its development in the future.

**Ecological aspects of *Gerrhonotus infernalis* in Parque Ecológico Chipinque, Nuevo León, México**

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Falso escorpión or Texas alligator lizard (*Gerrhonotus infernalis*) is an Anguillidae lizard, distributed from Texas in the southern United States of America throughout northeast in Mexico. Very little data exists on the ecology of this species, particularly in México. Herein it is reported data from July 2008 to November 2011 on the activity, sexual size dimorphism, reproductive period and home range size of *G. infernalis* in Parque Ecológico Chipinque, Nuevo León, México. Lizard activity and sexual size dimorphism were evaluated in oak, oak-pine and pine-oak forest and home range was estimated in oak-pine forest. The main lizard activity was during the fall and daily activity pattern varied seasonally. There was statistical difference in ambient, substrate, microhabitat and body lizard temperature among seasons. Lizard habitat preference was oak forest, frequently founded in leaf litter microhabitat. Reproductive period was determined considering preserved specimens from laboratory and some live collected specimens. *Gerrhonotus infernalis* mating from Parque Ecológico Chipinque is during the fall (October-November), eggs development is throughout winter and early spring, clutches are laid during spring (may) and hatchling is during the summer (later June and early July). There was significant difference in the dimensions of the head and body, showing that there is sexual dimorphism in *G. infernalis*. This sexual dimorphism is likely a consequence of intra- and inter- sexual selection. Home range size was estimated and no significant difference was found between reproductive seasons by sex.

**A translocated population of tuatara (*Sphenodon punctatus*) in a cool southern ecosanctuary: are females nesting?**

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Although tuatara (*Sphenodon punctatus*) were once widespread across New Zealand, populations have become restricted to about 32 offshore islands since the arrival of humans. The main causes of decline have been introduced mammalian predators and habitat destruction. Recent efforts to restore mainland populations have involved the translocation of tuatara from islands to fenced mainland ecosanctuaries. The most southerly translocation occurred between Stephens Island (Takapourewa) in Cook Strait and Ōrokonui Ecosanctuary near Dunedin in 2012. Ōrokonui is within the past latitudinal range of tuatara, but is notably cooler than Cook Strait. Ongoing study of this population has involved a close relationship between university researchers and ecosanctuary personnel, under a memorandum of understanding with iwi kaitiaki (guardians). We expected that the translocation would have some effect on the seasonal and reproductive behaviours of tuatara, an egg-laying ectotherm with temperature-dependent sex determination. We used a combination of night monitoring and trail cameras to assess the seasonal patterns of reproductive activity including whether and when nesting is occurring. To understand whether disturbed soil provided an attractive nesting substrate for tuatara, we also set up a manipulative soil experiment. We identified several instances of nesting behaviour, including oviposition, although not in our dug-over (manipulated) plots. We are continuing to assess the implications of nest temperatures for incubation times and sex ratios. Management issues that have arisen relate to the locations chosen by nesting females and to interference from another translocated species (kiwi). These issues are being addressed in ongoing discussion between the parties involved.

**Parasites driving host divergence? Recent research in a long term study of Australian sleepy lizards.**

**Gardner M<sup>1,2</sup>**, O'Reilly R<sup>1</sup>

<sup>1</sup>Flinders University, <sup>2</sup>South Australian Museum

The sleepy lizard is a large, long-lived (30 + years) lizard that occupies stable overlapping home ranges and is host to two species of ectoparasitic ticks (*Bothriocroton hydrosauri* and *Amblyomma limbatum*). The two tick species have allopatric distributions that abut at an abrupt parapatric boundary. We have annual data on the behaviour and ecology of both lizards and ticks from 1982 to 2019, from a site in the mid-north of SA, a unique 38-year study started by the late Professor Mike Bull, with subsequent continuation in the past three years by Mike Gardner and his team. Recent analysis on population genetic structure within the lizards at this site has uncovered host population genetic divergence that appears to be associated with the two tick species. Concomitant with the host population differentiation are host immune gene allele frequency differences, also correlated with the presence of the two tick species. Taken together, these preliminary data suggest a role for ticks or their associated parasite communities in driving the evolutionary diversification of their host species, the sleepy lizard. I will also provide recent data on investigations of the maintenance of the tick boundary.



**Where squamate sociality reaches its peak: *Egernia stokesii* research past, present and future.**

**Gardner M**<sup>1,2</sup>

<sup>1</sup>Flinders University, <sup>2</sup>South Australian Museum

Lizards of the subfamily Egerniinae have varied social systems with examples from relatively solitary living to extended family groups. *Egernia stokesii* were the first species confirmed to live in families. Here I will provide an overview of the research examining different aspects of this sociality from the past, present and future. This includes recent research on genes of the major histocompatibility complex (MHC) indicating that mate choice likely occurs at the formation of groups; and also research on the role that scat piling might play in how the lizards explore their environment. Current research is centred around how habitat manipulations may change social structures.

## **Microhylids of India: Could new discoveries from India untangle the colonization routes of Asian microhylids?**

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India is a leading center for global amphibian diversity, a recognition chiefly attributed to its unique geological history. After the initial break-up of Madagascar-Seychelles-India from Africa and Australia-Antarctica blocks (~140–130 Mya), followed by separation of the Indian subcontinent from Madagascar (~88 Mya) and Seychelles (~65 Mya), the northward-drifting Indian plate is considered to have been a “biotic ferry” during its course to unite with Eurasia (starting ~55 Mya). The ancestors of present-day frogs that moved along with the Indian landmass during Late Cretaceous or Palaeogene experienced long isolation resulting in the origin of endemic lineages. Subsequent connections with Eurasia provided opportunities for faunal exchange allowing several radiations to acquire wider distributions.

The family Microhylidae, a large neobatrachian radiation of Gondwanan origin with circumtropical distribution, is represented by higher number of taxa in Southeast Asia than India, where the subfamily Microhyliinae is dominant. Based on known diversity and distribution patterns, recent large-scale phylogenies suggest an into-India scenario for microhyline colonization. However, the phylogenetic relationships and divergence times of Microhyliinae and its major radiations, along with corresponding landmass movements, suggest otherwise. An out-of-India dispersal of microhylines through multiple Cenozoic land bridges between India and Southeast is likely to be more plausible. Evidence from new microhylids being discovered in Peninsular India highlights the possibility of several missing evolutionary links. Could such findings, complemented with extensive distribution data, connect the dots to untangle the colonization routes of Asian microhylid frogs and resolve biogeographical puzzles concerning several recent ranoid radiations? Additional discoveries will tell.

**Cross-Continental, Interlineage Competition and Virulence of *Batrachochytrium dendrobatidis***

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*Batrachochytrium dendrobatidis*, the fungal pathogen responsible for amphibian declines on all continents where amphibians occur, is composed of at least 4 distinct, deeply diverged genetic lineages. Two of these (BdGPL and BdCAPE) co-occur on both the African and European continents and between them are responsible for the majority chytridiomycosis-associated amphibian declines. We have been experimentally examining the potential for competitive interactions amongst these lineages using both African and European hosts and using isolates from each lineage derived from hosts on both continents. Here we present the results of these experiments, which describe both lineage effects and effects associated with the continents they were collected on. Perhaps most interestingly, competitive interactions were strongly influenced by host species, and in some cases order of exposure to the different isolates.

## **Anthropogenic changes in distribution and severity of disease caused by ranaviruses affecting UK common frogs**

Price S<sup>1,2</sup>, Balloux F<sup>2</sup>, Cunningham A<sup>1</sup>, Garner T<sup>1,2</sup>, Nichols R<sup>3</sup>

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Ranaviruses are responsible for disease-driven declines of common frogs in the UK and are emergent pathogens of European amphibians causing 21st century mass mortalities and declines in 6 other European countries. The spatiotemporal pattern of disease in UK common frogs has been tracked for 25 years as part of the Garden Wildlife Health project, and we used this data set, pathogen genomics and experiments to ascertain how the pathogen is spreading and how disease impacts may vary over time and space. Spatiotemporal models revealed that human population density significantly outperformed temperature when examining factors affecting spread. Interestingly, model fit adjusting human population density for a measure of income improved and suggested a role for increasing affluence in increasing the spread of infections in frog populations. Logistic models investigating disease outbreaks, however, supported a positive relationship between environmental temperature and the rate and severity of disease, a relationship further supported by both in vitro and in vivo studies of pathogen growth and, for the latter, virulence. Both approaches suggest interventions that, in combination, might serve to mitigate the impact of ranavirosis on common frogs.

## **The importance of film for communication and outreach in the field of herpetology**

**Garrett K<sup>1</sup>**

<sup>1</sup>*Freelance (National Geographic)*

Recently a paper was published in Science highlighting the extent to which amphibian chytrid fungus is decimating amphibian populations around the world. A colleague, Jonathan Kolby, suggested we produce a short video to help communicate this important message to audiences that might not read academic journals or lengthy media articles. Within days our video had thousands of views on our YouTube channel alone, and was then published by National Geographic and other media outlets, sharing the key points of this study to a huge diverse audience.

Reptiles and amphibians are not often well represented in traditional media, and we rarely see the kind of funding needed for big budget production. But the perception that video content with high impact is expensive does not have to be the case. The budget for the film described above was particularly low, and short, well thought out, accurate videos are easily achievable, and their reach can be enormous. I am focusing my career on innovating to create high-impact, low-budget productions about reptiles and amphibians that are both attention grabbing and scientifically accurate for complex topics. With a little creativity and, crucially, good connections with herpetologists to access the most cutting edge work, the possibilities are endless. Since attention spans are ever shortening and people expect information in colourful bite-sized chunks, it is important to work within these parameters to improve our science communication.

## Spatial ecology of an endangered amphibian *Litoria raniformis* within modified Tasmanian landscapes

Garvey T<sup>1</sup>

<sup>1</sup>Deakin University

Within Tasmania the growling grass frog (*Litoria raniformis*) has experienced a rapid contraction in distribution, primarily attributed to habitat loss through landscape modification and improved land drainage. Reductions in seasonal water-sources has placed increasing importance on permanent water bodies for reproduction and foraging. Rural Tasmanian landscapes often feature small artificial ponds utilised for watering livestock. An improved knowledge of how *L. raniformis* may exploit anthropogenic ponds is required to inform continued conservation management. Telemetric tracking of *L. raniformis* (n = 25) was implemented within agricultural and managed forestry sites, with tracking conducted periodically over the breeding season. We investigated (1) potential differences in habitat utilisation between agricultural and plantation sites, and (2) the post-breeding dispersal of individual frogs. Frogs were found to remain in close proximity to ponds throughout November/December, with individuals occupying vegetative depauperate water bodies beginning to disperse by January/February. Dispersing individuals traversed exposed plantation understory and agricultural pastureland in order to enter patches of native scrubland. By March/April all individuals captured at minimally vegetated ponds had retreated to adjacent scrub corridors. Animals found in ponds featuring dense riparian vegetation were not recorded to disperse. This behaviour was replicated in agricultural and forestry sites. The patterns of movement reported in this investigation emphasise the significant contribution of manmade water-bodies and remnant vegetation corridors towards the conservation of *L. raniformis* within modified landscapes. Loss of artificial dams or buffering scrubland in heavily altered landscapes could see the breakdown of the greater *L. raniformis* meta-population further threatening their regional persistence.

## **Radiocesium accumulation and spatial ecology of rat snakes (*Elaphe* spp.) in Fukushima Japan**

**Gerke H<sup>1</sup>**, Takase T<sup>3</sup>, Hinton T<sup>3</sup>, Beasley J<sup>2</sup>

<sup>1</sup>University Of Georgia, <sup>2</sup>Savannah River Ecology Laboratory, <sup>3</sup>Institute of Environmental Radioactivity

Despite being one of the largest anthropogenic releases of radioactive contamination in history, many questions remain regarding the ecological impacts of the 2011 nuclear accident in Fukushima, Japan. In particular, despite their high trophic status, limited home range sizes, and close association with soil where many radionuclides accumulate, few studies have investigated radiocesium accumulation in snakes. Here we present one of the most comprehensive radioecological studies of snakes to date. Using a combination of whole-body radiocesium counts, GPS transmitters, and optically-stimulated luminescence (OSL) chips attached to rat snakes (*Elaphe* spp.) in Fukushima, Japan, our objectives were to 1) quantify radiocesium activity levels of snakes within the Fukushima Exclusion Zone relative to nearby control areas, and 2) investigate snake movements, multi-scale habitat selection, and temporal dynamics of radiation exposure to elucidate the role of snake movement on radiocesium accumulation. Whole-body radiocesium levels for snakes were generally high compared to other species for which published reports are available from Fukushima. Furthermore, radiocesium levels were highly variable among individuals, suggesting local contamination levels and individual movements likely have substantial influence on exposure. Short-term home ranges were small with limited daily movements and included more grassland and less evergreen forest than expected given the proportions available on the landscape. Snakes also avoided evergreen broadleaf habitat within their home ranges, providing insight on potential radiation exposure since contaminant levels vary among habitats. Limited movement rates of snakes compared to more mobile species suggests snakes could be useful bioindicators of local contamination.

**Mitigating for development: where are we at and where are we going?**

**Germano J**<sup>1</sup>, Field K<sup>2</sup>, Griffiths R<sup>3</sup>, Clulow S<sup>4</sup>, Foster J<sup>5</sup>, Harding G, Linoff L<sup>6</sup>, Swaisgood R<sup>7</sup>

<sup>1</sup>*New Zealand Department Of Conservation*, <sup>2</sup>*US Fish and Wildlife Service*, <sup>3</sup>*University of Kent*, <sup>4</sup>*University of Newcastle*, <sup>5</sup>*Amphibian and Reptile Conservation*, <sup>6</sup>*Smithsonian Institute*, <sup>7</sup>*San Diego Zoo Global*

Despite rapid growth in the field of reintroduction biology, results from scientific research are often not applied to translocations initiated when human land-use change conflicts with the continued persistence of a species' population at a particular site. Such mitigation-driven translocations outnumber and receive more funding than science-based conservation translocations, yet the conservation benefit of the former is unclear. Because mitigation releases are economically motivated, outcomes may be less successful than those of releases designed to serve the biological needs of species. Translocation as a regulatory tool may be ill-suited for biologically mitigating environmental damage caused by development. Evidence suggests that many mitigation-driven translocations fail, although the application of scientific principles and best practices would probably improve the success rate. Lack of transparency and failure to document outcomes also hinder efforts to understand the scope of the problem. If mitigation-driven translocations are to continue as part of the growing billion-dollar ecological consulting industry, it is imperative that the scale and effects of these releases be reported and evaluated.



## Lizard Mitigation in New Zealand

Germano J<sup>1</sup>, Adams L<sup>2</sup>, Monks J<sup>3</sup>

<sup>1</sup>*Department Of Conservation*, <sup>2</sup>*Department of Conservation*, <sup>3</sup>*Department of Conservation*

New Zealand is home to over 100 lizards species with the majority being at risk or threatened with extinction. The greatest threats are introduced mammalian predators and habitat loss. While some legislation is in place to protect wildlife from the impacts of development (e.g. Resource Management Act 1993, Wildlife Act 1953), until recently, there has been little compliance in respect to the killing of lizards. This changed, when a private individual successfully prosecuted a District Council for consenting development with the full knowledge that it would kill protected wildlife. Since then, several Councils have started to consider the impact on lizards in relation to human development and the Wildlife Act has been applied in many of these cases. In these larger urban areas where Councils have their own biologists that can advocate for lizard protection/mitigation, attempts to mitigate have been increasing. However, despite this, we continue to see huge losses due to direct killing, fragmentation, habitat loss and degradation throughout the country. The greatest challenge to dealing with this is the lack of empirical knowledge and a lack of tested, reliable tools for predator control and habitat enhancement to grow and protect lizard populations. Without these tools, attempts to use translocations/salvage of individuals is for the most part likely to be futile. While the sheer volume of this issue has been great due to the diversity of NZ's lizard fauna, the same issues are likely to increase in the future for both lizards and native frogs found on the mainland.

## The future of Western Pond Turtles: what might climate change do?

**Germano D<sup>1</sup>**, Bury B<sup>2</sup>

<sup>1</sup>California State University, Bakersfield, <sup>2</sup>U.S. Geological Service (retired)

The Western Pond Turtle (*Actinemys marmorata*) has an extensive latitudinal range (1400 km long) along the Pacific coast of North America. Upland habitat is arid or desert in parts of its southern range contrasting with more mesic conditions from oak woodlands to conifer forests to the north. Models of climate change suggest that the average air temperatures will increase, and could be accompanied by less precipitation, although some models predict no change or even wetter conditions. There seems to be agreement that less snow will fall in the winter, at least in the Sierra Nevada of California. The species, especially in the southern portion of the range, may be harmed by prolonged droughts, especially if human-made water catchments (cattle tanks) dry for prolonged periods. However, the species evolved in arid western North America and survived past dry periods. We present data from the southern part of the range that supports the hypothesis that Western Pond Turtles can survive extended drought at small ephemeral sites. Of greater concern to the species is increasing human population growth and associated habitat loss, especially in California. Even then, because this species uses human-made water systems extensively, there may be sufficient numbers of water catchments of small to moderate size at lower elevations to support Western Pond Turtles into the next century.

## Comparison of population and life-history traits of the Western Pond Turtle across its linear range

**Germano D<sup>1</sup>**, Bury R<sup>2</sup>

<sup>1</sup>California State University, Bakersfield, <sup>2</sup>U.S. Geological Service

The Western Pond Turtle (*Actinemys marmorata*) occurs from near the Canadian border southward to Baja California over a distance of 2,500 km. They frequent wetlands that range widely in habitat from mesic coniferous forest to hot, dry desert. Our long-term studies at multiple sites across its range show marked variation in population parameters and life-history traits: size and age structure, adult size, growth rates, and clutch sizes. Most traits appear influenced by local or regional climate, and clinal variation by latitude. Adult size does not differ across the range. They have higher growth rates in southern populations, and it takes almost twice as long (12-16 y) to reach large sizes in northern populations. Also, because of higher growth rates, turtles in many southern populations reach sexual maturity in half the time as in northern populations. Clutch sizes, however, are lower in southern populations than northern ones, although many females double clutch in southern populations, which has been found only for one population of northern turtles.

**Resolving *Batrachochytrium dendrobatidis* lineage distribution and ecology in South Africa**

**Ghosh P<sup>1,2</sup>**, Verster R<sup>2</sup>, Brookes L<sup>3</sup>, O'Hanlon S<sup>1</sup>, Garner T<sup>2,3</sup>, Weldon C<sup>2</sup>, Fisher M<sup>1</sup>

<sup>1</sup>Imperial College London, <sup>2</sup>North West University, <sup>3</sup>Zoological Society of London

The lineage of *Batrachochytrium dendrobatidis* (*Bd*) associated with an amphibian population is an important epidemiological factor; only two lineages, BdGPL and BdCAPE are known to have precipitated amphibian declines in nature. However, the extent to which *Bd* lineage determines epidemiological outcomes and the significance of interactions amongst lineages as well as their distributions, remain largely unresolved. In South Africa, where both BdGPL and BdCAPE have been isolated, we used a novel lineage specific PCR based diagnostic to map the distributions of lineages across the country in the largest scale effort to delineate *Bd* lineage populations to date. We have identified evidence of lineage co-occurrence, interaction and hybridisation in wild amphibian populations and apparent segregation of lineages along environmental gradients. This work has also created a valuable baseline dataset with which to move forward in understanding the importance of *Bd* lineage ecology and distributions.

## **Agricultural intensification driven landscape modification as determinants of herpetofaunal abundance**

**Ghosh D<sup>1</sup>**, Basu P<sup>1</sup>

<sup>1</sup> *University of Calcutta, Department of Zoology*

Agricultural intensification has been cited as a major driving force for landscape modification around the globe. Vulnerability of herpetofauna from landscape modification is argued to be very high due to their low agility, reticent nature and high sensitivity to microhabitat changes. However, very little information exists regarding impact of agricultural intensification driven landscape modification on the herpetofauna community. We conducted our work along an agricultural intensification gradient in Eastern India to investigate how herpetofauna community is influenced by intensive agriculture driven landscape change at different spatial scales. The study looked into the effect of seven land-uses and landscape composition, on herpetofaunal abundance in four spatial scales from 125m to 1000m. Although species diversity remained unaltered between low and high intensity agricultural landscapes, abundance was lower in higher intensive areas compared to low intensive zones. Different land-use elements showed variable effect on herpetofaunal abundance. Landscape composition at patch level showed positive effect of intensification, semi natural vegetation and water body whereas plantation, human settlement and fallow lands had negative impact on abundance. At 250m radius intensification and agriculture positively affected abundance where as human settlement had negative impact. But agriculture and plantation showed negative impacts at 500m spatial scale while fallow land and curiously human settlement showed positive effect. At the landscape scale of 1Km none of the elements in the landscape appeared to have any effect on abundance. Our study provides an insight into the importance of managing land-uses in agricultural landscapes as a measure for restoring diversity of herpetofauna.

## **Captive management for conservation – ex situ interventions past, present and future**

**Gibson R<sup>1</sup>**, Adams L<sup>2</sup>

<sup>1</sup>*Auckland Zoo*, <sup>2</sup>*Department of Conservation*

The more than one hundred New Zealand lizard species, characterised by high levels of micro-endemicity and surviving in a vastly human-altered landscape over-run with recently introduced predators, present conservation managers with a significant challenge.

A pest-free mainland being many years in the future, insufficient research on effective survey/monitoring tools, poor understanding of predator-sensitivities, and a dearth of suitable pest-free islands for translocation of all threatened species, mean that lizard conservation in New Zealand requires a new paradigm.

Ex situ, or captive, management for conservation has been applied to different extents for a dozen or more native reptile taxa over the past 20 years. While some projects have been relatively successful in contributing to establishment of new wild populations, none has been without problems. Deficiencies in: planning and collaboration; husbandry knowledge and facility suitability; breeding management; knowledge of NZ reptile disease; and release and monitoring regimes, are all implicated among the projects reviewed.

Regardless of these drawbacks, there is a growing need to develop hybrid in situ/ex situ management models tailored to the unique and complex circumstances of each species. Ideally such models would be thoroughly tested but imminent threats to some species force our hand, and critically endangered lizards are now the test subjects for developing and understanding the benefits of partial versus complete predator removal, micro-habitat manipulation, and exploring the full continuum from in situ to ex situ management. Together these methods may offer credible new options for supporting lizard species survival.

## Optimising captive recovery for the critically endangered Baw Baw frog

Gilbert D<sup>1</sup>, Magrath M<sup>1</sup>, Byrne P<sup>2</sup>

<sup>1</sup>Zoos Victoria, <sup>2</sup>University of Wollongong

Food availability and temperature are known to independently trigger phenotypic change in ectotherms, but the interactive effects between these factors are only beginning to be considered. The aim of this study was to investigate the independent and interactive effects of water temperature and food availability on larval growth and development of the critically endangered Baw Baw frog, *Philoria frosti*. Larvae were reared at low (10-12°C) or high (15-17°C) water temperature in the absence or presence of substrate (food availability), and body size and time to metamorphosis were quantified. Growth and development of larvae was influenced by the individual effects of temperature and food availability: time to metamorphosis was lower in warm water treatment groups and in the presence of substrate. Water temperature and food availability did not have an interactive effect on either time to metamorphose or body size at metamorphosis. Under all treatment groups, metamorphic onset occurred once a developmental size threshold was reached, indicating that growth rate and body size are key factors controlling the metamorphic process in Baw Baw frogs (lending support to the Wilbur Collins model for ectotherm development). From an applied perspective, our findings have implications for amphibian conservation because they indicate that simple manipulations of temperature and food availability can be used to improve the rate at which frogs can be generated in captive breeding programs.

### How to Find a Frog in Forest- can detection dogs be used for conservation?

**Gilbert D<sup>1</sup>**, Magrath M<sup>1</sup>, Harnett C<sup>1</sup>, Cooper M<sup>3</sup>, Edwards L<sup>4</sup>, Byrne P<sup>2</sup>

<sup>1</sup>Zoos Victoria, <sup>2</sup>University of Wollongong, <sup>3</sup>Department of Environment, Land, Water and Planning,

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Amphibians (frogs, salamanders and caecilians) are declining more rapidly than any other vertebrate taxa with over one third of known species facing extinction. The causal factors for this decline are numerous, as are the conservation actions available to managers. One management option available to ensure against extinction and promote wild recovery is the establishment of ex situ conservation breeding programs. One priority goal for ex situ CBP is to establish a genetically healthy population, which is essential if wild recovery is a long-term objective. However, ex situ programs are often established when the population of the target species has already declined dramatically, making collection of sufficient material challenging. This is further complicated if the species is cryptic and found in complex habitat. The use of novel techniques to assist with detection of individuals should be explored to maximise chances of genetic collection. One technique that is widely used for the detection of cryptic objects is scent trained detection dogs. However, their reliability will depend on various factors, including strength of scent, wind, habitat complexity, sex and individual dog behaviour.

Here we test methodology and effectiveness of detection dogs trained to locate the highly cryptic Baw Baw frog (*Philoria frosti*), highlight challenges faced and recommend future uses for detection dogs in amphibian conservation management.



## Refinement of spermatozoa cryopreservation techniques in *Ambystoma tigrinum* for salamander conservation and management

**Gillis A**<sup>1</sup>, Guy E<sup>1</sup>, Marcec-Greaves R<sup>3</sup>, Kouba A<sup>2</sup>, Vance C<sup>1</sup>

<sup>1</sup>Biochemistry, Molecular Biology, Entomology & Plant Pathology, <sup>2</sup>Wildlife, Fisheries & Aquaculture,

<sup>3</sup>Detroit Zoological Society

The impact of cryopreservation techniques on caudate spermatozoa physiology and functionality are still being unveiled, such as effects of osmolality and toxicity of cryosolutions. Cryoprotectant (permeating and non-permeating) selection and freeze-thaw rates play a critical role in post-thaw survivability of sperm cells. Presently, 5% permeating dimethyl sulfoxide (DMSO), 5% non-permeating trehalose, and a thaw temperature of 20°C has been the most successful sperm freezing regimen for *Ambystoma tigrinum*, resulting in 28.7% average relative total motility and 92.4% morphologically normal sperm. In order to improve current cryopreservation protocols for caudate spermatozoa, we evaluated two permeating cryoprotectants, DMSO and DMFA (dimethylformamide), and two thaw temperatures of 20 or 40°C, on motility and morphology. Animals were injected with luteinizing hormone-releasing hormone to stimulate production of sperm, which was collected in the form of milt or spermic urine. Sperm samples were mixed 1:1 with one of two cryosolutions, 10% DMSO or DMFA with 10% trehalose and 1% BSA, loaded into 0.25 mL straws, cryopreserved over liquid nitrogen vapor, and evaluated after 12 hours of storage. Total motility and morphological abnormalities were examined pre- and post-thaw. An individual animal was designated as a block, and each straw an experimental unit. The average sperm initial total motility and morphological normality were 44.1% and 98.7%; whereas, average relative total motility and normality post-thaw were 13.6% and 88.9%. Head and tail abnormalities were similar within treatments. Outcomes from this experiment will benefit salamander conservation through improved understanding of spermatozoa physiology and cryopreservation methods.

## Evolution of caudal luring behavior in Crotalinae Opper, 1811 (Serpentes: Viperidae)

**Giozza A<sup>1</sup>**, Slobodian V<sup>1</sup>, Brandão R<sup>1</sup>

<sup>1</sup>University Of Brasilia

Caudal luring is one of the most striking behaviors displayed by snakes. This behavior is observed in cryptically colored snakes that wave their conspicuous tail tip to attract preys that feed on worm-like invertebrates. The caudal luring behavior is present in many species of different families, being very common in viperids, especially in the Crotalinae subfamily. This behavior is usually associated to other characters like ontogenetic shift in diet from ectothermic to endothermic prey, and a conspicuous tail tip that mimics a worm-like invertebrate. Since this behavior is widespread within Crotalinae, some authors often assume that species that show the beforementioned characters have caudal luring even when the behavior was not observed. Herein, we investigate the evolution of caudal luring in Crotalinae snakes, aiming to establish if this behavior is associated to ontogenetic shift in diet and presence of a conspicuous tail tip, with special focus on *Crotalus* genus. In order to do it, we conducted a bibliographic survey for the three characters on neotropical Crotalinae snakes, producing a character matrix which was optimized on a recent phylogenetic hypothesis. Our results suggest that caudal luring was lost in most *Crotalus* species, along with the loss of a conspicuous tail tip and ontogenetic shift in diet. Furthermore, our results indicate that the ontogenetic shift on diet is usually associated with the presence of caudal luring in Crotalinae snakes, however the conspicuous tail tip is not always a good indicator for the presence of caudal luring behavior.

## **Biogeography and the Timing of Anole Lizard Diversification**

**Glor R<sup>1</sup>**

<sup>1</sup>*University Of Kansas*

Anole lizards are an iconic example of an adaptive radiation with more than 400 species distributed across the neotropics. Prior studies recover extremely old ages for the onset of anole diversification; the most recent studies based on fossil calibration of molecular phylogenetic trees suggest that anole diversification began by the Eocene (roughly 50 Ma). We generate new estimates for the timing of anole diversification based on a phylogenomic dataset that includes comprehensive sampling of major anole clades and related pleurodont iguanian outgroup taxa. By applying nine well-established fossil calibrations to this dataset, we recover substantially younger ages for anole diversification than prior studies. Our results are in better agreement with the known geologic history of West Indian islands because they do not imply extensive diversification on islands that did not yet exist.

### **Aggregative behaviour in New Zealand's native lizards**

**Godfrey S**<sup>1</sup>, Nelson N<sup>2</sup>, Carter A<sup>3</sup>, Herbert S<sup>2</sup>, Irwin E<sup>4</sup>, Kikillus H<sup>2</sup>, Lennon O<sup>2</sup>, Price S<sup>2</sup>, Ramirez P<sup>7</sup>, Stenhouse V<sup>2</sup>, Woolley C<sup>2</sup>, van Winkel D<sup>6</sup>, Hare K<sup>5</sup>

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Social behaviour is poorly described among the world's herpetofauna. However, a growing number of examples demonstrate that herpetofauna can exhibit complex social organisation. Among the traits thought to favour the evolution of social behaviour, viviparity may be one precursor to the evolution of sociality in squamates. As all but one of New Zealand's 100+ species of endemic lizards (comprised of skinks and geckos) are viviparous, this group provides an ideal opportunity to investigate the frequency and nature of aggregative behaviour. We reviewed all known peer-reviewed publications and grey-literature recording observations of aggregation in both field-based and laboratory studies. Among the 29 species in which behaviour had been documented, aggregative behaviour (where two or more individuals were found in association) was described from more than 75% of species, ranging from adult-offspring associations and male-female pairs, to aggregations of more than 20-100+ individuals. Aggregative behaviour was most commonly noted in *Woodworthia* geckos, while *Naultinus* geckos (n=2) were exclusively territorial. Potential mechanisms driving aggregation behaviour in these species include aggregations based around resources (refuges and food resources). Evidence exists for complex sociality among New Zealand's native lizards, including family groups and parental care.

## **The Madagascar ploughshare tortoise programme – between recovery and extinction of an iconic species**

**Goetz M<sup>1</sup>**

<sup>1</sup>*Durrell Wildlife Conservation Trust*

The ploughshare tortoise (*Astrochelys yniphora*) is the world's rarest and most critically endangered tortoise and subject of a 30+ year conservation programme in the remote North-West of Madagascar. Early threats were mainly man-made bushfires, invasive pigs, habitat fragmentation and small population sizes which were successfully addressed through a combination of political and community engagement, habitat protection (creation of a national park) and in-country captive breeding, head-starting and release. But a surge in poaching for the international illegal pet trade has now brought the species to the brink of extinction in the wild. New responses to this threat had to be developed and tested hastily but were significantly hampered by financial constraints and an unstable political situation in Madagascar. This leaves a previously very successful recovery programme trying to adapt to a new, barely manageable human threat purely driven by financial motivations.

The talk will outline the main threats and past and present key interventions with outcomes and lessons learned and will give an outlook of ongoing, planned and hypothetical solutions.

## **Integrating underwater surveys, telemetry and citizen science to understand drivers of movement in sea snakes**

**Goiran C<sup>1</sup>**, Udyawer V<sup>2</sup>, Chateau O<sup>3</sup>, FGM Citizen Scientist Group<sup>4</sup>, Shine R<sup>5</sup>

<sup>1</sup>LabEx Corail & ISEA Université de la Nouvelle-Calédonie, <sup>2</sup>Australian Institute of Marine Science,

<sup>3</sup>Département scientifique et pédagogique, Aquarium des lagons, Nouméa, <sup>4</sup>The Fantastic GrandMothers Citizen Scientist Group, <sup>5</sup>Department of Biological Sciences, Macquarie University

As reef meso-predators, sea snakes play an important role in reef ecosystems and are bio-indicators for eel populations and overall reef health. Strong evidence suggests that marine snakes are able to identify changes in environmental conditions (i.e. tide, lunar phase, water temperature, air pressure) and use these as cues to alter behaviour to capture prey, avoid predators, find mates, and survive storm conditions. However, research on this topic has been restricted to changes in the observed abundance of snakes at different time periods, with few data on continuous movements and use of space of these animals over extended periods. In this study, we incorporated mark-recapture data collected using regular underwater surveys with passive acoustic telemetry methods and local citizen science initiatives to study the ecology of sea snakes at a higher resolution and identify environmental factors that influence their movements and home ranges in Noumea, New Caledonia. The increased resolution of movements and habitat use gained from the combination of methods of data collection highlighted strong tidal and seasonal patterns of residency in Olive-headed sea snakes (*Hydrophis major*). We also found strong species-specific differences in dispersal capacities across three species of sea snake commonly found in Noumea (*Emydocephalus annulatus*, *Hydrophis major*, *Aipysurus duboisii*), highlighting variable habitat use patterns within a single site. Movement and habitat use information can provide an insight into how sea snake populations may react to acute or chronic changes to the ecosystem.

## Sea snake research in New Caledonia

### Goiran C<sup>1</sup>

<sup>1</sup>*Université De La Nouvelle-calédonie*, <sup>2</sup>*Laboratoire d'excellence Corail*

New Caledonia hosts about fourteen species of sea snakes. They belong to both of the marine Elapidae lineages: laticaudinae or sea kraits, which are oviparous and amphibious, and hydrophiinae or true sea snakes, which are viviparous and fully marine. Some populations of these sea snakes species are exceptionally easy to access for researchers, as they inhabit shallow coastal waters near urbanized areas. However, they did not attract much interest from scientists before the twenty-first century. Less than 20 scientific articles about New Caledonian sea snakes were published before 2000, some of them only making a brief account of species presence. Sea snake research greatly increased in New Caledonia during twenty-first century, as more than 55 articles containing data about New Caledonia sea snakes were published to date. Professor Rick Shine is one of the major initiators of this increase, since he authored or co-authored at least 35 of these papers. These studies cover different aspects of sea snake ecology and evolution. This presentation aims to highlight some of the findings of his research about sea snakes in New Caledonia.

**Being cool is hot: substrate heat capacity influences ventral color evolution in lizards.**

**Goldenberg J<sup>1</sup>**, Massetti F<sup>2</sup>, D'Alba L<sup>1</sup>, Clusella-Trullas S<sup>2</sup>, Shawkey M<sup>1</sup>

<sup>1</sup>*Ghent University*, <sup>2</sup>*Stellenbosch University*

Colored integument plays a major role in animals' ecology. Not only for display, sexual selection or crypsis, colors can also have a significant impact on their thermoregulation. Ectotherms are highly dependent on their surrounding environment to regulate their body temperature, thus the colored integument may exert a key task in providing their optimal thermal balance. Previous studies primarily focused on the dorsal region of squamates as this is their most visible area. However, most squamates are confined to the ground and the effect of the substrate temperature can affect their thermal balance. In this research we investigated the effect of substrate reflectance and heat capacity (cp) on the ventral coloration in 12 South African lizards (Cordylidae). We hypothesized that bright ventral colors will be selected in low cp substrates to reflect and dissipate the heat transferred from the ground. We found a strong association between low cp substrates and bright ventral colors; nevertheless, substrate reflectance was not strongly associated with ventral brightness, suggesting that the cp of substrates has a major role in shaping the ventral coloration of cordylids. To understand the evolutionary path of ventral brightness we performed an ancestral state reconstruction. Our results show a rapid brightness diversification within the Cordylinae following its divergence with the Platysaurinae ~25 Mya; a period concurring with an aridification phase of Earth, further hinting for a thermoregulatory role of bright ventral colors. This study suggests for the first time that ventral coloration evolves independently of dorsal coloration, and under different selection pressures.



## **Anuran larvae disperse seeds and enhance germination of aquatic plants**

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Seed dispersal by frugivorous animals is key for plant reproduction and spatial distribution. In aquatic systems, birds and fish are thought to be the main seed dispersers. Here we show for the first time that anuran larvae that commonly feed on aquatic plants disperse seeds in temporary wetlands at a high frequency. Between 9 and 33% of tadpoles from five species retrieved from natural ponds in Southern Spain transported seeds. Some anuran larvae like Western spadefoot toads consume large quantities of plants, commonly feeding on their leaves but also eating their fruits. Spadefoot toad tadpoles damaged only about 22% of the seeds from the ingested fruits. Passage through the tadpole's gut broke dormancy of the ingested seeds, increasing five-fold their germination success and accelerating their germination time. Moreover, we found that even larvae of anuran species that do not feed on plants also disperse viable seeds under natural conditions, likely picking them while grazing from the pond sediment. Given the abundance of anuran larvae in freshwater systems worldwide, they may constitute major vectors of seed dispersal in aquatic environments. This overlooked ecosystem service provided by larval anurans is yet another reason to increase conservation efforts on the worlds' most threatened group of vertebrates.

## Sequencing and annotation of the Western Spadefoot toad (*Pelobates cultripes*)

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Amphibians are very important for our general understanding of evolution, as they have evolved a myriad of reproductive modes and alternative configurations of their ancestral complex life cycle. They have also adapted to widely divergent environments, succeeding to occupy most habitats on Earth. Amphibians are also of huge ecological relevance, providing fundamental ecosystem services to both terrestrial and aquatic systems affecting energy flow, nutrient cycling, and community composition. Unfortunately, amphibians are also currently the most threatened group of vertebrates, as they are highly vulnerable to habitat destruction, pollution, emergent diseases, and invasive species. Despite their importance across fields, the amphibian genomic resources available are still clearly lagging behind those of other vertebrate groups, due in no small part to their typically large genome sizes. Here we contribute the sequence and annotation of the Western spadefoot toad, *Pelobates cultripes*. This species is distributed in the Iberian Peninsula and Southern France and represents the most basal anuran for which the genome is available to date. The genome size of *P. cultripes* is estimated to be ~4.2 Gb in size, close to the average anuran genome size. We used different sequencing approaches, combining paired-end Illumina reads (PE480 at 82.5x coverage), mate pair libraries with insert sizes of 3Kb and 8Kb at ~40x coverage each, and ~22x coverage of long reads from Oxford Nanopore technologies. We obtained an assembly merging previous assemblies generated with MaSurCA and Flye and we then improved the scaffolding with Chicago and HiC data using Dovetail's HiRise software.

**Food, not friend: Tadpoles of the sandpaper frog, *Lechriodus fletcheri*, cannibalise on conspecific eggs**

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Although cannibalism has been considered an abhorrent behaviour, it may be one that incurs significant fitness benefits for those species in which it has evolved. Among the amphibian, cannibalism is commonly associated with tadpole species that exploit ephemeral systems, as they are temporarily inundated with high densities of conspecifics during breeding events that may serve as a novel food source. In this study, we provide evidence of cannibalisation of un-hatched embryos of the sandpaper frog (*Lechriodus fletcheri*) by conspecific tadpoles as a likely adaptation to poor nutrients availability within highly ephemeral pools. Field observations revealed late stage *L. fletcheri* tadpoles actively predated on conspecific oocytes of a recently oviposited spawn body, which were commonly consumed whole. When tadpoles were exposed to spawn for the first time in the laboratory, they quickly came to exploit it as a nutrient source, remaining at the site of the spawn for extended periods to gorge themselves on the available nutrients. These findings, along with the common occurrence of this behaviour in all pools in the field that contain older conspecific tadpoles, suggests that it is a significant source of pre-metamorphic mortality in this species. This appears to be the first description of this feeding strategy in *L. fletcheri*, but one that may be common among Australian anurans that exploit temporary aquatic systems.

### **Multiple kinds of success in amphibian conservation**

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In an era of rapid human-induced change, a central problem of research scientists, resource managers, and land management agencies is how to define conservation success, and to pinpoint why failures occur so that they can be prevented in future problems. Scientists are often tasked to develop decision-support frameworks to help managers and agencies navigate through conservation problems, with the explicit goal of identifying and supporting actions that address a set of defined objectives. Engaging in this process often results in improved conservation outcomes, but the presence of biological and management uncertainties and institutional constraints can result in actions that fail to achieve explicit conservation goals. Part of this failure may come from the lack of an integrated framework for considering the connections between the decision process and the conservation goal (e.g., species recovery and the maintenance of biodiversity). The decision process lays an important foundation that, if conducted appropriately, can increase the probability that conservation goals are reached. In addition, this process provides opportunities for “indirect” successes (e.g., improved collaboration, identification of critical research needs) that may also benefit conservation. As conservation science emerges as a new framework at the boundary of management and science, we recognize conservation success as a continuum.

### Conserving Panamanian Harlequin Frogs by Integrating Captive-breeding and Research Programs

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Captive breeding programs are a valuable conservation resource especially when integrated with research goals. Panamanian Harlequin frogs (genus *Atelopus*) serve as a case study for integrating captive breeding and research goals because they have experienced drastic chytridiomycosis-related declines and have large captive populations. We reevaluated all Panamanian *Atelopus* species through the IUCN Redlist and compiled occurrence records for Panamanian *Atelopus* species to create improved historical distribution maps. We model *Atelopus* habitat suitability to improve our knowledge of their likely range and to guide for future conservation and reintroduction efforts. Captive breeding efforts in Panama and the United States established secure ex-situ populations of *Atelopus certus*, *A. glyphus*, *A. limosus*, *A. varius*, and *A. zeteki*. *Atelopus chiriquiensis* is presumed to be extinct with no captive populations. The status of one undescribed species, *Atelopus aff. limosus*, has not been evaluated and no secure captive population has yet been established. Captive breeding efforts that produce a surplus of *Atelopus* are an important resource for disease mitigation research and have enabled release trials to begin adaptive management approaches to understand the factors limiting *Atelopus* reintroduction efforts. The recent proliferation of molecular tools, climate models, bio-banking, and reproductive technologies position us to address multiple applied and basic evolutionary questions such as: What factors cause differential disease outcomes? Do persisting populations have heritable traits associated with improved survivorship? Are there climatic refugia from disease? Ultimately, the answers to these questions will help us develop applied solutions and facilitate reestablishment of self-sustaining wild populations.

**Managing host density and habitat structure could influence contact rates and transmission of *Batrachochytrium salamandrivorans***

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*Batrachochytrium salamandrivorans* (*Bsal*) is an invasive fungal pathogen that is emerging in Europe and is highly pathogenic to some salamander species. Given its widespread distribution, the eastern newt (*Notophthalmus viridescens*) has the potential to significantly contribute to *Bsal*'s epidemiology if the pathogen emerges in North America. Thus, we designed two studies to estimate: 1) contact rates given different host densities and levels of habitat structure, and 2) the probability of transmission from infected to susceptible individuals as *Bsal* chytridiomycosis progressed. Using parameter estimates from these experiments, we modeled infection and disease outcomes for a population of eastern newts using a system of ordinary differential equations. *Bsal* transmission was very efficient between newts even at early stages of infection. For example, one contact of one-second duration between an infected and susceptible newt resulted in 100% transmission only 12 days after the infected newt was initially exposed to *Bsal* zoospores. Contact rates between newts were density dependent; reducing host density 4-fold reduced contact rates by 5 - 15X. At higher newt densities, adding plants to mesocosms reduced contacts by 3X. Our simulations show rapid transmission of *Bsal* among newts, resulting in >95% infection prevalence in one month and >80% mortality in three months, illustrating the potential for severe population impacts. Our results demonstrate that if *Bsal* is introduced into North America, eastern newts have the potential to play a major role in its epidemiology. Additionally, reducing newt density or increasing habitat structure at *Bsal* positive sites might reduce transmission and outbreak size.

## Broad host range of *Batrachochytrium salamandrivorans* equates to high invasion probability in North America

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*Batrachochytrium salamandrivorans* (*Bsal*) is a recently discovered fungal pathogen that has caused declines of wild Fire Salamander (*Salamandra salamandra*) populations across Europe. Risk models suggest that *Bsal* poses high risk to North American amphibian biodiversity, especially in the southeastern and northwestern USA. Our goal was to estimate the susceptibility of 29 North American amphibian species to *Bsal* infection and chytridiomycosis. Experimental animals were exposed to one of four *Bsal* zoospore doses ( $10^3$ - $10^6$ ), and their condition monitored for at least six weeks. We swabbed animals every six days to estimate *Bsal* infection. Approximately 75% of species tested became infected and 30% developed *Bsal* chytridiomycosis. Susceptible species that developed clinical *Bsal* chytridiomycosis and mortality included: *Chiropetrotriton* sp., *Aquiloerycea cephalica*, *Ensatina eschscholtzii*, *Aneides aeneus*, *Eurycea wilderae*, *Pseudotriton ruber*, *Notophthalmus viridescens*, *N. meridionalis*, *N. perstriatus*, *Taricha granulosa* and *Osteopilus septentrionalis*. Tolerant species that maintained low intensity *Bsal* infections over several successive swabs included seven caudates: *Ambystoma opacum*, *A. mexicanum*, *Cryptobranchus alleganiensis*, *Desmognathus aeneus*, *D. ocoee*, *E. lucifuga* and *Plethodon metcalfi*. Five anuran species were also tolerant of *Bsal* infection, including *Anaxyrus americanus*, *Hyla chrysoscelis*, *Lithobates chirichahuensis*, and *Scaphiopus holbrookii*. Resistant species that did not maintain *Bsal* infections after exposure included: *A. latarele*, *D. monticola*, *Hemidactylium scutatum*, *Necturus maculosus*, *L. sylvaticus*, *L. catesbeianus*, and *P. shermani x teyahalee*. Our results suggest that most North American amphibian communities will be composed of a combination of amplification, tolerant and resistant host species, which could facilitate the emergence, spread and maintenance of *Bsal* in the western hemisphere.

**Diurnal calling behaviour in the nocturnal túngara frog (*Engystomops pustulosus*) on Isla Taboga, Panama.**

**Gray H<sup>1</sup>**

<sup>1</sup>*Redpath Museum, McGill University*

The túngara frog (*Engystomops pustulosus*) is a common neotropical frog found from Mexico to northern South America. Throughout its native range, this species calls only at night. I investigated the temporal calling pattern of a population of túngara on Isla Taboga, Panama where I had found males calling and breeding during the day. Using recordings (Reconyx XR6), the time of day and number of males calling was determined. Abiotic data was also collected (temperature, weather, light condition). Túngara on Barro Colorado Island (BCI), Panama served as a control. There was significant diurnal calling by males on Taboga compared to the control population. This diurnal calling was not correlated to abiotic factors. The biotic factors such as predator and parasite risk as well as intra- and inter-specific competition are discussed as possible mechanisms for the diel shift seen on Isla Taboga.



## **The toad's year: environmental cues of amphibian phenology**

### **Green D<sup>1</sup>**

<sup>1</sup>*Redpath Museum, McGill University*

For animals living in strongly seasonal environments, timing transitions between periods of activity and periods of dormancy is critical and, to a large degree, in direct response to environmental stimuli. Thus, for every population, there should be particular conditions of temperature, precipitation, wind speed or other parameters that may qualitatively differ from prior environmental conditions and so trigger appropriate behaviours. For example, in Fowler's Toads, which hibernate deep underground, springtime emergence is predictably associated with certain surface conditions of temperature, rainfall, wind, and moon phase, which remains true whether the toads emerge relatively early or late in spring. However, this is preceded by early spring turnover in temperatures far underground, which induces the animals to rise to the surface layer where they can be affected by aboveground conditions. Conversely, the onset of cooler autumn weather induces pre-hibernation movement towards suitable sites, curtailment of regular nightly activity and deeper retreat into the sand where they can respond to the fall turnover of underground temperatures. The animals' seasonally appropriate responses to in situ environmental conditions enables the timing of springtime emergence and fall retreat to track unpredictable changes in climate.

**Amphibian decline in the Anthropocene: knowledge and uncertainties.**

**Green D<sup>1</sup>**

*<sup>1</sup>Redpath Museum, McGill University*

Mounting evidence of declines in abundance, disappearances of populations and even extinctions of entire species propelled a surge of research into amphibian population ecology and conservation over the past three decades. Despite some initial skepticism that the trends were real and irreversible, it is now clear that amphibians are declining worldwide in many ways in many places, and for many different reasons. The rapid growth of investigations into amphibian ecology has created considerable new, detailed knowledge, as well better comprehension of what we still do not know. Are the incidents of amphibian decline we are recording truly novel or are they periodic? To what extent are declines anthropogenically caused or anthropogenically exacerbated? How extensively are causative factors of decline inter-related and synergistic in their effects? Are declines in some populations in some places offset by increases in other populations in other places? Amphibians, having survived for hundreds of millions of years, are hardly just the relics of a distant, more glorious past. In evolutionary terms, modern amphibians are spectacularly successful, diverse and abundant. If such remarkable animals as these are facing declines and extinctions due to human activity, then many others are too.

**Toads less travelled: an overview of the ecology of cane toads in New South Wales.**

**Greenlees M**<sup>1</sup>, Shine R<sup>1</sup>

<sup>1</sup>*Macquarie University*

For invasive or potentially invasive species, an understanding of how they function in a particular environment is fundamental to any attempt at predicting likelihood and anticipating characteristics of an invasion. More crucially if they are established, such understanding is necessary for maximising effectiveness of management, and assessing outcomes. Cane toads are one of the more notorious invasive species in Australia though until quite recently, the majority of our understanding of their biology and ecology has come from studies conducted on populations in a limited proportion of their invaded range – namely, in the wet-dry tropics of the Top End. For the past six years we, along with various honours and post-graduate students have been working to improve our understanding of the invasion of cane toads in (more of) Australia by studying various aspects of their biology and ecology in northern New South Wales, a region with a mosaic of different environments and an invasion front. We will provide a brief overview of some of these studies and summarise what we've learned thus far.

### Through the heart: ventricular septum and cardiac conduction system across Sauropsida

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The extent of ventricular septation and development of atrioventricular conduction axis in vertebrates are connected. The atrioventricular bundle and branches of the cardiac conduction system (CCS) reside in the ventricular septum of mammals and birds and can be identified, among other markers, by Human Natural Killer-1 (HNK-1) antibody. We identified putative CCS elements by HNK-1 and we validated activation patterns by using Optical Mapping (OM). By this approach we were able to investigate developing reptilian ventricles, and elucidate the presence of CCS-like structures among Sauropsida. We studied species from clade Archelosauria (*Mesoclemmys heliostemma*, *Crocodylus siamensis*, *Crocodylus mindorensis*, and *Gallus gallus*) as well as several species of Squamata (*genus Varanus*, *Pogona vitticeps*, *Pantherophis guttatus*, *Eublepharis macularius*). HNK-1 specifically labeled epicardium, atrioventricular canal, mesenchymal structures, and a subset of myocytes in the interventricular septum in crocodiles, and ventricular septum in *Mesoclemmys*, *Varanus*, *Pogona* and *Pantherophis* in contrast to *Eublepharis*. OM of both crocodylian species detected similar observations – there was a transition in development from base-to-apex to apex-to-base conduction. In turtle, we only observed base-to-apex propagation. In Squamata, OM detected difference in the impulse propagation among chosen species. The most distinct detection was observed in genus *Varanus* in the area of the forming ventricular septum on the left side of ventricle. In *Pogona*, OM revealed the primitive base-to-apex activation similar to *Eublepharis*. In *Pantherophis*, activation was propagated caudo-cranially in ventricular extensions in later stages. Taken together, primordial CCS-like structures are linked with ventricular septation in crocodylians, and also in varanids.

PROGRES Q38

**Population assessment and distribution of the Madagascan golden mantella in the face of climate change**

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Successful conservation outcomes require multiple approaches at different scales. For Critically Endangered species this is often difficult, because there is often a paucity of data available at any scale on which to construct a conservation plan. The charismatic golden mantella is unusual in that it is Critically Endangered within a very small range in Madagascar, but is widely bred in captivity by zoos and herpetoculturists. As part of a broader conservation plan, we assessed the population status of golden mantellas at local and regional scales in Madagascar. In addition, we used species distribution modelling to predict the implications of climate change on the range of the species. We batch-marked mantellas with VIE tags, and developed a new statistical model that combines features of both open and closed populations to assess changes in abundance over the wet season, detectability and associated covariates. Confined to relatively small patches of forest in central-eastern Madagascar, less than 30% of suitable habitat is currently contained within protected areas. Under a climate change scenario, the climatic envelope for the species will shift to the south-east, to areas outside the protected area network and where the habitat may be less suitable. Long-term conservation of the species therefore requires a dynamic approach that take account of the needs of the species at multiple scales.

## **Past and present status of development mitigation for herpetofauna in the UK**

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Mitigating impacts of development on protected amphibian and reptile species presents significant challenges. This is particularly the case for widespread species that frequently come into conflict with development. Over the past thirty years management interventions to mitigate the impact of development on native species in the UK have evolved, but are still poorly underpinned by rigorous protocols based on evidence. There is now greater emphasis on habitat restoration, creation and management and a better understanding of the importance of connectivity to maintain viable populations within the landscape. In some species, wholesale translocations from development footprints have declined, and have been replaced by interventions aimed at maintaining/enhancing existing populations instead. Nevertheless, many development mitigations fail to maintain viable populations, and post-mitigation monitoring shows that some populations go extinct despite costly interventions. The reasons for failure of mitigation actions are varied, and include presence of non-viable populations pre-mitigation, inadequate mitigation actions, cumulative effects of further development, and emergence of new threats. Where translocation is used, immediate dispersal of animals from the release site may contribute to very low recapture rates in post-translocation monitoring. Maintaining animals in temporary enclosures at the release site may reduce the initial dispersal from the site. We recommend refinements to current interventions to improve success, including better connectivity and improved landscape-level management that utilizes newly emerging techniques and evidence.

### Independent evolution of habitat preferences in 238 species of *Cyrtodactylus* Gray, 1827

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Stochastic character mapping was used to infer the evolution of eight different habitat preferences across a robust BEAST phylogeny composed of 238 species of *Cyrtodactylus*. The analysis recovered a general habitat preference as the widespread ancestral condition throughout the tree that initially gave rise to all other habitat preferences: terrestrial (three times), cave (four times), karst forest (15 times), swamp forest (two times), granite forest (nine times), trunk (four times), and arboreal (three times). Major independent radiations of karst forest species dominate Myanmar and Indochina with lesser radiations in Vietnam and Malaysia. Two independent radiations of species with a terrestrial habitat preference occur in southern India and the Ayeyarwady Basin of Myanmar. A number of large Wallacean species specialize in inhabiting the trunks of trees. The remaining habitat preferences arise independently in single species or small clades throughout the tree. Terrestrial, arboreal, and cave habitat preferences lead to dead-end morphologies so extreme that they can no longer invade and adapt to any other habitat.

## Tolerance and resistance in defence against amphibian chytridiomycosis

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Animal defences against infection involve two distinct but complementary mechanisms: tolerance and resistance. Tolerance measures the animal host's ability to limit detrimental effects from a given infection, whereas resistance is the ability to limit the intensity of that infection. The study of host tolerance to infection is a burgeoning area in the field of animal disease ecology. Unlike resistance, tolerance does not affect pathogen fitness, and hence does not promote antagonistic counter-adaptation. There is a vast range in amphibian responses to infection with the main fungal agent of the worst vertebrate disease, chytridiomycosis (*Batrachochytrium dendrobatidis*; *Bd*). Here, we quantify measures of tolerance and resistance across the amphibian chytridiomycosis literature, comparing species, life-stages and individual level variation. Infection tolerance is important for the dynamics and co-evolution of *Bd* infection within amphibian communities. Some frogs die from chytridiomycosis infection loads that others can tolerate without detrimental effects. Furthermore, some frogs harbour intense infections of > 8 million zoospore equivalents, consistent with the concept of superspreading. Tolerant amphibian life-stages such as tadpoles can harbour chytridiomycosis in their mouthparts but do not die from disease. Such tolerant tadpoles can then rescue a population despite high mortality of adults, or alternatively act as reservoirs for infection. Sympatric species, and tolerant superspreaders may also act as infection reservoirs, promoting pathogen persistence, maintaining high force of infection, and driving less tolerant hosts to extinction. Improving our understanding of infection tolerance and resistance promises powerful new strategies for understanding and mitigating infectious diseases.



## Sea Snakes of the Sahul Shelf, Spatial Temporal Changes and Threats, 1993 to 2017

### Guinea M<sup>1</sup>

<sup>1</sup>*Charles Darwin University*

The Sahul Shelf occupies the Australian continental shelf between Western Australia and Indonesian waters. Sea snake surveys were conducted over seven reefs of which four had emergent islands between 1993 and 2017. Surveys were sporadic in timing but enabled comparisons to be made with previous datasets. The most complete surveys were in 2012 and 2013 to assess the impact of hydrocarbon release from the Montara gas field. These produced indices of diversity and density of sea snake populations at assumed impacted and non-impacted reefs. Ashmore Reef displayed the greatest decrease in sea snake populations over a period of about two decades before a slight recovery highlighting possible causes for their decline. Seringapatam Reef displayed the greatest change in density but not diversity over a twelve-month period following seismic surveys and nearby exploratory drilling.

Based on the changes in sea snake populations in response to passed human activity on the Sahul Shelf, a possible trajectory of population changes to planned developments are of concern and need to be addressed as early as possible to minimise their impact. Planned activities will increase maritime vessel noise, enhance risk of unscheduled releases of hydrocarbon and the impact of the response, physical impaction by marine craft and subsidence of marine habitats along with the impacts of changing climate and warming seas. This region of northern Australian continental shelf that was once the cradle of sea snake evolution has been seriously depleted by unintended consequences of human activity and may face an even bleaker future.

## **How temperature variation drives latitudinal differences in ectotherm sensitivity to heat extremes during warming**

**Gunderson A**<sup>1</sup>

<sup>1</sup>*Tulane University*

Climate change is increasing the frequency of heat extremes, and therefore the overheating risk of wildlife. Overheating risk should be dictated by interactions between mean habitat temperatures, habitat temperature variation, and organismal heat tolerance limits; however, how these factors combine to dictate overheating risk and responses to warming is not well explored. I used simulations to predict how mean temperature and temperature variation should jointly influence overheating risk and tested the predictions using data on daily habitat temperatures and physiological heat limits for terrestrial reptile and amphibian populations across a 120° latitudinal range. Simulations predicted that overheating risk should increase non-linearly with warming, and that overheating risk is higher at a given mean temperature when thermal variation is greater. However, organisms from low-thermal variation habitats should experience more rapid increases in overheating risk with warming once overheating thresholds are reached. Empirical data mirror these predictions. Despite living in habitats with mean temperatures further from their thermal limits, temperate taxa currently have greater overheating risk than tropical taxa and will experience greater increases in overheating risk with warming because of differences in thermal variation. These results show that characterizing thermal variation and incorporating it into predictions of global change responses is critical, as doing so can alter predictions about global patterns of vulnerability to global change. In addition, non-linearity in overheating risk means that organisms may experience sudden shifts to dangerous thermal regimes despite linear increases in environmental temperature.

## Conservation Management of New Zealand Frogs

**Haigh A<sup>1</sup>**

<sup>1</sup>*Department Of Conservation*

The three archaic endemic leiopelmatid frogs of New Zealand are all threatened with extinction, *Leiopelma archeyi* being the top species on the Amphibian EDGE List. Key threats to *Leiopelma* frogs include introduced mammalian predators, habitat loss, disease, climate change and conflicts with human land use. Native frog conservation recovery is guided by an expert advisory group (Native Frog Recovery Group) and a New Zealand threatened species Recovery Plan (Native Frog Leiopelma Recovery Plan). The long-term management goal is to secure and recover all *Leiopelma* taxa by 2050 so that they are no longer threatened. This requires refining predator control techniques. Greater involvement in management of land use activities in or near frog populations through land management legislative processes is needed to protect two species on the North Island. Translocations to predator-free sites and ex-situ captive breeding are also important for persistence and recovery of *Leiopelma* species. Strengths and successes include some of the best long-term datasets in the world, successful translocations, improved understanding of predator control, disease resistance to chytrid, secure populations on predator-free islands, and captive breeding success. Challenges are the limited focus on frogs by conservation managers, severe Archey's frog declines, further developing captive husbandry, and some translocation failures. Required for recovery are raising profiles of *Leiopelma* research and management amongst conservation managers, increasing support from the public and businesses, a predator control regime which increases populations, cost-effective and robust monitoring tools, a better understanding of *Leiopelma* ecology (e.g. Hochstetter's frog), more translocations and consistently successful captive breeding.

### **Sex in the city: urbanization broadens the reproductive season of lizards**

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<sup>1</sup>Auburn University, <sup>2</sup>University of Rhode Island, <sup>3</sup>Washington University

Urbanization is a global phenomenon that is expected to increase in the future. Urbanization affects wildlife in a myriad of ways; however, most studies have focused on animal behaviour or wildlife management. Much less attention has been given to population ecology, even though such data are necessary to understand how populations will respond to urbanization. Moreover, most urbanization studies have focused on birds and mammals; therefore, there is a large knowledge gap concerning how urban environments influence the population ecology of non-avian reptiles. One effect of urbanization observed across diverse taxa is phenological shifts (e.g. advancement of flowering date in plants); however, very few studies have considered how urban environments influence reproduction in non-avian reptiles. Several conditions known to influence reptile reproduction differ between urban and natural habitats (e.g. temperature, artificial light at night). To bridge this knowledge gap, we routinely captured female brown anole lizards (*Anolis sagrei*) across an entire year from two urban and two natural populations. We measured body size, egg size, and abdominal fat mass for each female, and assigned each to a reproductive stage according to established methods. We found that urbanization results in a broader reproductive season compared to natural habitats: urban females are reproductive for about 1.5 months longer during the year than females from natural sites. Thus, urban environments can significantly alter important aspects of reproduction in lizards. Assessing the influence of urbanization on reproduction and other aspects of population ecology will be critical to understand its diverse effects on reptiles.

### **Factors affecting the thermal tolerance of reptile embryos: lessons from *Anolis* lizards**

Hall J<sup>1</sup>, Warner D<sup>1</sup>

<sup>1</sup>*Auburn University*

Aspects of global change (e.g. urbanization, climate change) expose reptile populations to unnatural thermal conditions; thus, many species face physiological challenges due to extreme temperatures. Most studies of thermal sensitivity use adults, but embryos are often more sensitive to thermal agitation. Moreover, studies often utilize unrealistic temperature treatments that do not reflect the stochastic nature of real thermal environments. We have conducted multiple studies using lizards from the genus *Anolis* to understand the factors that contribute to thermal tolerance in reptile embryos. Across these studies, we have exposed embryos to different frequencies and magnitudes of thermal stress at various points during embryo development. Our thermal treatments are extreme fluctuations in nest temperatures recorded from within the urban-heat island, and, thus, are ecologically meaningful treatments to explore how aspects of global change influence egg survival. By synthesizing results from these studies, we draw broad conclusions about the factors that influence thermal tolerance in reptile embryos. Exposure to increased magnitudes and frequencies of thermal stress have compounding, negative effects on egg survival, embryo physiology, and hatchling morphology and survival. Additionally, relatively old- and young-aged embryos are more vulnerable to thermal stress than embryos of intermediate age. Moreover, these effects can differ widely among closely-related species. We conclude that responses of reptile embryos to thermal stress induced by global change will depend heavily on the magnitude and frequency of exposure, the timing during development at which exposure occurs, as well as unique aspects of each species' natural history.

## Habitat Selection and Spatial Ecology of a Hot Spring Endemic Toad

**Halstead B<sup>1</sup>**, Kleeman P<sup>1</sup>, Rose J<sup>1</sup>

<sup>1</sup>*U.S. Geological Survey*

The increasing urgency of climate change has led to the rapid development of clean and renewable sources of energy. As these alternative sources of energy are developed, it is important to consider their potential effects on reptiles and amphibians. Dixie Valley toads (*Anaxyrus williamsi*) are endemic to a small system of springs in Nevada, USA, and their restricted range makes them vulnerable to environmental perturbations. Proposed geothermal energy development could affect the volume and temperature of water reaching the surface in this spring system, but it is unknown how Dixie Valley toads use their spring-dependent habitat. We used radio telemetry to study the habitat selection and spatial ecology of Dixie Valley toads during the spring breeding season and in fall as they selected overwintering sites so resource managers could anticipate potential effects of geothermal energy development on the toads. We found that the toads were selective with regard to water temperature, using a relatively narrow range compared to what was available in the environment. In the spring, toads were dispersed broadly throughout the wetlands created by springs, but in the fall, toads were more concentrated where water remained in dense bulrushes or near springheads. Toads overwintered in the mud in dense bulrushes or in burrows near wetlands. Accounting for seasonal variation in habitat use and thermal selectivity are important considerations for conserving this rare toad.

**Proximate Factors that Elicit Centipede-Eating Behavior of a Japanese Pit Viper, *Gloydius blomhoffii***

**Hamanaka K<sup>1</sup>**, Mori A<sup>1</sup>

<sup>1</sup>*Graduate School of Science, Kyoto University*

A Japanese pit viper, *Gloydius blomhoffii*, is a venomous snake distributed on Hokkaido, Honshu, Shikoku, and Kyushu islands in Japan. This snake mainly feeds on vertebrates such as frogs, but also occasionally eats centipedes. Among invertebrates only centipedes have been reported as diet of the snake. Our previous study showed that the venom of *G. blomhoffii* is not effective enough to incapacitate or kill centipedes. However, there is no research on feeding behavior of *G. blomhoffii* on centipedes. Here, we examined proximate factors that *G. blomhoffii* relies on to recognize centipedes as prey and how the snake eats them. We revealed that snakes do not respond to chemicals from body surface of centipedes but well respond to chemicals of body fluids of centipedes. In the laboratory encounter experiment, snakes did not react to intact centipedes that actively moving around but reacted to injured or dead ones. Two snakes swallowed dead centipedes. These results suggest that some chemicals of body fluids elicit centipede-eating behavior of *G. blomhoffii*. We presume that this viper avoids the risk of retaliatory bite of centipedes by eating only weakened or dead individuals instead of killing them by the venom because centipedes are highly resistant to the venom of *G. blomhoffii*.

**Sexual dimorphism in external and skeletal morphology of Japanese lentic breeding salamander (Amphibia: Urodela: Hynobiidae)**

**Hara S<sup>1</sup>**, Nishikawa K<sup>1</sup>

<sup>1</sup>*Kyoto University*

We examined the sexual dimorphism in external and skeletal morphology of Japanese lentic breeding salamander, *Hynobius setouchi*. We evaluated data sets that included 24 external and 37 skeletal characters of 45 adult males, 39 females and 24 juveniles (16 males, eight females) using univariate method. Skeletons were visualized, observed, and analyzed by using the radiographs and the Whole-mount double stain using Alcian blue for cartilage and Alizarin red for mineralized bone. Results showed that sexual dimorphism in external morphology included not only body size (females have a larger snout-vent length than males), but also shape (males have a larger head size, limb size, and tail size than females). These sexual dimorphisms could be observed not only in external morphology but also in skeletal morphology. Especially, the male humerus was larger than the female and differed in shape. We also found significant differences in caudal parts between male and female. There are three types in the shape of first haemal arch: Flat, Bent and Straight. Female has the thin and bent first haemal arch. Furthermore, we found larger number of caudal-sacrum in females than males. These sexual dimorphisms were surmised to be related to reproductive behavior and strategy, thus must have been attributed to sexual selection. For examples, the larger head size in male must be attributed to male-male competition, and longer limbs in male seem to be beneficial for reproductive success. Furthermore, the fecundity selection promotes sexual difference such as greater abdominal volume and larger number of caudal-sacrum in females.



## What have we learned from the tree of herps?

Harmon L<sup>1</sup>

<sup>1</sup>*University Of Idaho*

Extensive effort over the past decades has led to an unprecedented view of the phylogenetic relationships of reptiles and amphibians. This knowledge can be harnessed to learn about the tempo and mode of speciation and trait evolution across clades. So, what have we learned from this endeavor? In this talk, I will use examples to summarize three main findings from phylogenetic comparative methods: (1) evolutionary rates vary tremendously across different times, places, and clades; (2) rapid evolution is typically triggered by the interaction between species traits and the environment; and (3) the persistence of "young" species is a critical constraint on diversification that can explain massive differences in diversity across clades.

## **Optimising habitat management for amphibians: from simple models to complex decisions**

Scroggie M<sup>1</sup>, **Heard G<sup>1</sup>**

<sup>1</sup>*Arthur Rylah Institute For Environmental Research, Victorian Department Of Environment, Land, Water and Planning*

Habitat loss, degradation and fragmentation remain leading causes of amphibian declines across the globe. To mitigate these impacts, conservation managers may protect core habitats and pursue habitat creation or enhancement actions, including construction of artificial wetlands, manipulation of wetland hydroperiods, removal of invasive species or restoration of aquatic and riparian vegetation. Yet management budgets are universally tight. When planning such actions, managers face the fundamental and complex problem of choosing where and when to invest limited resources to maximise the likelihood of species persistence. Here, we extend our previous research on this problem, and demonstrate the utility of coupling occupancy models with optimisation algorithms to identify preferred habitat management schemes across multiple, disjunct habitat networks. Our real-world case study, completed in close collaboration with conservation managers, focused on optimal habitat creation schemes for a threatened Australian frog in a rapidly urbanising region. Our new technique identified clear priorities for investment in wetland construction both among and within seven disjunct habitat networks, solving a spatial prioritisation problem that entailed millions of potential solutions and which was otherwise intractable. Such complex, multi-scale spatial prioritisation problems are pervasive in amphibian conservation. Coupling occupancy models with spatial optimisation algorithms represents a promising avenue to solve these problems and design habitat protection, creation and management schemes that maximise the chance of species persistence.

## Evolutionary Loss of Drinking Response in Marine Snakes

Lillywhite H

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Contrary to long-held belief, sea snakes do not drink seawater in spite of having functional extrarenal salt glands. Relatively recent research has demonstrated that > 10 species of marine snakes dehydrate in seawater and require drinking fresh water to remain in water balance. Some species will drink brackish water < 50% seawater, and generally < 30% seawater. Moreover, the distribution and abundance of marine snakes has been shown to correlate with sources of fresh water. The threshold of dehydration at which snakes are stimulated to drink fresh water has been measured in various terrestrial, semi-aquatic, estuarine, and marine species of snakes. These data demonstrate that the threshold correlates with the tendency to marine habits, irrespective of phylogeny. Thus, despite the requirement for fresh water, it seems that the evolutionary suppression or drinking response is related to association with marine environments. Data further suggest that some species might have lost a drinking response altogether. Water balance is not understood and requires further investigation in these species.

## The Paradox of Stenotopy and Eurytopy in the Yellow-Lipped Sea Krait, *Laticauda colubrina*

### Heatwole H<sup>1</sup>

<sup>1</sup>*University of New England*

*Laticauda colubrina* is stenotopic in the marine environment (restricted to coral reefs except for the lake-locked population on Rennell Island, Solomon Islands), but eurytopic in the terrestrial environment (under vegetation; under rocks; in caves, limestone pillars, treeholes, and burrows in sand, and even occupies terrestrial habitats associated with unoccupied marine habitats if the latter are adjacent to coral reefs, e.g. mangroves). This paradox arises from the facts that (1) coral reefs are widely distributed in the tropics, which allows *L. colubrina* also to have a wide geographic distribution, despite its marine stenotopy, and (2) the terrestrial habitats associated with coral reefs are very diverse, which means that *L. colubrina* needs be terrestrially eurytopic in order to fully occupy the geographic extent of its available marine habitat. The dual occupancy of habitats may have a bearing on the colour pattern of this species since the selective forces posed by the array of background shapes and colours on a reef may be quite different from those on land; the resulting pattern may be a compromise between these two selective contexts. The guilds of potential predators upon snakes are different in the two environments with perhaps reliance to varying extents on different sensory perceptions and responses to evocative stimuli. To what extent do the relative roles of background matching, visual illusions, and warning colouration differ between the reefal and terrestrial environments and their effects on the corresponding suites of predators? Ways of addressig these questions are discussed.

## Evaluating Aerial Descent in the Flap-legged Geckos (Genus *Luperosaurus*) of the Philippines

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Gliding or parachuting behavior has evolved numerous times in squamate reptiles, including independently in several lineages of geckos. Morphological and behavioral traits associated with aerial descent and gliding have been especially well studied in the “flying” geckos, *Ptychozoon* spp., and in the flat-tailed house gecko, *Hemidactylus platyurus*. In contrast, only anecdotal evidence of gliding or parachuting exists for other putative gliding gecko lineages. *Luperosaurus* is one such genus containing putatively gliding species. *Luperosaurus* species are Southeast Asian arboreal geckos closely related to or nested within the genus *Lepidodactylus*, but differ from members of this genus in having skin flaps and webbing that may aid in aerial descent. On a recent field expedition in the Philippines, we collected individuals of two *Luperosaurus* species (*L. macgregori* and *L. angliit*), as well as exemplars of the arboreal gecko genera *Gehyra*, *Gekko*, *Hemidactylus*, and *Lepidodactylus*, and observed aerial descent behavior in each of these taxa. Our data suggest that at least the *Luperosaurus* species found in the northern Philippines do not have gliding capabilities equivalent to those of the better known *Ptychozoon* spp. and *Hemidactylus platyurus*. Phylogenetic, morphological, and behavioral correlates of gliding in geckos are discussed.

### Is habitat enhancement for reptile conservation a viable strategy in pest-invaded landscapes?

**Herbert S<sup>1</sup>**, Kelly F<sup>1</sup>, Hartley S<sup>1</sup>, Nelson N<sup>1</sup>

<sup>1</sup>Victoria University Of Wellington

Habitat restoration and reconciliation (hereafter, “enhancement”) are promising strategies for reptile mitigation and conservation because the Reptilia are extremely sensitive to habitat loss. Globally, reptiles frequently use, and reproduce in, enhanced habitats. However, evidence of positive population-level outcomes is relatively lacking, and 95% of studies are performed in continental environments. We sought to evaluate whether habitat enhancement could protect an island-endemic lizard fauna threatened by the introduction of mammalian predators.

Lizard communities in the Wellington region of New Zealand were surveyed to examine their responses to variation in habitat characteristics and mammal species presence. Lizard populations at a further three mammal-invaded sites were monitored, and compared with historical data to examine the long-term consequences of co-existence with mammals.

Two skink species, *Oligosoma aeneum* and *O. kokowai*, were commonly encountered in mammal-free environments but were very infrequently detected in mammal-invaded environments. The gecko *Woodworthia maculata* was abundant in mammal-free environments, but was restricted to a sub-set of habitat types in the presence of mammals. The skink *O. polychroma* was most commonly encountered in mammal-invaded environments that lacked high abundance of other lizard species. Most of the monitored populations of *O. polychroma* and *W. maculata* co-existed with mammals at stable or increasing densities over time periods spanning 6-49 years. Our results suggest that habitat enhancement will benefit some, but not all, of Wellington’s lizard species in the absence of mammal eradication or intensive control. A before-after-control-impact (BACI) habitat enhancement experiment is currently underway to test the validity of this conclusion.

## Comparative studies of egg tooth development in squamate reptiles

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<sup>1</sup>*Department of Animal Histology and Embryology, University of Silesia in Katowice*

The egg tooth is an evolutionary trait of great importance characteristic for all squamates. It plays major role during hatching from calcified eggshells after end of prenatal development. General structure of the egg tooth is similar to structure of typical tooth characteristic for all vertebrates. Egg tooth however, due to function it performs, exhibit structural differences in relation to regular teeth. Most of the squamates posses single unpaired egg tooth except geckos and dibamids which possess double egg teeth. This study involves a detailed comparison of morphology of the developing egg teeth in embryos of two Unidentata representatives: brown anole *Anolis sagrei* and grass snake *Natrix natrix*, and two representatives of Gekkota: leopard gecko *Eublepharis macularius* and mourning gecko *Lepidodactylus lugubris*. We found that egg teeth of investigated unidentates have different morphology (most notably different shape, spatial orientation and presence of cutting edges) and are differently attached to the premaxilla in comparison to egg teeth of investigated geckos. In addition, egg teeth morphology and their spatial orientation differ between analyzed species of geckos which might be the result of different hardness of their eggshells.

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## High throughput microCT scanning of small fossils: an example using Australian herpetofauna

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High-resolution X-ray microcomputed tomography, or microCT, has become an increasingly valuable tool for the non-invasive visualization of small objects. MicroCT is particularly beneficial for the study of museum collections, which often contain thousands if not millions of small, delicate, and unique items not amenable to traditional preparation. Despite the increasing use of microCT in systematic biology, a major challenge remains in the practical imaging of high numbers of small specimens within a project scope, for example in the context of large-scale analyses of community structure. This has proven difficult because of the need for specimens to remain motionless during scan time (typically minutes to hours) and because each individual must be digitally labelled to match the specimen's identity, and hence retain important information (e.g., collection date, locality, deposit). Here we outline steps developed for the high throughput microCT scanning of small fossils ( $\leq 5$ mm), meant to facilitate advanced exploration of museum collections, and allow researchers with limited access to microCT facilities the opportunity to maximize their investments. We illustrate our method using herpetofaunal fossils from Capricorn Caves in Queensland, Australia, to identify changes in morphology, ecology, and distribution over time. We succeed in maximizing the quantity of samples per scan in order to obtain high quality 3D models for comparisons with their modern counterparts. These steps should also be applicable to any small, dry objects of similar properties and size, including geological material and invertebrates. This work was supported by an Australian Research Council DECRA DE180100629 to C.A.H..



## Two contrasting incursions of *Lampropholis delicata* in New Zealand's South Island

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*Lampropholis delicata* has been well established in the North Island since the 1960s, and is now widespread in the northern 2/3 of the island and spreading rapidly. Dozens of hitch-hiking individuals have been found in the South Island but there was no evidence of breeding there until late 2017 when an established population was discovered at the Riverlands industrial estate near Blenheim. Initially the population appeared to be very localised and intensive trapping with insect sticky traps gave promising results. However, subsequent broader delimitation work showed the population to be established over at least 80 hectares between the Riverlands and Cloudy Bay estates, although much more abundant within the estates than in the intervening rural area. Eradication over such a large area is not considered feasible. Preliminary genetic results suggest this is a new incursion from Australia. A few months after the discovery of the Riverlands population, scores to hundreds of plague skinks arrived in Havelock in a shipment of used mussel farming rope from Coromandel. An eradication was begun very quickly, but not before tens of skinks had been released into a neighbouring DOC-owned field in the belief that they were native skinks. Dozens of skinks were killed during unpacking of the ropes, others were subsequently trapped in the yard where the ropes were unpacked, and trapping is now underway on the adjacent DOC land block, which has also been fenced to stop dispersal of skinks. Very few skinks are now being trapped, and eradication appears feasible.

**The role of variable temperatures on acclimation of thermal and performance phenotypes.**

**Hodgson M<sup>1</sup>**, Schwanz L<sup>1</sup>

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Reptile's across the globe are feared to be at risk of extinction under climatic warming because temperature has such a dramatic impact on the performance of ectotherms. Thermal behavioural and performance traits show an extensive ability for phenotypic flexibility. This acclimation often benefits individuals in new environments and may buffer the impact of novel conditions on populations. Previously, many have investigated this in a lab environment by acclimating animals under constant thermal treatments. However, this may lead to false conclusions as such experimental conditions fail to capture the daily temperature variation these animals experience in nature. We use an experimental approach in the Australian jacky dragon (*Amphibolurus muricatus*) to investigate the role that diel variation in environmental temperature has on several measures of performance as well as behavioural thermal phenotypes.

***Allobates femoralis*: an optimal model species for studying anuran social behavior and cognition**

Ringler E<sup>1</sup>, Pasukonis A<sup>2</sup>, Ringler M<sup>1</sup>, Hödl W<sup>1</sup>

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Poison frogs are characterized by a highly complex social and reproductive behavior, involving territoriality, female choice, prolonged courtship, and parental care. Most of our research efforts occurred on the dendrobatid frog *Allobates femoralis*, which has proven to be an optimal model species for studying poison frog reproduction and parental behavior. In 2012 on a river island in the heart of French Guyana we have established an experimental population of *A. femoralis* by the controlled introduction of 1800 morphologically measured and genetically sampled tadpoles. The possibility to continuously study these frogs in the field in a closed system as well as to perform experiments under controlled laboratory conditions (at the university of Vienna) allows us to address a wide array of research questions, ranging from animal cognition to population ecology. Methods include focal observations, individual tracking, molecular parentage analysis and video monitoring. Our findings show, that these little forest-floor frogs are very thoughtful parents, exhibit excellent navigation and orientation abilities, as well as behavioural flexibility across various contexts.

Within this presentation a (very) short summary on the results deriving from several Master and PhD theses as well as Post-Doc field studies will be presented.

### Understanding why endangered frogs survive or perish on a tiny scale

**Hoffmann E<sup>1</sup>**, Mitchell N<sup>1</sup>

<sup>1</sup>*The University Of Western Australia*

Identifying the causes of population declines is central to effective management of threatened species. Statistical models of habitat and species occurrence can provide inferences on the drivers of decline as well as identify key habitat characteristics to inform selection of translocation sites. *Geocrinia alba* and *G. vitellina* are two threatened frog species with very small distributions in southwestern Australia. Both species show extreme site philopatry, and are specialised terrestrial breeders in headwater streams. Over half of the known populations of *G. alba* have gone extinct in recent decades and other populations are continuing to decline. Attempts to translocate head-started juveniles of both species have had limited success. A current management challenge is a lack of understanding of site characteristics that allow both natural and translocated populations to persist. Here we show that very fine-scale characteristics relating to hydrology and microclimate may be key to population persistence. We found that sites where larger populations persist were associated with higher soil moisture, and were more buffered from summer temperature extremes compared to immediately adjacent areas and sites where frog populations are extinct. We are also developing a mechanistic model to understand how frog's physiological thresholds may be challenged by changes in soil moisture and temperature. These insights will allow us to identify potentially unsuitable translocation sites, and to prioritise sites most likely to support viable populations in a drying climate.

**MHC associated differential survival to infection of two strains of *Batrachochytrium dendrobatitis* in common toads**

**Hoglund J<sup>1</sup>**

<sup>1</sup>*Uppsala University*

While both innate and adaptive immune system mechanisms have been implicated in resistance against the chytrid fungus *Batrachochytrium dendrobatitis*, studies on the role of specific MHC haplotypes on *Bd* infection are rare. Here, we studied latitudinal variation in MHC Class IIB loci along a latitudinal gradient from southern to northern Sweden in common toads, *Bufo bufo*. Swedish toad populations had fewer MHC Class IIB haplotypes compared to a previous study of populations in Britain. Furthermore we found MHC diversity to decline from south to the north within Sweden. The low diversity may compromise the ability of northern populations to fight emerging disease, such as the chytrid fungus *Bd*. In a laboratory experiment, we infected newly metamorphosed toads with two strains of the Global Pandemic Lineage of the fungus (*Bd*-GPL) and compared survival with sham controls. We found *Bd*-infected toads had lower survival compared to controls. Survival was dependent on *Bd*-strain and whether experimental toads were collected in the south or the north of Sweden with lower survival in northern individuals. MHC diversity was lower in toads of northern origin, all northern animals being monomorphic for a single MHC haplotype, whereas we found seven different haplotypes in southern animals. Survival of infected animals was dependent on both *Bd*-strain and MHC haplotype suggesting differential infection dynamics depending on both *Bd*-strain and host MHC characteristics.

**Habitat use by *Leiopelma archeyi* in disturbed and undisturbed sites on the Coromandel Peninsula, Aotearoa**

**Hotham E<sup>2</sup>**

<sup>1</sup>*Boffa Miskell Ltd*, <sup>2</sup>*Massey University*

The world's most Evolutionary Distinct and Globally Endangered ('EDGE') amphibian species, the Archey's frog (*Leiopelma archeyi*), is now restricted to three locations in New Zealand. These populations remain vulnerable to disease and predation, with the Coromandel Peninsula population further threatened by habitat disturbance from a range of human activities. To make informed management decisions, it is important to understand the ecology of a species. This study therefore examines the micro- and macro-habitat use of Archey's frogs at 16 sites on the Coromandel Peninsula that were disturbed by vegetation clearance from exploration mining and housing/roading, in comparison to 16 paired sites that were considered undisturbed. The results of this study include an assessment of abundances at disturbed and undisturbed sites using closed-mark recapture, frog microhabitat use with respect to vegetation structure, and the effect of disturbance on those structural characteristics. I discuss implications for post-disturbance management to encourage frog recolonization.

## Cost-effective amphibian captive breeding using biobanking to meet IUCN 100-year, 90 percent heterozygosity criteria

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Captive breeding remains an integral component of many threatened species programs despite criticisms regarding genetic issues and economic costs. In addition, given the limited number of individuals usually involved, these programs are unlikely to approach IUCN targets of retaining >90% of original genetic diversity after 100 years. Using iterative costing and genetic models, based on real-world data, we modelled biobanking as a complementary tool for captive breeding of Oregon spotted frogs (*Rana pretiosa*). We determined reductions in live colony numbers and program costs possible when genetic backcrossing is performed using frozen founder sperm at different generational intervals in theoretical captive populations designed to meet IUCN targets. Meeting this target without backcrossing, requires >1800 live frogs and >C\$156 million total 100-year program costs. Backcrossing every 10th generation, 5th generation and each generation reduces required colony numbers and program costs by 57%, 86% and 97% respectively. The optimal scenario is backcrossing every generation, reducing live colony requirements to 58 individuals, C\$18 million in 100-year program costs, minimal start-up costs (C\$89k), and no loss in genetic diversity after 100 years, exceeding IUCN targets. Backcrossing costs are minimal (2% of total program costs), therefore could be incorporated across existing amphibian captive breeding programs now, without significant deviation from current operating costs, and deliver long-term cost reductions and genetic viability. Biobanking may leverage resources to generate species-specific protocols for biobanking, create time to address in-situ drivers and boost global capacity to address the quantum of threatened species in need of captive management.

## **An Update and Taxonomic Review of Snakes Housed in the Herpetological Collection from Auckland Museum**

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Data inferred from preserved specimens in herpetological collections have contributed immensely to the modern understanding of the biology of snakes. Even small museum collections can contain historical specimens that have inestimable value, providing a source of vouchered information for research. Here we report on our recent efforts to update the taxonomy of the snake collection at Auckland War Memorial Museum. Specimen records, including the scientific name of the species, site and collection date, collector, voucher number, geographic distribution and previous records demonstrate this small collection consists of over 160 specimens from all continents except the polar zones, belonging to eight families. Approximately 50 specimens of snakes were examined and 11 actual taxonomic changes are recommended. The best-represented families are Viperidae with seven species of Vipers and Pit Vipers, followed by Boidae, with five species, mainly of the genus *Candoia*, and Elapidae, with two species of Sea Snakes, *Hydrophis platurus* and *Laticauda cf. colubrina* (occasional visitors of New Zealand's shore) and the family Pythonidae with one species of Python. The Elapid *Loveridgelaps elapoides*, known as Solomons's small-eyed snake, presents a very interesting discovery, as specimens are extremely rare with few descriptions in the literature for this Vulnerable (VU, IUNC Red List) species. Updates of taxonomic designation for even small museum collections is extremely important to enable ongoing studies of the taxonomy, natural history and conservation of snakes which are threatened globally at local, regional, and continental scales.



## Sexual Dimorphism and Reproductive Ecology of the South-American Rattlesnake, *Crotalus durissus* in the Brazilian Cerrado

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The rattlesnake (*Crotalus spp*) is frequently considered as a model system for ecological studies. Species of rattlesnakes are usually widely distributed covering several habitats, including deserts, flooded areas, forest environments and open habitats. *Crotalus durissus* is restricted to South America. In Brazil, it is widely distributed, except for the states of Acre and Espírito Santo. In addition, there are isolated populations in savannas within the Amazonian Forest. Here we investigated patterns of sexual dimorphism and reproductive ecology in the South-American rattlesnake, *Crotalus durissus* from the Brazilian Cerrado. Sexual dimorphism is pronounced, with males having body size, snout-vent length and tail length relatively larger than females. Clutch size ranges from 4 - 20 embryos and is significantly correlated with snout-vent length. Among females, vitellogenesis peaks between the end of the dry season (May to September) and onset of the rainy season (October to April). The very low frequency of reproductive females suggests a biennial reproductive cycle. Males are reproductively active year-round, but testis volume peaks in a few months (March and September). Our results suggest the existence of a pattern of sexual dimorphism indicating the intra-sexual competition between males through combat rituals to determine dominance in relationships as well as the existence of seasonality in the reproductive cycle.

## Using movement to inform conservation corridors for Mojave Desert Tortoises

**Hromada S<sup>1</sup>**, Esque T<sup>2</sup>, Vandergast A<sup>3</sup>, Mitchell C<sup>1</sup>, Dutcher K<sup>1</sup>, Nussear K<sup>1</sup>

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Providing corridors for gene flow between populations is crucial to ensuring the survival and recovery of threatened and endangered species. As human activity continues to fragment animal populations, characterizing natural corridors is important to establishing and maintaining suitable corridors amongst anthropogenic development. The Mojave Desert tortoise (*Gopherus agassizii*) is a threatened species inhabiting a variety of habitats in the Mojave Desert, USA. Urban expansion, incursion of recreation (e.g. OHV use), and utility scale solar facilities are all increasing in tortoise habitat. Desert tortoises are corridor-dwellers, and understanding how they use suitable habitat can be crucial to describing and identifying corridors. To elucidate how tortoises traverse available habitat and interact with potentially inhospitable terrain and human infrastructure, we used GPS dataloggers to infer fine-scale movement of individuals (~130) at ten 1-km<sup>2</sup> study sites centered in the greater Ivanpah Valley area, along the California/Nevada border. Our sites encompass a variety of habitats, including mountain passes that serve as important corridors connecting neighboring valleys, and have a variety of anthropogenic impacts (e.g. roads, fencing). We used path selection functions to quantify tortoise movements and develop resistance surfaces based on landscape characteristics and anthropogenic alterations. Our results indicate that both natural and human-created features shape tortoise movement patterns. Understanding these influences can help to identify protected corridors to compare with resistance surfaces determined through species distribution modeling, and inform future land management strategies.

## **Integrating ecotoxicology and art to understand and communicate how human activities influence amphibian disease susceptibility**

**Hua J<sup>1</sup>**

<sup>1</sup>*Binghamton University*

Human activities influence amphibians throughout the globe, including altering disease dynamics. To understand the complex interaction between human activities and disease, this talk emphasizes three priority actions from the Amphibian Conservation Action Plan (Ecotoxicology): (1) predicting amphibian susceptibility to diverse pollutants; (2) understanding low levels of pollutants and their interactive effects with other stressors (parasites); (3) developing initiatives to educate young people on the issues surrounding pollutants and amphibians. Specifically, we evaluated how environmentally-relevant levels of seven anthropogenic stressors (an insecticide, road salt, light pollution, leaf leachates from invasive species, an antimicrobial, cold-temperature variability, and toxins from algal blooms) influence amphibian susceptibility to a common trematode (*Echinostoma* sp.). We found: Four (insecticide, road salt, light pollution and invasive leaf litter) of 7 stressors increased wood frog susceptibility to trematodes. The mechanisms driving patterns of susceptibility varied but anthropogenic stressors that reduced swimming behavior typically resulted in tadpoles with higher parasite loads. Next, to better understand how artists and scientists can work together to generate public understanding and support for amphibian conservation efforts, we created an art exhibit and developed a children's book and lesson plan depicting amphibian disease and ecotoxicology research. Participation in the art exhibit increased student recognition of conservation issues by 12% and improved the likelihood students would support wetland conservation by 20.8%. Completing the lesson plan improved 3rd grade student understanding of amphibian-trematode interactions by 38%. Collectively, this work suggests that an integrated approach that combines ecotoxicology and art may be an effective for amphibian conservation efforts.

## Climate change drives reptile community collapse and adaptation on Orchid Island

**Huang W<sup>1</sup>**

<sup>1</sup>*National Museum Of Natural Science*

It is now well documented that climate change is impacting individual species and causing local population extinctions. However, less attention has been paid to understand how the loss of those populations then can propagate through the trophic community in unexpected ways. Here, we use two decades of data on the occurrence and trophic interactions of several reptile species native to Orchid Island to highlight how the loss of one species locally can have profound, long term effects on several others. On Orchid Island, the green sea turtle became locally extinct when its nesting habitat was destroyed by an unusual series of successive typhoons. The nesting sea turtles had supported a high density of kukri snakes on Orchid Island, a reptile-egg-eating snake that had subsisted almost wholly on sea turtle eggs. After the nesting beach was lost in 2002, sea turtles never returned to the local population. The snakes subsequently switched their primary food source towards the eggs of other local reptiles (primarily lizards). We found that seven species of reptile with the non rigid eggs consumable by kukri snakes have declined due to the negative impact of kukri snakes on nesting success. Our research shows that even a relatively small local extinction can sometimes cascade through the community, resulting in significant ecological changes in biotic communities that would be difficult to predict a priori.

## Zoo-led collaboration for conservation- Gecko monitoring on Matiu/Somes Island

Huang C<sup>1</sup>

<sup>1</sup>Wellington Zoo

From 2007-2012, 90 Barking Geckos (*Naultinus punctatus*) and 77 Ngahere Geckos (*Mokopirirakau* “southern north island”) were released onto Matiu/Somes Island in Wellington Harbour. Since November 2016, Wellington Zoo Trust and the Department of Conservation have been collaborating under a memorandum of understanding on a follow-up survey of both species. The purpose of the project is to assess whether the introduction has been of success, as well as to train Zoo staff in reptile surveying techniques. The Department of Conservation provides funding, accommodation and in-situ expertise, and Wellington Zoo Trust provides trained staff and field equipment. This presentation will expand on the collaborative process between the partners, what conclusions can be drawn from the project and its significance to gecko conservation on Matiu/Somes and to the Zoo's ex situ work with native geckos. We look forward to continuing our successful collaboration in the name of reptile conservation.

**Testudo: Using protected artificial nest structures to protect Bell's turtle (*Myuchelys bellii*) nests from invasive red foxes (*Vulpes vulpes*)**

**Hughes G<sup>1</sup>**, Burns A<sup>1</sup>, McDonald P<sup>1</sup>

<sup>1</sup>*University Of New England*

Red foxes (*Vulpes vulpes*) are an invasive species in Australia that are responsible for declines of native fauna. Foxes are partially accountable for reduced recruitment of several Australian turtle species, including the endangered Bell's turtle (*Myuchelys bellii*), through unnaturally high rates of nest depredation. Numerous methods of protecting turtle nests have been deployed to date, including poison baiting, shooting, trapping, and caging of turtle nests. These methods typically show limited or short-term success, and are highly labour-intensive. We therefore investigated the effectiveness of a nest protection method adapted from North American deployments; constructing semi-permanent artificial nesting structures that can protect both the nests and nesting females. These structures consisted of a wooden frame covered with wire fencing except for the entrance; soil in the interior was tilled to entice females to nest inside. A nearby (<20 m) patch of soil was similarly tilled to serve as a control for any aversion to the structure. An electrified wire was placed along the top of the entrance at a height that would give a painful shock to any foxes attempting entry, whilst allowing access to nesting turtles. We placed six of these structures at Bell's turtle sites prior to commencement of nesting activity. We tested whether i) Bell's turtle females were willing to use the structures and ii) success at fox exclusion. This technique may provide a cheap, low-labour alternative/supplement to existing nest protection strategies that will improve recruitment for both this species and a range of similar turtle species.

## Mitigating chytrid fungus: successful translocation of a highly susceptible frog species to an environmental refuge

Hunter D<sup>1</sup>, Scheele B<sup>2</sup>, Gilbert D<sup>7</sup>, Marantelli G<sup>3</sup>, Gillespie G<sup>4</sup>, West M<sup>5</sup>, McFadden M<sup>6</sup>

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Despite *Batrachochytrium dendrobatidis* (hereafter *Bd*) being a leading cause of frog declines globally, there are few examples of successful mitigation strategies to conserve susceptible species in the wild. We trialled the use of assisted colonisation to establish a population of the Spotted Tree Frog (*Litoria spenceri*), an endangered riverine species from south-eastern Australia that is experiencing ongoing declines associated with *Bd*. We hypothesised that streams with warmer micro-climates and fewer reservoir host species may provide an environmental refuge and allow Spotted Tree Frogs to coexist with *Bd*. We identified a potential release site outside the historic range of *L. spenceri* that was significantly warmer (more than double the number of hours above 28 degrees Celsius during Dec-April) and which supported no other riverine frogs. Starting in 2014, we released 400 frogs into the candidate site, and 200 frogs to a control site where the species recently was extirpated due to *Bd*. Post-release survivorship and breeding was vastly different between the two sites. The new site achieved high survivorship (> 0.8 annual survivorship) and subsequent recruitment, which has resulted in an overall increasing population. By contrast, the historic control site achieved very low survivorship (< 0.3), no observed recruitment, and as a result the population has declined to fewer than 10 individuals. While it is too early to claim we have established a long-term, self-sustaining population of *L. spenceri*, our study demonstrates the potential for assisted colonisation be used as a management tool to secure wild populations of *Bd* susceptible species.

**Competition and resources shape multidimensional trophic niche overlap in Brazilian thin-toed frogs (*Leptodactylus* sp).**

**Ingram T<sup>1</sup>**, Costa-Pereira R<sup>1,2,3</sup>

<sup>1</sup>University Of Otago, <sup>2</sup>Universidade Estadual Paulista (UNESP), <sup>3</sup>McMaster University

Competition plays a central role in the maintenance of biodiversity. Local coexistence of competitors is expected to be favoured by niche contraction or divergence, but this effect should depend on the available diversity of resources, particularly when resources vary in multiple ecological dimensions. Here, we investigated how available resource diversity and competition together shape multidimensional niche variation and niche overlap in thin-toed frogs (*Leptodactylus* sp.) in the Brazilian Pantanal. We measured diets and available invertebrate resources for 42 frog populations in two key trophic dimensions: prey size and carbon stable isotopes. We found 14-fold variation in multidimensional niche width across populations, most of which was accounted for by within-individual diet variation. Individual niche breadth increased with resource diversity and decreased with the number of congeneric competitors. These ecological gradients also interact to influence the degree of niche overlap between species, which surprisingly decreased with population total niche width. Our results show how competitive interactions and local resource breadth combine to drive patterns of trophic niche variation in tropical frogs.



## Regional characteristics of toxic components in Japanese toads

**Inoue T**<sup>1</sup>, Nakata R<sup>2</sup>, Ujiie R<sup>3</sup>, Yoshinaga N<sup>1</sup>, Savitzky A<sup>4</sup>, Mori A<sup>5</sup>, Mori N<sup>1</sup>

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Many species of the genus *Bufo* synthesize and accumulate bufadienolides (BDs) in their parotoid glands. BDs are known as cardiotoxic steroids and play an important role in defense against their predators. Two species and one subspecies of toads, *Bufo japonicus formosus*, *B. japonicus japonicus*, and *B. torrenticola*, are distributed in Honshu, the mainland of Japan. BDs of *B. j. formosus* have been studied for several decades, but those of the other two have not been reported yet. Thus, we analyzed BDs and compared the composition of BDs among these species/subspecies collected from several regions over Honshu by LC / MS analyses. We found species-specific and subspecies-specific BDs, and there were few BDs having high commonality among them. Therefore, the BD composition was different among the species/subspecies. The sampling localities were divided into 16 areas, and BD composition was analyzed by NMDS, which is a quantitative method for indicating the level of dissimilarity. As a result, we found that some of the dissimilarity values of the BD composition within the species/subspecies were larger than those among the species/subspecies. Therefore, the BD composition showed clear regional differences in some areas. Two regionally specific BDs (which significantly increase the dissimilarity values) and four widely distributed BDs (which slightly increase the dissimilarity values) were isolated from *B. j. formosus/japonicus* using HPLC and were identified by NMR analyses. One of the latter BDs was identified as a novel compound.

## Metamorphosing strategy of stream tadpoles.

Iwai N<sup>1</sup>

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The size and timing of metamorphosis (metamorphosing strategy) has been well documented in pond tadpoles and known to have high plasticity within and among species. The strategy in stream tadpoles has not been well studied although there can be different adaptive meanings than those of pond tadpoles. I aimed to understand the metamorphosing strategy of stream tadpoles by examining the size and timing of metamorphosis under different growth history. I applied a Gompertz growth function and hypothetical fitness contour model to six species and compared its parameters between stream and pond species and between overwintering and annual species. The relationship between larval period and size at metamorphosis was negative in *Babina subaspera* and *Buergeria buergeri* while positive in *Rana rugosa*, *Odorrana amamiensis*, and *Rana tagoi*, which was explained neither by habitat (stream/pond) nor overwintering. The ratio of “the effect of size at metamorphosis on fitness” to “the risk of longer larval period” was high in *O. amamiensis*, medium in *Ba. Subaspera* and *R. rugosa*, and low in *Bu. Buergeri*, which was also not explained by habitat or overwintering. The model suggested that for the overwintering stream species, growing larger by metamorphosing later might not give much advantage, but rather, metamorphosing at the right season might be more important.

## Optimizing phylogenomics with Rapidly Evolving Long Exons: Examples from all squamates and New Caledonian geckos

**Jackman T<sup>1</sup>**, Karin B<sup>2</sup>, Gamble T<sup>3</sup>, Keating S<sup>3</sup>

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We introduce Rapidly Evolving Long Exon Capture (RELEC), a new set of loci that targets single exons that are both rapidly and relatively long in length, while at the same time avoiding paralogy across amniotes. We compare the RELEC dataset to other reduced representation data sets in squamate reptiles by aligning and analyzing orthologous sequences from 17 squamate genomes, composed of ten snakes and seven lizards. The RELEC dataset (180 loci) outperforms other data sets by maximizing per-locus genetic variation while maintaining presence and orthology across a range of evolutionary scales. RELEC markers show high phylogenetic informativeness and RELEC gene trees show high similarity to the overall species tree. Furthermore, with fewer loci, RELEC remains computationally tractable for full Bayesian coalescent species tree analyses. We contrast RELEC to and discuss important aspects of comparable methods and demonstrate how RELEC may be the most effective set of loci for resolving difficult nodes and rapid radiations. We also show that the RELEC data set in New Caledonian geckos obtained using baits and sequencing show the same properties as the RELEC loci across squamates as a whole, with highly informative individual loci tractable using full Bayesian and coalescent species tree analyses.

**Contrasting sociality in two sympatric skink species; *Oligosoma otagense* and *O. grande***

**Jacks P<sup>1</sup>**, Monks J<sup>1,2</sup>, de Sá Rocha Mello R<sup>1</sup>, Godfrey S<sup>1</sup>

<sup>1</sup>University Of Otago, <sup>2</sup>Department Of Conservation

Little is known about the aggregative behaviour of New Zealand's lizards. We applied photo-identification and social network analysis techniques to a wild population of Otago skinks (*Oligosoma otagense*), a species known to form aggregations, as well as the grand skink (*Oligosoma grande*), a sympatric species sharing the same rock tor structures. We recorded approximately 2000 observations over a three-month period, with 10% of these observations being interactions between 2 or more lizards. Two opposite social tendencies were revealed: among Otago skinks these interactions were primarily peaceful aggregations of up to five individuals, whereas grand skinks displayed predominantly antagonistic behaviours. In contrast, interspecific interactions all resulted in submissive behaviour from the grand skinks. The findings from this study are foreseen to enhance conservation decisions concerning the population management and translocation of these two nationally endangered species.

## **Examining variation in sperm response in divergent populations of red-eyed treefrogs.**

**Jacobs L<sup>1</sup>**

<sup>1</sup>*San Diego Zoo Institute for Conservation Research*

The non-lethal collection of sperm from live males is an important component for multiple captive breeding techniques, including ART protocols, sperm cryopreservation and in vitro fertilization. However, the type of hormone and amount used is highly variable among taxonomic families as well as closely related species. To date, no studies have examined the sperm response to exogenous hormones across highly differentiated populations within a species. We examined sperm viability and counts to the hormone LHRH among four divergent populations of an iconic Neotropical treefrog. We hypothesized that these highly differentiated populations would show variability in sperm count and viability in response to two dosages, 2 µg/g and 4 µg/g, of the hormone LHRH. We collected spermic urine 3 hr post injection (PI). We found that although sperm count did not vary among populations, at least one population showed decreased sperm viability in response to LHRH. We then examined spermiation variation at 3, 7, 12, and 24 hours PI of LHRH for two allopatric populations of treefrog. We found no difference in sperm viability or counts. However, we did detect a downward trend in sperm viability in both populations 3 hours PI.

In conclusion, because ART often focuses on threatened species with small, isolated populations, the potential for evolutionary processes such as local adaptation and drift to act is high. The population-level differences in sperm viability we observed demonstrate that practitioners of ART should consider whether divergent responses to hormones may affect study designs and animal receptivity to ART protocols.

**Assembling the *Rana temporaria* genome to inform the evolution of Anuran sex chromosomes.**

**Jeffries D<sup>1</sup>**

<sup>1</sup>*University of Lausanne*

Anurans are emerging as a powerful model for studying the evolution of sex chromosomes, particularly those with little differentiation between gametologs. We recently showed that in true frogs (Ranidae), sex chromosome transitions occur at an extremely high rate, however the mechanisms and drivers behind these rapid transitions are not known. Our leading hypothesis is that new sex chromosomes in this system quickly accumulate deleterious mutations due to the general lack of recombination in males and the high proportion (at least 60%) of transposable elements in the genome, most of which appear active. This mutation load on the existing sex chromosome would then favour a transition to a new sex chromosome system.

Here we present the assembly of the 4.5 Gb *Rana temporaria* (European common frog) genome, which we then anchored to a high-density linkage map. We will use this resource to characterise repeat content and dynamics in the genome and specifically on the sex chromosome. In doing so, we hope to be able to estimate the repeat insertion rates on new sex chromosomes and provide insights into how this aspect of genome evolution might affect the large scale patterns of sex determination system evolution we observe in this system.

## Uncovering the mechanisms and drivers of the rapid transitions among frog sex chromosomes

**Jeffries D**<sup>1</sup>

<sup>1</sup>*University of Lausanne*

Most amphibians, and especially frogs, have homomorphic sex chromosomes, where differentiation between gametologs (e.g. X and Y) is difficult to see. We have previously shown that one major reason for this in true frogs (Ranidae) might be the rapid rate of transitions between sex chromosome systems. However the mechanisms of these transitions and, perhaps more importantly their evolutionary drivers, are not known. To address the former, we performed targeted sequencing of 12 genes known to be important for sex determination, across 6 species, in order to generate hypotheses for the mutations underlying the sex chromosome transitions between them. As for their evolutionary drivers, based on somewhat anecdotal evidence, we have hypothesised that they are driven primarily by rapid mutation load build-up as a result of rapid loss of recombination across incipient Y chromosomes. One major source of deleterious mutation is repeat element insertion and we know that frog genomes are full of highly active transposable element (TE) families. In light of this, we also attempted to quantify the extent and speed of repeat element build-up on new frog sex chromosomes following a transition using a new genome assembly and pooled sequencing data of males and females from the European common frog (*Rana temporaria*). I will discuss these results in the context of how amphibian sex chromosomes help us understand the evolution of sex chromosomes and sex determination more generally.

## Genomic signatures of population structure and disease susceptibility in common toads

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The genomic makeup of individuals and populations is determined by neutral and adaptive processes, and plays a key role in studies on evolution, ecology and epidemiology. Pond-breeding amphibians are characterised by a particularly deme-structured lifestyle, which makes them well suited to investigate how population subdivision affects the spatial distribution of fitness-related traits. Here, we employ a combination of microsatellite and SNP genetic markers to characterise a set of common toad (*Bufo bufo*) populations in coastal Scotland, before relating genomic inferences to outcomes of experimental infections with *Batrachochytrium dendrobatidis* (*Bd*) in this putatively disease-naïve setting. As predicted from neutral expectations, more isolated populations were genetically more distinct and less diverse. At the level of individuals, *Bd* susceptibility was predicted by body size, but unrelated to heterozygosity and not consistently linked to outlier loci as identified with ~11 000 SNPs. *Bd* susceptibility was nevertheless significantly associated with population of origin, suggesting that even a large panel of loci cannot reveal the complex quantitative genetic basis for performance during experimental infection. The finding that population-level *Bd* susceptibility was unrelated to population-specific measures of genetic variation challenges assumptions of a universal link between genetic variation and fitness in isolated populations.



**Population structure of the Morelet's Crocodile (*Crocodylus moreletii*) in the region of Calakmul, Campeche, Mexico**

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In the region of Calakmul (Campeche, Mexico), Morelet's crocodiles (*Crocodylus moreletii*) inhabit semi-temporary and therefore highly dynamic natural ponds (aguadas) sustained by rainfall, possibly leading to a spatial population structure which is different from other studied populations. Local and total abundance estimations were performed by the standard method used in Mexico (Visible Fraction - VF, Sánchez-Herrera et al. 2011) and by using a Zero-Inflated Poisson (ZIP) N-mixture model approach, conducting spotlight surveys in 43 waterbodies across the region twice a year between 2018 and 2019. To investigate size class distribution of crocodile encounters, we used a Chi-Square analysis and tested against the null hypothesis of even distribution of size cohorts. Local abundance estimates through VF and ZIP averaged  $6.5 \pm 2.2$  (zero – 73) and  $8.97 \pm 1.92$  ( $6.93 \pm 1.54$  –  $43.91 \pm 15.67$ ), respectively. Total abundance in the region was estimated at 9499 individuals (8886 – 10154) using TM and as 12426 individuals (9683 – 15950) using ZIP. The overall size structure was significantly different from the theoretical equal distribution of size classes ( $\chi^2 = 31.52$ ,  $df = 3$ ,  $p$ -value < 0.001). Approaches based on N-mixture models seem better suited for Calakmul as they incorporate more information and allow for superior inferences compared to traditionally used techniques. Demographic structure can vary between seasons but generally follows a pyramidal scheme with a common structure of 1-2 adults and an increasing number of smaller size class individuals.

## Overcoming Human-Herpetofauna conflict using snakebite as a case-study

### Jesudasan A<sup>1</sup>

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The biggest threat to some species of herpetofauna is society's inability to overcome conflicts between humans and herpetofauna. Worldwide, Crocidilians cause approximately 1000 human deaths annually. This is dwarfed compared to deaths caused by snakebite – between 81,000 to 138,000. In India, 50,000 people die of snakebite annually. The reasons for high incidence of snakebite deaths in India are many – poor quality of venom (used for the production of antivenom), poor quality of anti-venom, geographic variation of venom in same species, inadequate supply of antivenom, reliance on quack treatments, lack of widely disseminated first aid and treatment protocols, lack of knowledge about snake behavior, inadequate training of clinicians in dealing with snakebite. We have been working to overcome these challenges for about half a decade and had laid a solid foundation to strategically overcome all issues. What helped us the most was the decision of World Health Organization to declare snakebite envenomation as a class-A Neglected Tropical Disease. This decision helped us open conversation with various government agencies to take concrete steps on snakebite. We now have a set-up with the state government to monitor snakebite related cases in all hospitals in Tamil Nadu. We are in conversation with the national medicine regulators to set higher standards for the quality of antivenom. A vibrant educational program is underway to educate people about snakebite avoidance and appropriate treatment for snakebite and is showing tremendous results. Our project shows how we have to think beyond herpetology to overcome daunting conservation problems.

**Is competition causing character displacement and niche segregation in a radiation of cryptic species?**

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The role of biotic interactions on species distribution, abundance, and ecological traits is usually overlooked in most of research dealing with realized niches. Biotic interactions can restrict the distribution of species, and also drive the evolution of relevant traits. Interspecific competition can block geographic expansions of species and, through ecological character displacement, drive shifts in key traits of species and community composition.

Here we present research utilizing recently developed methods for inferring joint effects of competition from phylogeographic and trait data to explore whether long-term estimates of competition strength translate to recent and contemporary interactions. We chose the evolutionary radiation of Australian *Gehyra* geckos, with more than 60 lineages, often morphologically cryptic. These lineages, identified using phylogenomics, correspond to 30 named taxa, with more taxonomic revisions in progress. Like many low-dispersal organisms across northern Australia phylogenomic analyses have revealed many deeply divergent phylogeographic lineages within species of *Gehyra* and several centres of endemism. Australian *Gehyra* show highly evolvable body size within and across species. However, many sympatric *Gehyra* lineages differ in body size and habitat use. Using an integrative approach combining morphometric measures of museum specimens, and macroevolutionary and biogeographical inference methods, we assess the biogeographical effects of competition (such as range blocking and character displacement) among rock-dwelling *Gehyra*. Parapatric distributions of similar sized lineages, together with sympatry of different sized lineages, suggests that competitive exclusion plays an important role shaping their current communities.

## **Premating reproductive isolation between allopatric populations promoted by reproductive character displacement in geckos**

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Secondary contact of closely related species is considered to enhance premating reproductive isolation, shifting the mode of mating traits in a manner that increases character differences between species to avoid maladaptive interspecies crossing (reproductive character displacement; RCD). Since this shift does not occur in allopatric populations, RCD may result in incidental reproductive isolation between sympatric and allopatric populations within single species. In the Central Ryukyus, Japan, two gecko species, *Gekko hokouensis* (GH) and *Gekko* sp. (GS) are distributed sympatrically in several islands, but GH solitary occurs in many other islands. To test this idea, we examined male's mating calls and female preference for the sympatric and solitary GH populations. Our analysis revealed that GH calls consisted of significantly shorter inter-pulse intervals than GS calls, and that inter-pulse intervals in the sympatric GH populations were even shorter than the solitary populations. Playback experiment suggested that females of both populations could discriminate between conspecific and heterospecific calls. Females of the sympatric populations discriminated calls of its own populations and preferred males with shorter inter-pulse intervals within the population, whereas females of the solitary populations were not concerned with difference in inter-pulse intervals between the populations and among males within the population. These results strongly suggest that the change in mating calls and the female preference resulted from RCD, and accuracy of species recognition was raised in the sympatric populations. Overall, RCD against GS has promoted reproductive isolation of the sympatric GH populations from conspecific allopatric populations to some extent.

## Nasal GnRH administration elicits sperm production in both bufonidae and ranidae families

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Intraperitoneal (IP) injection of either human chorionic gonadotropin (hCG) in the family bufonidae or a cocktail of both hCG and gonadotropin-releasing hormone (GnRH) in the family ranidae stimulate reproductive behaviors in males, but these hormones are costly and IP injection requires training or veterinary assistance. Due to low reproductive output in many endangered anuran species, interest in affordable, less labor-intensive assisted reproductive technologies (ART) has risen. We recently found that nasal administration of small dosages of GnRH elicits sperm production comparable to GnRH and hCG IP injections in the bufonid *Anaxyrus fowleri*. Here, we demonstrate similar responses in a species of ranid. The current study compared the responses of the ranid *Lithobates chiricahuensis* (n=8) and the bufonid *Anaxyrus fowleri* (n=15) to nasal GnRH treatments. Wild-caught males from each species were nasally administered 10 µg of GnRH. Spermic urine was collected hourly for 3 hours and analyzed for concentration (sperm/mL), forward progressive motility (FPM), and total motility (TM). Overall, spermiation occurred in 100% of *L. chiricahuensis*, and 93% of *A. fowleri*. Neither FPM nor TM differed between the groups ( $p>0.05$ ), but *L. chiricahuensis* produced significantly higher ( $p=0.01$ ) concentrations of sperm ( $2.34 \times 10^6$  sperm/mL) compared to *A. fowleri* ( $3.06 \times 10^5$  sperm/mL). These results show that sperm can be collected from both ranidae and bufonidae using a single nasal GnRH administration with minimal differences between the two families of anurans. Overall, these results support the use of nasal GnRH administration as a fast, minimally-invasive tool for ART in anurans.

## Comparative embryology of the VNO and associated structures in squamate reptiles: a 3D approach

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Unlike in other tetrapods, the vomeronasal organ (VNO) in squamate embryos loses its direct connection with the nasal cavity, and in adult forms it enters the oral cavity exclusively. Thus, the organ's sensory function depends on chemicals collected by the tongue, such as pheromones or prey odors. In this study we investigated the embryonic development of the VNO and associated structures in representatives of three large clades of Squamata: Gekkota (mourning gecko, *Lepidodactylus lugubris* and leopard gecko, *Eublepharis macularius*), Iguania (brown anole, *Anolis sagrei*) and Lacertoidea (sand lizard, *Lacerta agilis*). Based on histological studies and 3D reconstructions based on X-ray microtomography, our visualisations show various aspect of embryo morphology, such as the shape of the VNO and the nasal cavity, the relation of the choanal groove to the VNO duct, association of the choanal groove to the lacrimal duct, and variation in anatomy of the nasal gland. Developmental data obtained here shed light on the mechanism of formation of various adult conditions and may provide an important contribution to resolving the deepest branches of the squamate phylogenetic tree. Moreover, because many predictions about chemoreceptive abilities are based on tongue morphology or its movements, our findings are also important to understanding the function of squamate olfactory systems.

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## Urban plums and toads: do fleshy fruits affect the post-metamorphic growth of amphibians?

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The aim of the study was to analyse the influence of fleshy fruits on the post-metamorphic growth and feeding behaviour of the green toad *Bufo viridis*. We tested the following hypotheses: juveniles of the green toad are characterised by faster growth in conditions involving fallen plums *Prunus cerasifera* due to the associated presence of more varied food such as invertebrates; toads exhibit more active feeding behaviour in the presence of fleshy fruits.

A total of 120 toad metamorphs were assigned to one of four groups: two experimental groups with fleshy plums and two other groups as controls (without fruits). Groups were kept in an enclosure to which invertebrates had free access. Each toad was measured for snout-vent length and body mass every other day for 30 days. The number of active and hidden (under an artificial shelter) individuals was also noted. Toads from enclosures with plums were characterised by more rapid growth than individuals from the control treatments. Simultaneously, in the enclosure with fleshy fruits, greater species richness of wild invertebrates was observed. No differences in active feeding behaviour were noted between control and groups with plums.

Fleshy fruits, attract many types of invertebrates; thus they may represent good dietary supplements for juvenile amphibians. Therefore, the presence of fruit trees close to a breeding site might influence the post-metamorphic growth of amphibians, but not their feeding behaviour. The results are important, since, due to political and social pressure, numbers of fruit trees are currently being reduced in the city.

## **Editors symposium**

### **Kaiser H**

This workshop is designed for colleagues, who wish to learn more about the publishing process in general and who want some pointers for how to streamline manuscript preparation - and perhaps increase their published output as a consequence. Topics will include a technical review of the editorial process (manuscript preparation, tasks and functions of editors) and the peer-review process (conceptual overview, benefit, anonymous vs. non-anonymous, etc.), a discussion of what is publishable and how big or small a paper is appropriate, as well as some specific topics in herpetological publishing (nomenclature, open access, preparation of figures and tables, etc.).



**The status of caecilian (Amphibia: Gymnophiona) collections at the Natural History Museum, London, UK.**

**Kamei R**<sup>1</sup>, Streicher J<sup>1</sup>

<sup>1</sup>*The Natural History Museum*

The Natural History Museum, London (NHM) houses one of the largest and oldest herpetological collections in Europe. The Museum's collection contains over 100,000 amphibian specimens. However, the precise number of specimens and their modern taxonomic identities are yet to be determined—the taxonomy has not been updated since the early 1900s for most of the collection. As the first step towards resolving these issues, we have been conducting a major curatorial effort on the caecilian collections. I will talk about our curatorial work including (1) archiving historical labels that feature the handwriting of early curators (e.g. Gray, Günther, Boulenger) from specimen jars, (2) issuing a newly developed barcoded label system, (3) physically rearranging the collection based on current taxonomy, and (4) georeferencing all caecilian specimens from the collection.

To date, our work has demonstrated that the collection contains ~700 caecilian specimens including ~100 type specimens of which ~70 are primary types. The Museum's collection is richer than previously thought—in both the number of specimens and the number of taxa represented in the Museum: nine/ten families and 28/32 genera. Our work has led to the discovery of a genus (*Luetkenotyphlus* Taylor, 1968) that was previously unknown in the collection, and has added multiple types establishing the NHM as one of the most taxonomically and geographically complete caecilian collections in the world.

## **Beam forming as an acoustic tool for population counts in amphibians**

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<sup>1</sup>*Xishuangbanna Tropical Botanic Garden, Chinese Academy Of Science*

Amphibians as a group is under severe threat with over 40% of species estimated to be under threat by IUCN, an impediment to the development of effective conservation plans is the lack of baseline information available for a high number of species. Acoustic monitoring is used increasingly around the world, providing large amounts of data useful for studying fields such as occupancy and acoustic niches at a relatively low cost. However, the way the data is collected makes it difficult to use it for accurate population counts as it is not possible assess the location of a call. Acoustic techniques like beam forming have been used for population counts in avian and marine studies. Beam forming involves multiple microphones in a line and is used to estimate the location of a sound very accurately. This technique requires species specific adjustments but could be advantageous when you have a complex habitat with many species. In this study we tested the technique on multiple amphibian species in a tropical setting using a self-built system. We report on issues with the set-up as well as logistical challenges with implementation and give recommendations on use. We also present analyses on the type of data this technique delivers and what conclusions you can draw from it. We conclude that it is a useful complement to standard acoustic monitoring, which is especially useful for species that are hard to count using classical survey techniques.

## Captive incubation boosts recovery of a relict tuatara population

**Keall S<sup>1</sup>**, Daugherty C<sup>1</sup>, Walle R<sup>2</sup>, Nelson N<sup>1</sup>

<sup>1</sup>Victoria University Of Wellington, <sup>2</sup>Department of Conservation

Conservation programmes for tuatara have involved artificial incubation and head-starting of resulting juveniles. These techniques are sometimes controversial, but we chose to use them because they can increase survival of eggs and juveniles compared to incubation in natural conditions, and therefore enable faster potential recovery of populations. Tuatara (*Sphenodon punctatus*) are the sole living representatives of the reptilian order Rhynchocephalia and survived only in New Zealand where their natural range was restricted to offshore islands due to predation by introduced mammals. Hauturu (Little Barrier Island) is a 3,000 hectare Nature Reserve on which a remnant population of tuatara was found during surveys in 1991 and 1992. Eight adult tuatara (4 females, 4 males) were taken into captivity on the island to protect them from Pacific rats (*Rattus exulans*). Eggs laid by these tuatara were artificially incubated, and the resulting 290 juveniles were head-started in captivity on the island. Following eradication of Pacific rats, from 2006 these juveniles were released into locations around the island where adult tuatara were either captured on the original surveys, or seen since. In 2015 a population survey recaptured healthy head-started individuals that were larger than when released. Evidence was found of successful recruitment in the wild and that further individuals of the original population had survived. Successful re-establishment of the Hauturu population will take decades to confirm, but without use of these conservation techniques the population would likely have disappeared.

## A Snake in the Grass: A Molecular Study of the Widespread *Psammophylax* Genus

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<sup>1</sup>Rhodes University, <sup>2</sup>Port Elizabeth Museum, <sup>3</sup>University of Texas at El Paso

*Psammophylax* (Fitzinger 1843) is a widespread yet poorly studied genus of African grass snakes. Grass snakes or 'skaapstekers' as they are sometimes referred, are terrestrial, diurnal, active foragers that can often be found in grassland and savanna. A genetic phylogeny of six of the seven species was estimated using both Bayesian and Maximum Likelihood Inference. To support the genetic analyses, we conducted morphological analyses on the body (traditional morphology) and head (geometric morphometrics) separately. Phylogenetic analyses recovered a similar topology to past studies, but with better resolution and node support. We found substantial genetic structuring within the genus, supported by significantly different head shapes between *P. a. acutus* and other *Psammophylax*. *Psammophylax a. acutus* was recovered as sister to its congeners, and sequence divergence values, bGMYC analysis and morphometric analysis supported its recognition as a new genus, described in honour of the late Bill Branch. Increased sampling in East Africa (Tanzania, Kenya and Ethiopia) revealed that *Psammophylax multisquamis* is polyphyletic, necessitating the description of a new, morphologically cryptic species from northern Tanzania. The distribution of *P. multisquamis* sensu stricto is likely restricted to Kenya and Ethiopia. The study has resolved multiple aspects of *Psammophylax* systematics, including the taxonomic validity of two central African subspecies. The inclusion of specimens from the more remote parts of Africa, in future analyses, may result in the recovery of additional diversity within *Psammophylax*.

**Testing the Enemy Release Hypothesis: The Global Spread of Cane Toads as an Accidental Experiment.**

**Kelehear C<sup>1</sup>**, Torchin M<sup>2</sup>, Saltonstall K<sup>2</sup>, Shine R<sup>3</sup>

<sup>1</sup>*Sul Ross State University*, <sup>2</sup>*Smithsonian Tropical Research Institute*, <sup>3</sup>*Macquarie University*

Invasive species are the second greatest threat to biodiversity, yet we know little about what drives their success. The “enemy release hypothesis” posits that release from native competitors, predators, and parasites facilitates invader success in a new environment. The cane toad, *Rhinella marina*, is a toxic amphibian native to South America that has been widely introduced. It has reached pest status in many new ranges, poisoning domestic pets and native wildlife. Because of its extensive invasion history and unparalleled documentation of introductions, the cane toad provides an ideal “natural” experiment to test the potential influence of enemy loss on invasion success. We traced the geographical pathway of toad introductions back to their native origins to investigate whether the enemy release hypothesis explains the cane toad’s prosperity as an invasive species. First, to determine if cane toads exhibit features consistent with increased population success in invasive ranges we compared two proxies for fitness (body condition, reproductive output) in replicate invaded and native toad populations. Second, to investigate whether fitness is a function of enemy release, we compared diversity and intensity of parasitic infections in invasive vs native toad populations. The results of our study are discussed.

### Effect of male phenotype and nest quality on mating success in the northern corroboree frog

**Kelleher S<sup>1</sup>**, Silla A<sup>1</sup>, Keogh S<sup>2</sup>, Hunter D<sup>3</sup>, Scheele B<sup>4</sup>, Endler J<sup>5</sup>, Byrne P<sup>1</sup>

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Amphibian biodiversity loss currently exceeds that of any other vertebrate class, with an estimated 41% of species now threatened with extinction. Captive breeding and reintroduction programs are one of the main management actions recommended to assist the recovery amphibian species at imminent risk of extinction. However, the success of amphibian captive breeding programs has been variable, explained in part by a general lack of knowledge about the reproductive biology of target species. This study aimed to investigate predictors of male mating success in a wild population of the critically endangered Northern Corroboree frog, *Pseudophryne pengilleyi*, in order to inform the captive breeding and reintroduction program for this species. In this terrestrial breeding anuran, males construct nests in sphagnum moss and use calls to attract prospective mates. Theoretically, females might choose their mates based on male phenotypic traits that signal a male's potential to supply offspring with genes that improve their viability, or male nest qualities likely to influence offspring survival. Data was collected in the field, with twelve phenotypic characteristics and eight nest-site characteristics measured for one of the largest remaining wild populations of this species. Results showed that 66% of males (n=35) received matings, though there was significant variation in the number of matings per male. Both male phenotypic and nest-site characteristics contributed to this variation, and this knowledge will help direct the multi-institutional captive breeding program for this species. Gathering information about species' reproductive ecology is essential to improve the propagation and viability of captive amphibian colonies globally.

**Is rapid colour change in Raukawa geckos (*Woodworthia maculata*) an adaptation to predator avoidance?**

**Kelly F<sup>1</sup>**, Herbert S<sup>1</sup>, Nelson N<sup>1</sup>

<sup>1</sup>Victoria University Of Wellington

Reptilian colouration is important for camouflage and thermoregulation. Thermoregulation in herpetofauna underpins all physiological processes, including the ability to digest food and evade predators. Camouflage is important to avoid visually hunting predators such as birds and reptiles, New Zealand's main lizard predators before the introduction of mammals. New Zealand has over 110 endemic lizard species, including at least 42 gecko species, of which 88% are threatened or at risk. New Zealand lizards are generally not brightly coloured by international standards, however rapid physiological colour change has been observed in at least seven species of geckos to date. The triggers for colour change in New Zealand geckos have not previously been fully investigated, despite their potential importance. Internationally, there are many species of lizards with brightly coloured tails, thought to be an adaptation to predator avoidance given their ability to autotomise their tails. We investigated the colour change responses of three New Zealand gecko species to various stimuli. Raukawa geckos (*Woodworthia maculata*) rapidly changed colour when handled, becoming paler in colour. However, the colouring of their tails became brighter and the patterning more contrasted. By simulating the presence of predators, we propose this is an adaptation to the anticipation of predator attack. When a gecko cannot otherwise escape, rapid colour change can assist in directing the predator's attention to the autotomisable tail, thus potentially increasing survival probability. Determining the triggers for colour change in geckos is important, as these can affect conservation management decision-making around habitat changes and climate change.

## Detecting selection in urban dragons with a comparison of neutral markers and quantitative trait divergence

**Kent N<sup>1</sup>**, Littleford-Colquhoun B<sup>1</sup>, Strickland K<sup>1</sup>, Frere C<sup>1</sup>

<sup>1</sup>*University Of The Sunshine Coast*

Environmental heterogeneity can induce diverse patterns of genetic and phenotypic differentiation between populations, through both neutral (e.g. drift) and selective processes. As highly heterogeneous environments, city parks have been shown to act as islands for phenotypic differentiation in contemporary island biogeography. The Eastern Water Dragon (*Intellagama lesueurii*), has shown strong morphological differentiation in parallel with substantial genetic divergence in city parks when compared to native riparian habitat. This suggests that these dragons are likely experiencing altered selective processes in city parks. Here, we used microsatellites as neutral genetic markers and compared this with the divergence in morphological traits in the Eastern Water dragon. In what is often referred to as a Qst-Fst comparison, we were able to determine if adaptive evolution is occurring on morphological traits in urban environments, the type of selection (stabilising or directional) and the patterns among city park populations and between city parks and native riparian populations.



### **City life alters the gut microbiome and stable isotope profiling of eastern water dragons**

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<sup>1</sup>University Of The Sunshine Coast, <sup>2</sup>Australian Centre for Ancient DNA, Department of Genetics and Evolution, The University of Adelaide

Urbanisation is one of the most significant threats to biodiversity, due to the rapid and large-scale environmental alterations it imposes on the natural landscape. It is, therefore, imperative that we understand the consequences of and mechanisms by which, species can respond to it. In recent years, research has shown that plasticity of the gut microbiome may be an important mechanism by which animals can adapt to environmental change, yet empirical evidence of this in wild non-model species remains sparse. Using an empirical replicated study system, we show that city life alters the gut microbiome and stable isotope profile of a wild native non-model species – the eastern water dragon (*Intellagama lesueurii*) in Queensland, Australia. City dragons exhibit a more diverse gut microbiome than their native habitat counterparts and show gut microbial signatures of a high fat and plant rich diet. Additionally, we also show that city dragons have elevated levels of the Nitrogen-15 isotope in their blood suggesting that a city diet, which incorporates novel anthropogenic food sources, may also be richer in protein. These results highlight the role that gut microbial plasticity plays in an animals' response to human-altered landscapes.

**The powerhouse of asexual cost? Endurance and mitochondrial efficiency in parthenogenetic whiptail lizards (genus: *Aspidoscelis*)**

**Klabacka R<sup>1</sup>**, Parry H<sup>2</sup>, Yap J<sup>2</sup>, Cook R<sup>1</sup>, Herron T<sup>1</sup>, Horne M<sup>3</sup>, Maldonado J<sup>4</sup>, Álvarez G<sup>3</sup>, Kavazis A<sup>2</sup>, Oaks J<sup>1</sup>, Fujita M<sup>4</sup>, Johnson J<sup>3</sup>, Schwartz T<sup>1</sup>

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The advantage of passing 100% of an individual's genetic material to progeny should lead to an abundance of asexual organisms. However, the indisputable scarcity of asexual vertebrates alludes to an inherent cost incurred in asexual reproduction within this clade. Studies measuring the effect of asexuality on physiological performance have generally focused on whole-organism physiology, and may be missing nuances concealed within cellular processes. Cellular respiration, the mechanism by which eukaryotes generate energy in the form of ATP, functions by harnessing subatomic energy via an electron transport system made up of co-evolved protein subunits of mitochondrial and nuclear origin. Asexual lineages lack the ability to efficiently filter variation via genetic recombination, and thus are predicted to gradually lose compatibility between mitochondrial and nuclear genomes. The universal need for energy among eukaryotes suggests that this intragenomic network may underpin the scarcity of asexual vertebrates. Alternative hypotheses include genomic incompatibilities due to the hybrid origins of essentially all parthenogenetic vertebrates. With asexuals constituting one third of its ~45 species, the genus *Aspidoscelis* (whiptail lizards) is a great model system for testing costs of asexuality. Asexual whiptails may possess lower endurance capacity compared to congeneric sexuals, a trait predominantly affected by cellular respiration and potentially associated with the high energy demands of these actively foraging lizards. Here we measure endurance capacity in five species of *Aspidoscelis* and examine markers of mitochondrial efficiency between sexual and asexual species within a phylogenetic context using enzyme activity assays (ETS complexes), live mitochondria respirometry, and DNA sequence data.

**Reintroduction efforts for two species of Panamanian Harlequin frogs (Genus: *Atelopus*) threatened by chytrid fungus**

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The emergence of the amphibian chytrid fungus in Panama resulted in catastrophic population declines or extinction in the six species of *Atelopus* that occur within the country. The Panama Amphibian Rescue and Conservation Project is an ex-situ captive breeding project that was established to prevent the extinction of the most susceptible Panamanian amphibians. Captive assurance populations of five *Atelopus* spp. (*Atelopus certus*, *Atelopus glyphus*, *Atelopus limosus*, *Atelopus varius*, and *Atelopus zeteki*) have been successfully maintained and bred in Panama. Release trials with captive bred F1 individuals of two species, *Atelopus limosus* and *Atelopus varius*, were completed to understand dispersal patterns, survivorship, susceptibility to chytrid. We equipped several adult *A. limosus* and *A. varius* with VHF radio transmitters to track daily movements, obtain weekly weight measurements, sources of mortality, and monitor chytrid status post release. We attempted mark and recapture surveys with animals that did not have a radio transmitter with limited success. Soft release enclosures to determine if there was difference in survivorship between hard and soft release individuals. These initial efforts will guide the development of future reintroduction strategies.

### **Can increased environmental salinity facilitate the reintroduction of a chytrid-susceptible species?**

**Klop-toker K**<sup>1</sup>, Callen A<sup>1</sup>, Stockwell M<sup>1</sup>, Pizzatto L<sup>1</sup>, Valdez J<sup>1</sup>, Fardell L<sup>3</sup>, Edgar M<sup>1</sup>, Clulow S<sup>2</sup>, Clulow J<sup>1</sup>, Mahony M<sup>1</sup>

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Reintroductions for conservation should only be considered if the threat that caused the target species' decline has been reduced or removed. This makes reintroductions for chytrid-susceptible amphibians problematic, as the pathogen is almost impossible to remove from the landscape. However, laboratory and small-scale field trials have shown water salinities > 3ppt reduce the growth and movement of *Batrachochytrium dendrobatidis* and improve the survival of infected amphibians. Therefore, increased pond salinities may be a potential management strategy to facilitate amphibian survival in the presence of this landscape persistent pathogen. To assess the feasibility of using salt to mitigate chytrid within a landscape, we ran a series of experiments testing: frog behavioural and tadpole physiological responses to increased salinity; the effect of increased salinity on other pond biota; the levels of pond salinity over time; and survival of reintroduced frogs. We found that salinity levels between 3 to 5ppt were readily used by our target species, increased their size at metamorphosis, supported two released frog populations, but reduced the abundance of some aquatic invertebrate and non-target frog species. Salinity levels also varied with environmental conditions (i.e. changes in water levels due to drought/rain). These findings suggest that the application of salt to some waterbodies in a habitat mosaic may improve the viability of chytrid-susceptible populations in reintroduction programs, but would need to be assessed on the basis of site and species suitability.

### Comparative skull morphology of Leptotyphlopidae via Micro-CT images

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The threadsnake family Leptotyphlopidae consists of 14 genera with 141 fossorial species that are rarely detected in the field. These head-first burrowing snakes have a reduced body plan with much of their divergence and evolutionary success associated to modifications of their tiny heads (often just 2-3 mm in length). Most species and even genera look superficially alike, and only limited suite of diagnostic characters for differentiation are available.

Until now almost only external morphological traits have been consistently used for differentiation, but the ranges of variation usually overlapping among many closely related species. Thus, there is a strong need for the establishment of further diagnostic characters of systematic and taxonomic value, which can be visualized with non-destructive methods due to the rarity of some species. Former studies provided evidence that the arrangement of dorsal skull bones is useful for the diagnosis of genera and in some cases even species. Nevertheless, skeletal characters have rarely been used for distinction and of so far only 43 species and 9 genera of Leptotyphlopidae the cranial morphology has been examined although at different levels of detail.

The aim of the present study was to contribute substantial information on the skulls of the family to allow a better understanding of this complicate snake lineage and to facilitate species identification even for non-specialists in this taxon. Generic and interspecific differences in skull morphologies were examined with the aid of high-resolution computerized microtomography ( $\mu$ -CT). Whenever available, primary types or topotypes built the basis of the examined material.

### Putative mutualistic symbiosis between an ant and a snake

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Nests of ants are inhabited by many organisms that are specialized for association with ants. Vast majority of such inhabitants is arthropods, and examples among vertebrates are relatively scarce. Our radio-telemetric research on a Madagascan snake, *Madagascarophis colubrinus*, demonstrated that *M. colubrinus* frequently shelters in the nests of a particular species of ants, *Aphaenogaster swammerdami*. In the field experiment we confirmed that *A. swammerdami* was least aggressive toward *M. colubrinus* among six species of syntopic snakes that were presented at the ant nest entrance. In the behavioral experiment *M. colubrinus* exhibited predatory behaviors toward cotton swabs carrying the odor of a blindsnake, *Madatyphlops decorsei*. This blindsnake is a major predator of *A. swammerdami*, which intrudes ant nests and gorges on its larva and pupa. The ant would be able to reduce this risk of predation by sharing the nest with *M. colubrinus*, a predator of the blindsnake. Our results suggest a unique, mutualistic relationship between the ant and the snake, where the host provides a favorable sheltering habitat, and the guest may serve as a 'guardian'.

## **Using social media to benefit herpetology, your career, and public perception of reptiles and amphibians**

**Kolby J<sup>1</sup>**

<sup>1</sup>*James Cook University*

The internet has become an extremely inexpensive, highly accessible, rapid tool to exercise science communication. In a way unlike ever before, multiple platforms stretching across Facebook, Instagram, Twitter and Tumblr provide us with an audience at our fingertips, reaching into the millions. Keeping in mind some basic strategies, we can use these platforms to share our love for herpetology, raise attention for our academic achievements, and build new relationships that can produce fruitful collaborations. I first approached social media with strong skepticism that it could help me as herpetologist, both academically and personally. Over the past 3 years, I've been amazed by the opportunities it has provided for me including producing videos for National Geographic, being interviewed multiple times about breaking news stories, and sparking a dramatic increase in the number of times my publications have been read and cited. In this presentation, I will help you unlock your potential and share what I've learned from my experiences diving into all of these social media platforms simultaneously. There are now many simple things that each of us can do online to help engage with the public and build a stronger global herpetological community.

## **Temporary translocation to dodge the chytrid bullet: The Honduras Amphibian Rescue and Conservation Center strategy**

**Kolby J<sup>1</sup>**

<sup>1</sup>*James Cook University*

Emerging infectious diseases now pose a growing threat to reptiles and amphibians around the world. Translocations are traditionally performed to protect animals from habitat threats, but now, habitat protection is no longer sufficient to protect species from extinction because diseases don't respect protected area boundaries. Accordingly, novel conservation strategies must be implemented to prevent disease-driven declines. One potential avenue is the temporary or permanent translocation of animals to evade diseases. The spread of amphibian chytrid fungus has already caused declines in at least 500 amphibian species and pushed 90 into extinction. In this presentation I'll explain our conservation response at the Honduras Amphibian Rescue and Conservation Center. Our frog rescue team has developed a head-start and reintroduction program to prevent the extinction of three endangered species in the cloud forest of Cusuco National Park centered around temporary translocations. In this effort, tadpoles highly susceptible to chytrid infection and chytridiomycosis will be removed from the rainforest and transported to our frog rescue laboratory 100 miles away. Here, animals will be protected from developing chytridiomycosis and raised until they pass the most vulnerable period of metamorphosis. Then, they will be returned to the forest possessing the more resilient immune system of an adult frog. Our goal is to supplement the population of adult reproductive frogs persisting in the wild to boost the volume of offspring produced in-situ. By repeating this year after year, we aim to assist natural selection and evolution while preventing extinction as species adapt to co-exist with chytrid.



## The source of reptile and amphibian pandemics in the Anthropocene

Kolby J<sup>1</sup>

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The world has recently become irreversibly connected by rapid technological advancements in communication and transportation. This globalization phenomenon has brought together people, places, and wildlife, bridging physical and historical boundaries in a way hardly imaginable 50 years ago. Although some of these changes have been positive and support societal and economic development, significantly negative consequences have emerged as well. The most negative impact of globalization with respect to amphibian conservation is the spread of emerging infectious diseases, and most notably the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*). Discovered in 1998 and formally described in 1999, much work has been done over the past 20 years to understand this pandemic, but unfortunately very little has been accomplished to halt the continued global spread. In this symposium, I will explain my PhD work to evaluate the role of the international wildlife trade in the spread of emerging infectious diseases. This presentation will primarily focus on chytrid fungus, but data on ranavirus will also be provided. I will present the results from my trade surveillance and then offer recommendations to effectively manage the uncontrolled movement of a pathogen now responsible for the most severe wildlife disease event in recorded history.

## Genomic approaches to amphibian conservation

Kosch T<sup>1</sup>

<sup>1</sup>*Massey University,* <sup>2</sup>*University of Melbourne*

Amphibians are the most threatened group of vertebrates with over one-third of species vulnerable to extinction. Two of the largest threats that amphibians face, emerging infectious diseases and climate change, do not currently have any long-term solutions leaving many species dependent on captive breeding and reintroduction programs for their continued survival. For such immitigable threats, facilitated evolution through genetic manipulation may be the only way to produce self-sustaining, resilient wild populations. Historically, genetic manipulation has been mostly restricted to model and agricultural species, but recent advances in genomic technologies and increased availability of reference genomes from resources such as the Vertebrate Genome Project make genetic manipulation for wildlife conservation amendable for the first time. I will discuss how genetic manipulation can be applied to amphibian conservation to improve fitness in a changing landscape and some pros and cons of these approaches.

## **Status of the U.S. National Amphibian Genome Bank and future efforts to conserve anuran biodiversity**

**Kouba A**<sup>1</sup>, Julien A<sup>1</sup>, Gillis A<sup>1</sup>, Guy E<sup>1</sup>, Barber D<sup>2</sup>, Kouba C<sup>1</sup>

<sup>1</sup>*Mississippi State University*, <sup>2</sup>*Fort Worth Zoo*

Amphibian genome resource banks (AGRBs) are gaining importance as tools in the field of conservation biology, enabling investigators to store and access large numbers of catalogued samples for population management. While some labs are storing amphibian cell cultures, the majority of programs are focused on gamete and embryo preservation, such that live animals can be produced from the preserved samples. With the advancement of sperm cryopreservation techniques and assisted reproductive technologies to produce offspring, the benefits of these biorepositories are beginning to be realized by captive breeding programs. Multiple species of anurans and two species of caudates have now been produced by cryopreserved sperm. These proof of concept and success stories suggest it is time to scale-up and expand the collection and storage of a significant portion of the world's amphibian biodiversity as a hedge against extinction. Several countries, including Australia, Russia and the United States have labs dedicated to advancing the science and curation of amphibian biodiversity through AGRBs and their associated reproductive technologies. Most of these labs would indicate they are far from reaching their full potential and true conservation value. For example, there are still numerous obstacles that prohibit the collection, efficient sharing of, and access to these accessioned samples. Some of these barriers include financial, logistical, ethical, and legal issues. In this presentation, we will provide a description of advances in AGRBs, future opportunities for network expansion, barriers to be overcome and discuss key themes that are needed for the future of this discipline.

### **Proteus conservation project in Croatia**

Jalžić B<sup>4</sup>, Jelić D<sup>1</sup>, Koller-Šarić K<sup>3</sup>, **Kovac Konrad P<sup>2</sup>**

<sup>1</sup>Biota j.d.o.o., Braće Radića 128/A, <sup>2</sup>Zagreb speleological union, Radićeva 23, <sup>3</sup>Assosiation Hyla, I.Lipovac 2, <sup>4</sup>Croatian biospeleological society, Demetrova 1

Croatia is one of the hotspots for cave biodiversity in the World. New species of cave animals are still discovered on regular basis in over 10 000 recorded caves in Croatia. In the past 10 years cave divers, cavers and biologists have been doing intensive cave diving research in numerous caves. In their explorations they came across a unique cave vertebra, the biggest predator in European caves, *Proteus anguinus*. *Proteus* lives in underground waters of Dinaric karst, from Italy, through Slovenia, Croatia and Bosnia and Herzegovina and is the only European troglobiont vertebrate. Although *Proteus* was studied in laboratories in France, Germany and Slovenia there is still no sufficient data on *Proteus* behaviour in the subterranean environment observed directly by cave-divers. Almost no information is gathered deeper than 40m until exploration in cave Zagorska Peć to 120m depth. For 7 years through a project funded by MAVA foundation, National park Krka, Croatian electricity company (HEP), and conducted by Association Hyla and Biota cave divers are studying behaviour and population distribution of *Proteus* on 6 different locations in Croatia. Since then started some basic research in the caves to try to establish the size of population in 6 caves, get some insight in why *Proteus* prefers only one part of cave system, why population chooses microhabitat inside the cave system, what are the stress factors etc. Genetic study and EDNA testing has been done.

## Spatio-temporal architecture of pancreatic islets in grass snake embryos

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Pancreatic islets in vertebrates are composed of mainly four types of endocrine cells: A, B, D and PP cells which release glucagon, insulin, somatostatin and pancreatic polypeptide. Within islets pattern of endocrine cell arrangement differs among vertebrate species. The aim of this study was to investigate spatial and temporal changes in structure of pancreatic islets in grass snake embryos with usage immunohistochemical, immunocytochemical and immunofluorescence methods. Results of this study indicated that just after egg laying, at stage II, A, B and D cells were located in unarranged manner within dorsal pancreatic bud where they formed large islets. At this time A and B cells were numerous. From stage IV A cells become the most abundant islet cell type and started to form smaller units containing also other endocrine cell types. At stage VI pancreatic islets were formed mainly by A and D cells which were intermingled, and B cells were only sporadically found. From stage IX B cells started to increase in number which was correlated with decreasing number of D cells. At the time of hatching A cells formed large islets located in the dorsal part of pancreas. Within them and in islet periphery B cells and single D cells were found. This architecture of grass snake islets was very similar to that of pancreatic islets in primates. During entire embryonic development pancreatic polypeptide was not detected in pancreas of *Natrix*.

## Sex chromosomes in reptiles are more stable than thought

**Kratochvil L<sup>1</sup>**, Rovatsos M<sup>1</sup>

<sup>1</sup>*Charles University*

In contrast to mammals and birds, sex determination evolution is thought to be labile and rapid in non-avian reptiles. However, our systematic tests of the homology of sex chromosomes based on gene content of sex chromosomes demonstrated high evolutionary stability of sex chromosomes comparable to endotherms in several groups of non-avian reptiles. ZZ/ZW sex chromosomes are very stable in caenophidian snakes, Anguimorpha lizards (monitors and beaded lizards), lacertids and softshell turtles. XX/XY sex chromosomes are stable across iguanas and pygopodid geckos. We showed that phylogenetic studies reporting divergent sex determination even in closely related species were often based on inaccurate data, which led to overestimated number of shifts among sex determination systems. For example, we showed that there is little evidence for environmental sex determination (ESD) and turnovers of sex chromosomes within varanids and lacertids, but also for female heterogamety in geoemydid turtles. The variability in sex determination in reptiles can be at least partially attributed to their ancient divergence times and independent origins of sex chromosomes (maybe directly from the ancestral ESD) rather than to general evolutionary lability in sex determination.

## Context-dependent effects of non-native vegetation on amphibian development in prairie ecosystems

**Kross C<sup>1</sup>**, Dodd A<sup>2</sup>, Mariage P<sup>1</sup>, Willson J<sup>1</sup>

<sup>1</sup>University of Arkansas, <sup>2</sup>Lyon College

Many natural landscapes have been altered for agricultural or urban land-use, often altering vegetation composition surrounding wetlands. However, the mechanisms by which vegetation alteration impacts amphibian reproduction are poorly understood, particularly for grassland-associated species. We used a mesocosm approach to investigate how vegetation composition (native grassland or non-native agriculture-associated vegetation [tall fescue grass; *Festuca arundinacea*]) surrounding breeding wetlands and timing of oviposition affected development of larval Crawfish Frogs (*Lithobates areolatus*), a declining grassland species in the United States. We found that larval survival differed between vegetation types in the 7-days post-vegetation addition oviposition treatment, with nearly 100% mortality in the fescue grass treatments. Conversely, survival rates were similar across vegetation types in the 22-days post-vegetation addition oviposition treatment (~43%), and larvae reared in fescue metamorphosed more quickly and were larger post-metamorphosis than larvae raised in grassland vegetation. To investigate mechanisms for mortality in fescue treatments, we conducted a subsequent experiment examining the effects of vegetation type on dissolved oxygen (DO) concentrations and hatching success. We found that very low DO concentrations (<2.0 mg/L) in fescue treatments persisted for at least a week post-vegetation addition resulting in high egg mortality, however, aeration completely ameliorated low DO effects on mortality. Microbial respiration was almost 3 times higher in fescue compared to grassland vegetation, which was likely influenced by nutrient content. These results suggest that amphibian breeding ponds in agricultural landscapes can support successful breeding, but also that interpreting the effects of non-native vegetation on amphibian survival and development is complex and context-dependent.

### Populational dynamics of the stream-dwelling frog *Boana poaju* in Southern Brazil

Campos L<sup>1</sup>, Carvalho-Rocha V<sup>1</sup>, dos Santos K<sup>1</sup>, Geisler E<sup>1</sup>, Kusterko Novaes S<sup>1</sup>, Neckel S<sup>1</sup>, Oliveira S<sup>1</sup>  
<sup>1</sup>Florianópolis

Frogs are the most threatened vertebrates due high sensibility to habitat loss and climate changes. Reports on frog local extinctions and populations decline are increasing worldwide, especially because the emerging disease chytridiomycosis. Frogs from streams seems to be more susceptible to this disease, given cooler temperature needed to fungus grows, and current water that transport spores. The tree frog *Boana poaju* (Anura: Hylidae) is known to occur exclusively on few streams of southern Brazilian Atlantic Forest, and may be susceptible to chytrid infection and consequent population declines. Therefore, we asked how abundance vary through time and how big is *B. poaju* population. We monitored, from August 2017 to May 2019, one population of *B. poaju* in a stream at Serra do Tabuleiro State Park, Santa Catarina, Brazil. We marked four females (not included on posterior results) and 147 males, of which 52 (35%) were recaptured at least once. We estimated the population size with the Robust Pollock's Design (368 individuals  $\pm 15,02$ , n=147 males) and a survival index of 28%. *B. poaju* was more abundant on November 2018, when 26 individuals were found, 7 of them being recaptures. Not a single individual was found on May and July 2018. *Boana poaju* showed an annual cyclic pattern of abundance, probably associated to temperature seasonal variation, as they are less found at colder months. As the monitoring continues, we will be able to observe the population fluctuation through time and assess the status of conservation of the species.



## Evolutionary trajectories of a shell-adapted respiratory apparatus: the turtle dilemma(s)

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The chelonian shell is a versatile safeguard, but it could only evolve in concert with several major reorganisations of the respiratory apparatus. The recent discovery and identification of a number of potential stem turtles allows to slowly piecing together the evolutionary sequence of skeletal modifications towards a fully developed shell, and hence also the associated functional constraints on, and the potential changes of the respiratory faculty. With this contribution I will address the progressive evolution of an obligatory obliquus- or flank-driven inspiratory ventilatory mechanism, which eventually allowed the formation of fused and thus akinetic ribs. Furthermore, I will discuss the influence of a fully developed shell on the morphology of the airways and lungs among the different lineages of extant turtles, with special emphasis on the evolution of the different kinds of neck retraction. The pulmonary diversity among cryptodirans will be addressed with regard to its developmental plasticity and phylogenetic significance. Overall, the chelonian respiratory apparatus displays a mixture of unique adaptations in the form of fundamental structural reorganisations, along highly conservative elements, which vary only slightly but consistently between taxa and thus are of high systematic relevance. With this contribution I intend to provide an overview of the evolutionary trajectories that brought about this exceptional situation in turtles.

**Linking in-situ and ex-situ populations of the Southern Rocky Mountain boreal toad (*Anaxyrus boreas boreas*)**

Vance C<sup>4</sup>, Willard S<sup>4</sup>, Langhorne C<sup>1</sup>, Calatayud N<sup>2</sup>, Smith T<sup>3</sup>, Kouba A<sup>4</sup>

<sup>1</sup>MSU affiliate, <sup>2</sup>Native Animal Researchers and Conservationists, <sup>3</sup>Native Aquatic Species Restoration Facility, <sup>4</sup>Mississippi State University

The Southern Rocky Mountain boreal toad is the target of intensive captive breeding efforts due to the species continuing decline in the wild. Inconsistent breeding, coupled with an aging population, could compromise the boreal toad captive breeding programme (CBP) through the loss of valuable founder individuals. Therefore, the introduction of gametes from corresponding in-situ regional population segments could substantially benefit the captive colony by restoring valuable genetics without the need to remove animals from the wild. This study explored the feasibility of developing a genetic linkage strategy between a captive boreal toad colony and their corresponding in-situ regional population segments. Sperm was collected from twenty-four hormonally induced wild-caught toads and concentration and motility parameters were evaluated in a mobile field station prior to either cold storage or cryopreservation. The functional competence of the sperm was assessed by artificial fertilisation using eggs collected from captive females. Ninety-six percent of male toads injected with hCG produced sperm 2 hr post-hormone administration with a mean sperm concentration of  $3.5 \times 10^6$  /mL. Eggs fertilised using cold-stored or cryopreserved sperm produced a total of 493 and 92 tadpoles, respectively. This study highlights the potential for in-situ sperm collection from wild-caught anurans and the capacity to maintain viable sperm by applying lab-based gamete collection, cryopreservation, and cold storage techniques in the field. The effective application of these methods offers a potential strategy for introducing genetic diversity into CBPs through the collection and transportation of gametes between fragmented wild populations and their captive counter-parts.

### Forays into understanding mate choice in herps

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<sup>1</sup>Pennsylvania State University, <sup>2</sup>State University of New York

My introduction to mate choice in herps came when Rick Shine took me to the garter snake dens of Manitoba when I was a graduate student. This was a truly formative experience (involving female-mimicking male snakes and unveiling of previously unknown sexual strategies) and really opened my eyes to how many interesting questions there are in this field. Although not my main research focus, my research group has continued to study mate choice and I will share some of the highlights of this work. First, I will discuss findings of a body of research examining traits used by male and female eastern fence lizards (*Sceloporus undulatus*) when choosing and competing over mates, including the importance of female mate choice and evidence of a Napoleon complex in small males. Second, I will present work on mate selection in wood frogs (*Rana sylvatica*) that have a scramble mating system, which suggests that sperm is not limiting and mate preference may not be all it's cracked up to be. Those two years at the garter snake dens both spoiled me (I will never again be able to catch 100 study animals in a matter of minutes) and continues to shape my research.

## Thermoregulation strategy of the invasive Asian House Gecko (*Hemidactylus frenatus*)

Lapwong Y<sup>1</sup>

<sup>1</sup>University of Technology Sydney

The Asian house gecko (*Hemidactylus frenatus*) is a tropical gecko species that has invaded colder subtropical regions of East Asia and Australia. Potentially, shifts in thermoregulatory strategies may enable this species to survive in novel habitats. In this study I investigated whether geckos from introduced populations in southeastern Australia displayed different patterns of thermoregulation compared to geckos from native populations. In the lab, I used data loggers to record the body temperatures of individuals in a cost-free thermal gradient. I further explored whether geckos showed a thermophilic response to feeding. I also compared thermoregulation of *H. frenatus* with its non-invasive congener, *H. platyurus*, which occurs in sympatry in Thailand. Despite being tropical species, both *H. frenatus* and *H. platyurus* displayed a diel thermoregulation pattern. Geckos maintained higher body temperatures during mid-afternoon and at dusk, but selected cooler temperatures during the night. Mean selected body temperatures were 31.62°C and 31.30°C for *H. frenatus* and *H. platyurus* respectively. Introduced populations of *H. frenatus* showed a similar diel thermoregulation pattern, but their mean preferred body temperatures were lower (29.66°C in summer and 27.17°C in winter). In its native range, individuals of *H. frenatus* did not shift their body temperature after being fed. By contrast, individuals from southeastern Australia preferred higher body temperature after being fed. In conclusion, my study shows that invasive geckos have shifted their preferred body temperatures downwards in their non-native range, which may have facilitated the spread of this tropical species into colder climates.

**Implementing of environmental DNA detection method in the surveillance of amphibian pathogen  
*Batrachochytrium salamandrivorans***

**Lastra González D**<sup>1</sup>, Baláž V<sup>2</sup>, Vojar J<sup>1</sup>

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The recently discovered pathogen *Batrachochytrium salamandrivorans* (*Bsal*) has severe impact mainly on European salamanders and newts. New presence of *Bsal* has been confirmed for the first time in the wild in Spain by this team. These findings confirm the great area of occurrence of the fungus. There is an urgent need for a better focus on this country, because Spain has a sizable community of exotic pet keepers and the country plays an important role in the amphibian pet trade. Moreover, this part of Europe represents a perfect zone for the spread of *Bsal* with severe potential impact due to the high endemism of newts and salamanders in this area. Environmental DNA (eDNA) allows us to monitor the fungus from earlier stages due to its higher sensitivity, avoiding a direct contact with the disease host and increasing the time frame for the monitoring. We have already tested the methodology in the Czech Republic for *Bd* successfully and showing same results than swabbing, even in small volumes. Together with that, we implemented eDNA methodology for the detection of *Bsal* in Spain to establish the first approach of this technic against this chytrid fungus in wild. Thus, eDNA and its proved usefulness against non-indigenous species is a powerful tool which may contribute to stop the spreading of the salamander killer fungus and establishing early alarm monitoring system. The knowledge of pathogen's current geographic range is essential to carry on effective measures in affected areas and prevention in *Bsal*-free regions

## Determinants of phenotypic and genetic variation along a latitudinal gradient in a widespread amphibian

**Laurila A<sup>1</sup>**, Luquet E<sup>2</sup>, Meyer-Lucht Y<sup>1</sup>, Rödin-Mörch P<sup>1</sup>, Cortazar-Chinarro M<sup>1</sup>, Richter-Boix A<sup>1</sup>, Höglund J<sup>1</sup>  
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While adaptive divergence along environmental gradients has repeatedly been demonstrated, the role of postglacial colonization routes in determining genetic and phenotypic variation along gradients has received little attention. Here we focus on the roles of adaptive and neutral processes in shaping phenotypic and genetic variation in moor frog (*Rana arvalis*) populations along a 1700 km latitudinal gradient across northern Europe. This species has colonized Scandinavia via two routes with a contact zone in northern Sweden. By using genomic data from 15 populations, we found strong regional clustering and that most of the variation along the gradient occurred between the two colonizing lineages. Similarly, a common garden experiment showed little phenotypic divergence within the lineages, however, all phenotypic traits were strongly diverged between the southern and northern colonization routes, with higher growth and development rates and larger body size in the north. Interestingly, we found indications of temperature-dependent adaptive phenotypic divergence close to the contact zone between the two colonizing lineages and that the patterns of gene flow over the contact zone differed between neutral and putatively adaptive SNPs. Genetic variation was reduced in high latitude populations, and this was the case even in variation in two candidate genes linked with developmental rate (transcription factor *C/EBP-1*) and immune defense (MHC class II), suggesting that high-latitude populations may be more vulnerable to environmental change. These results indicate that lineage-specific variation may account for much of the adaptive divergence along a latitudinal gradient.

## Evolution of sex-chromosomes within species – a case study in the Australian Marbled Gecko

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Sexually reproducing organisms require both males and females for species survival. However, different species have evolved a variety of mechanisms to establish the sex of their offspring – e.g. genetically, via sex-specific genes or sex chromosomes, such as human XX females/XY males; or environmentally, such as incubation temperature. Geckos represent a model system for studying evolution of sex-determining mechanisms as both genetic- and temperature- driven systems occur. The Australian Marbled Gecko (*Christinus marmoratus*) determines sex genetically, but unlike mammals (and more like birds) the female has different sex chromosomes (ZZ males/ZW females). Chromosomal studies of *Christinus* and the Japanese gecko (*Gekko hokouensis*) indicate that though both species have a ZZ/ZW system, they are not homologous, and have evolved independently in each species. The independent evolution of sex chromosomes in geckos prompted us to investigate the homology of the *Christinus* ZW. We compared RADseq data from male and female eastern *C. marmoratus*, to detect differences between the genetic complements of each sex, allowing identification of sex-specific molecular markers (sequences found only on the female W). By sequencing and assembling a modest genome for *C. marmoratus* we could then match female-specific sequences to larger genomic scaffolds. Identification of genes on these scaffolds revealed homology of *Christinus* sex chromosomes with different chicken chromosomes to those of *G. hokouensis* whose sex chromosomes are homologous with the avian ZW. We confirmed these bioinformatic results using PCR. Further, these sex-specific PCR markers can be used to investigate conservation of this sex-determination system in other *Christinus* species.

**Skull variation within a clade of miniaturized snakes from Australia (Squamata: Typhlopidae: *Anilius*)**

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The Australo-Papuan genus *Anilius* contains 47 species of round-snouted snakes. To date, species identification has relied on scalation, however, head morphology is highly variable across species, hence we have attempted to quantify their skull variation. We selected five groups (*A. australis*, *A. grypus*, *A. leptosoma*, *A. longissimus*, and *A. obtusifrons*) to investigate skull morphology using microCT data (18 specimens, 19 3D landmarks). Principal Component Analysis revealed remarkable morphological variation. Along PC1, skulls change from relatively short and tall to elongated and depressed. We found further diagnostic traits in the skull morphology for an undescribed species morphologically similar in skull elongation to *A. longissimus*. Sutures changed across species, with variation in degree of fusion or separation of supratemporal and exoccipital, parietals, and nasals. Fusion on midline elements was absent or incomplete in the majority of species, and unpaired sutural bones appear in different positions, especially in *A. longissimus* and the new form. *Anilius grypus* exhibits high phenotypic variation, and our results suggest at least five morphologically distinct clusters, ranging from pointed- to blunt-snout. MicroCT data generates qualitative and quantitative data with high potential for species diagnosis, especially in small species, which are challenging to study under more invasive preparation methods. Particularly, in cases with morphologically cryptic complexes like the *A. grypus* group, microCT data could become highly important in future resolution of species diversity.



## Enteric bacteria in sleepy lizards vary in richness, prevalence and co-occurrence patterns

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Disease infection is one of the major costs of group living and animals are often exposed to multiple pathogens at the same time. While parasite and pathogen co-occurrence has been well studied, we have a poor understanding of commensal bacteria and their co-occurrence pattern in wild animals. The aims of this research are to describe the host-bacteria relationship of the sleepy lizard and investigate co-occurrence between bacterial strains. We hypothesized that bacterial occurrence varies over time and that some bacterial strains co-occur more frequently than expected, while other strains co-occur less frequently than expected within hosts. We considered positive associations (observed > randomized) in occurrence between two strains as facilitation, and negative association (observed < randomized) as avoidance/inhibition. The project builds on the well-studied sleepy lizard (*Tiliqua rugosa*) system, and their enteric bacteria. First, we compared the richness (number of bacterial strains per lizard) and prevalence (number of lizards carrying bacteria) between six sampling occasions, and found that richness and prevalence significantly increased over time, and with ambient temperature. Our two sites differed significantly in richness and prevalence but there was no difference between sexes. Second, we used a network approach to calculate the bacterial co-occurrence and whether co-occurrence happened more or less than expected. While most strain combinations did not differ from random, some strains showed a positive association (facilitation), and fewer showed a negative association (inhibition). Our findings could improve our understanding of bacterial dynamics within animals, and multi-infection disease networks.

## Life on the Edge - Green and Golden Bell Frogs at in a Coastal Lake

Lemckert F<sup>1</sup>

<sup>1</sup>Australian Museum, <sup>2</sup>Eco Logical Australia

Baseline monitoring was conducted for the Green and Golden Bell Frog (*Litoria aurea*) (GGBF) completing four replicate surveys of 30 transects around Meroo Lakes in southern NSW. Sites were typically swamplands on the margins of a large lake, but included some freshwater ponds. The location, sex and maturity of any GGBF detected were recorded along with a range of habitat variables including vegetation and water chemistry parameters. 183 GGBF were located, including 118 adults and 65 sub-adults, and 24 of the 30 monitoring sites were occupied. During dry conditions adults typically inhabited dense saw sedge (*Gahnia* spp.) away from the lake margins, but juveniles inhabited sedge and salt marsh around lake edges. After flooding rains adult frogs concentrated within the areas of sedge. The lakes were saline prior to rain (> 30 ppt), with frogs observed sitting within these waters. The central lake area remained saline after the rain (> 20 ppt), but the margins became fresh (< 2 ppt), where calling males concentrated. Fish were present in the lakes, but avoided areas of flooded sedge. One of 43 frogs sampled tested positive for the amphibian chytrid fungus. The population at Meroo appears to be robust and widespread and with few obvious threats. The presence of saline conditions may be the key to mitigating the impacts of the chytrid fungus in this population and large coastal lakes may be key to the long-term survival of this species.

## Permanent water body provides micro-refuge for frogs in a fire prone landscape

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Frog habitats consist of aquatic and terrestrial components, and connectivity between these is important to their ecological function. Vegetation structure of both is important, providing foraging resources and shelter. In the event of fire, both emergent aquatic and terrestrial vegetation can be altered structurally or eliminated. Although fire can destroy emergent and terrestrial vegetation in and around water bodies, the question arises as to how frogs persist in landscapes prone to fire. Are frogs eliminated from habitats and recolonise landscapes post fire or do they have mechanisms to survive fires and persist in the post-fire landscape? We investigated this question opportunistically post-fire in a landscape occupied by the endangered Green and Golden Bell Frog (GGBF), *Litoria aurea*. A fire event on Kooragang Island (Hunter Estuary), NSW, Australia occurred in January 2019, in a landscape containing one of the largest known populations of the GGBF. Post-fire surveys found a significantly increased number of individuals in a known populated permanent water body compared to counts of frogs in the same habitat from previous surveys. The data indicated GGBF persisted in the margins of the waterbody (despite extensive burning of that vegetation under conditions of an intense burn) but no live animals were found in the post-fire terrestrial landscape. These data suggest that this species persists in a fire-prone landscape consisting of a terrestrial and wetland mosaic after intense burns through the micro-refuge function of the wetland vegetation component.

### **Does mandating mitigation translocations result in conservation benefits?**

**Lennon O**<sup>1</sup>, Wittmer H<sup>1</sup>, Nelson N<sup>1</sup>

<sup>1</sup>*Victoria University Of Wellington*

Anthropogenic habitat loss and degradation adversely affect wildlife worldwide. One strategy employed to reduce these negative impacts is mitigation translocation, the movement of wildlife away from development sites. However, mitigation translocations generally aim to fulfill regulatory requirements rather than to provide conservation benefits. I investigated whether mitigation translocations can also be used to meet conservation goals. I collaborated with indigenous, private, and state stakeholders on a mitigation translocation of endemic lizards at a major road construction in New Zealand. Lizards were salvaged over several years, released, and the release sites monitored over two years. Low numbers of individuals salvaged and deviations from management plans hindered the pre-release phases of this project, leading to a released population with uncertain viability. Additionally, I revisited mitigation translocations of New Zealand lizards and took surveys of populations at the release sites to determine their success over the mid/long-term (7 – 14 years post-translocation). I found success rates were lower than previously estimated. Contracted ecologists are well aware of conservation best practices, but often lack a mandate to implement these in mitigation translocations, due in part to the differences between mitigation and conservation goals. Collaborative mitigation translocations represent a huge opportunity for conservation and restoration due to their increasing prevalence and the opportunity to access a different stream of funding. However, we need to ask the question: are mitigation translocations worth the current investment, or would it be better, when circumstances permit, to focus on supporting existing conservation priorities?

## Species-dependent amplification of Ranavirus prevalence and infection severity in multi-host Amphibian communities

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In recent years the effects of biodiversity loss on pathogen prevalence have garnered a great deal of attention, from zoonotic human pathogens to wildlife diseases. The concept of a dilution effect has received particular attention and several host-pathogen systems have shown a direct relationship between an increase in host diversity and a decrease in infection prevalence. Nonetheless, the opposite mechanism (viz. amplification effect) has also been observed, whereby higher host diversity amplifies the disease risk. It is thus clear that the specific processes by which pathogens are shaping host communities and vice-versa are still poorly understood. To address this issue, our study focused on the dynamics of the multi-host pathogen Ranavirus in low-diversity amphibian communities in north-western Canada. Among 16 localities with communities of up to three hosts (wood frog *Rana sylvatica*; boreal chorus frog *Pseudacris maculata*; Canadian toad *Anaxyrus hemiophrys*), we observed strong amplification effects on infection prevalence and severity, positively linked to the number of species in the respective amphibian community. Our study underlines the importance of assessing whole systems, instead of focusing on selected interactions, because the relationship and interactions between host biodiversity and pathogens are complex and often context-dependent.

**The bioaccumulation of contaminants across an urban gradient in a wetland snake (*Notechis scutatus*).**

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<sup>1</sup>Curtin University, <sup>2</sup>CNRS

Wetlands provide resources and habitat for biodiversity; this is especially important in urban areas. However, urbanisation degrades wetlands by modifying their structure, hydrology, and chemistry through pollution, which can result in the loss of fauna. The development of reliable long-term pollution bio-indicators is, therefore, crucial. The bioaccumulation of contaminants (mostly heavy metals) has been studied in several species of wetland snakes, but research is lacking from Australian species. The metropolitan development of the city of Perth, Western Australia, has resulted in the isolation of a once connected chain of wetlands. These wetlands now vary in degree of modification and pollution through urbanisation. We chose tiger snakes (*Notechis scutatus*) as the model species for bioaccumulation due to their preference for living in wetlands and predation on frogs, a taxa known to accumulate contaminants. We analysed the sediments and whole livers from tiger snakes of four wetlands across an urban gradient for 17 heavy metals/metalloids, 14 polycyclic aromatic hydrocarbons and 21 organochlorine pesticides. We also analysed the foetuses of pregnant snakes from three of the sites to evaluate the maternal transfer of contaminants. Sediment samples indicate Arsenic, Copper, Lead, Mercury, Selenium and Zinc at levels higher than trigger values recommended by the Australian and New Zealand Sediment Quality Guidelines, as well as 16 other contaminants close to trigger values. Small sample sizes and large intra-site variation advocate for caution in identifying what contaminants are present at levels of concern. Contaminant concentrations in adult snake livers and foetus are presented.

## Effects of experimental warming on hibernation behavior of amphibian populations

Li Y<sup>1</sup>

<sup>1</sup>*Institute Of Zoology, Chinese Academy Of Sciences*

Phenological shifts due to climate warming might lead to temporal mismatches in food webs, exacerbating species vulnerability and affecting allocation life-history trait trade-offs. Yet insights into these changes through experimental approaches are still scarce in amphibians. Here, under controlled warming conditions, we report a critical, but poorly studied, life-cycle stage (i.e., hibernation) in frogs inhabiting subtropical latitudes and the allocation strategy of reproduction and growth for adult anuran individuals involving a reproductive stage. Using outdoor mesocosm experiments, we examined the effects of temperature (ambient vs. + ~2.2/2.4 °C of pre-/post-hibernation warming) and food availability (normal vs. 1/3 food) on the date of entrance into/emergence from hibernation and the trade-offs of growth and reproduction in *Pelophylax nigromaculatus*. We found temperature was the major factor determining the hibernation period, which showed a significant shortening under experimental warming (6–8 days), with delays in autumn and advances in spring. Moreover, the timing of hibernation was not affected by food availability, whereas sex and, particularly, age were key factors in the species' phenological responses. Specifically, male individuals emerged from hibernation earlier, while older individuals also entered and emerged from hibernation earlier. Feeding rate was the major factor influencing reproductive status of females, clutch size, and variation of body size, showing significant positive correlations between feeding rate and reproductive status, clutch size, or variation of body size. Our results suggested that that climate warming have effects on the timing of amphibian hibernation and that amphibians could adjust their reproductive output to cope with climate warming.

## Genomic and transcriptomic insights into the molecular basis of sexually dimorphic spines in *Leptobrachium leishanense*

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Sexual dimorphism is important in sexual selection and species survival, yet the molecular basis of sexual dimorphism remains elusive, especially in amphibians, in which sexually dimorphic traits have evolved repeatedly. Here, we chose the Leishan moustache toad (*Leptobrachium leishanense*), a species from Megophryidae in which males typically develop nuptial spines on their maxillary skin, to elucidate the molecular basis for spine production using genomic sequencing and transcriptomic analyses. We assembled de novo a 3.5 Gb genome with a contig N50 of 1.93 Mb and scaffold N50 of 395 Mb. Recent bursts of increases in the copy number of transposable elements were observed, causing the accumulation of repetitive sequences in the *L. leishanense* genome. Comparative genomic analysis revealed significant expansion of intermediate filament gene families in *L. leishanense*, which include numerous keratin genes. Within these genes, a cluster of duplicated hair keratin genes exhibited male-biased and maxillary skin-specific expression patterns, suggesting that these genes play important roles in the development of keratinized nuptial spines in *L. leishanense*. Furthermore, a module of coexpressed genes, including multiple keratin genes and other candidate genes, such as the melanin synthesis-related tyrosinase (*tyr*) gene, was identified to be significantly associated with nuptial spines formation. Additionally, hormones such as androgen, thyroid hormone, prolactin and relaxin are also likely involved in the regulation of nuptial spines development. This study not only presents a new high-quality anuran genome but also provides a reference for further studies on skin-derived sexually dimorphic traits in amphibians.



## The diversity of Short-legged Toad (Anura: Megophryidae : *Megophrys*) in China

Li Y<sup>1</sup>

<sup>1</sup>*Sun Yat-sen University*

The Short-legged Toad *Brachytarsophrys* within the genus *Megophrys* widely distribute in southern and southeastern Asia. There are five valid *Brachytarsophrys* species, namely *Megophrys* (*Brachytarsophrys*) *canrinense*, *M. (B.) feae*, *M. (B.) chuannanensis* and *M. (B.) popei*, and a controversial species *M. (B.) platyparietus*. In this study, phylogenetic trees were constructed from abundant *Brachytarsophrys* specimens collected from western and southeastern China using concatenated DNA sequences of the mitochondrial COI and CYTB gene. We found that *M. (B.) platyparietus* represented a separated evolving lineage, and the specimens from southern Jiangxi Province clustered into a monophyletic branch with strong supports. Subsequently, combined with morphological comparison and advertisement call analysis, *M. (B.) platyparietus* and the specimens from southern Jiangxi Province can be distinguished from other known congeners respectively, so we resume the validity of *M. (B.) platyparietus* and describe the specimens from southern Jiangxi Province as a new species. Currently, the subgenus *Brachytarsophrys* contains seven species, six of which occurring in China.

**Does malaria mediate the reliability of testosterone and dewlap coloration of *Anolis sagrei*?**

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The costs of ornaments are assumed to be essential for maintaining the reliability of sexual signals and the underlying sex hormone from the exploitation of dishonest signalers. Therefore, the ecological heterogeneity of the costs should drive concomitant changes in these sexual traits. Here we used *Anolis sagrei* and the malaria parasite in central Florida, a system that the signal, hormone, and parasite are all well-studied, to test this cost-mediated reliability of ornament and hormone at the inter-population level. We examined the effect of malaria infection on the dewlap coloration, level of plasma testosterone, and the reliability of these two traits among 147 individuals across 7 populations. Comparing the uninfected populations, we found marginally higher testosterone level, redder and brighter dewlap center, and higher hue difference between the central and edge region of dewlap in the infected population. Moreover, the positive relationship between body condition and testosterone level only appeared in the population with malaria, indicating that testosterone was more reliable in the infected population. The redness of dewlap center also tended to be more honest in the infected population than in the malaria-free populations, implying the malaria influence on signal honesty as well. Despite the limit sample size, our study is one of the firsts to suggest parasite-mediated reliability of sexual ornament and sex hormone under natural context.

**Quantity discrimination of an Asian freshwater turtle (Geoemydidae: *Mauremys sinensis*)**

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<sup>1</sup>*School of Life Science, National Taiwan Normal University,* <sup>2</sup>*The Thinking Dog Animal Behaviour Service*

Quantitative ability plays a key role in foraging, defense, and microhabitat choosing. Diverse fish, birds, and mammals had been proved their numerical abilities; yet there exists a profound gap of this knowledge in reptiles. In the present study, we adopted the wooden cubelets (15 mm length, 15 mm width, and 10 mm height) painted with red acrylic color as the 3D stimuli to test the numerical abilities of an Asian geoemydid freshwater turtle, *Mauremys sinensis*. During the pre-training and training phase, subjects were rewarded to recognize three cubelets on a randomly chosen white board, rather than one cubelet on the other side. After a total of 120 pre-training and training trials, they underwent sequential series of tests according to Weber's law, which considers the increased difficulty as the ratio between the two quantities increased: 1 vs. 3 (0.33), 2 vs. 4 (0.50), 3 vs. 4 (0.75), 4 vs. 5 (0.80), and 6 vs. 7 (0.86). Our results revealed that five among the six turtles in this study passed the tests of 4 vs. 5, and four turtles passed 6 vs. 7 ( $p < 0.05$  in all these cases). This is one of the first cases using training procedure to prove that the freshwater turtles can discriminate up to seven.

## **Challenges, desperation and hope: a review of herpetofauna conservation translocations**

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<sup>1</sup>*Smithsonian Conservation Biology Institute & National Zoological Park*, <sup>2</sup>*Department of Conservation | Te Papa Atawhai*

Herpetofauna reintroductions and other conservation translocations are often extremely challenging, multi-disciplinary, and have limited chance of success. However, the necessity to implement conservation translocations shows no signs of abating. Although reptile and amphibian translocations are still largely experimental, dozens of countries now host active herpetofauna translocation initiatives involving numerous species. The vast diversity within amphibians and reptiles, makes generalisations very difficult, but many common themes exist in regard to conservation translocations. We will review the current trends in herpetofauna conservation translocations, highlight examples of state of the science research, and the recent development of translocation guidelines. We will conclude by emphasizing current and future challenges that should be prioritized to continue developing a robust set of tools and strategies to successfully implement herpetofauna translocations.

**The potential for artificial selection in a reintroduction program: exploring individual variation in chytridiomycosis susceptibility**

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<sup>1</sup>*Smithsonian Conservation Biology Institute*, <sup>2</sup>*Smithsonian Tropical Research Institute*

The Panama Amphibian Rescue and Conservation Project has established captive populations of twelve highly threatened amphibian species that experienced catastrophic declines because of the amphibian chytrid fungus. Understanding how much variation in susceptibility to chytridiomycosis exists between individuals is useful to understand the potential for adaptation and in mechanistic studies of disease tolerance. Assessing variation of disease susceptibility has typically proved difficult without lethal disease exposure trials. Thus, understanding susceptibility in highly threatened species is problematic due to the rarity and conservation value the animals. Our study first utilizes a small number of surplus captive-bred offspring (N=20) in experimental live-pathogen exposure trials paired with a newly developed non-invasive assay of mucosome effectiveness to inhibit the chytrid fungus to correlate predicted to observed disease susceptibility. We then non-invasively profile the mucosome effectiveness of hundreds of frogs (N >400) within the captive breeding program. We are currently working to rank our captive species by disease susceptibility and investigate individual variation of mucosome effectiveness throughout the populations. Additionally, we are exploring whether observed differences in skin secretion effectiveness are transferred from parent to offspring by comparing effectiveness within known pedigrees of captive-bred animals. By identifying individual variation in skin secretion effectiveness, and demonstrating if these traits can be inherited, it may provide a theoretical framework to selectively-bred lines of frogs with greater disease tolerance. Our results may have broad conservation implications for managing captive amphibian populations.

## Evaluation of a camera trapping system for detecting Eastern Massasauga Rattlesnakes

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<sup>1</sup>*Ohio State University*

The Eastern Massasauga Rattlesnake (*Sistrurus catenatus*; hereafter, EMR) is a federally threatened pit viper native to the Midwest of the United States. The loss of wet prairie habitat has been a principle driver of declines, and wildlife agencies routinely require areas to be surveyed prior to development projects. Surveys for this cryptic species can be time consuming and costly. The goal of our project is to test the feasibility and efficiency of using camera traps along with drift fences (the Adapted Hunt Drift Fence Technique; "AHDriFT") to detect the presence of the EMR. The method uses inverted-bucket camera traps systems, specially designed to capture species-quality images of herpetofauna and small mammals, placed at the ends of drift fences. Original development in Florida was successful in capturing species diversity while dramatically lowering person-hours compared to traditional surveying. Our objectives include: (1) identifying technical issues with applying AHDriFT to wet prairie habitats and testing necessary modifications; (2) comparing the number and frequency of EMR images captured to the encounter rate of previous surveys and baseline population data; (3) determining the covariates and effort related to detection of EMR using AHDriFT to the desired confidence of absence; and, (4) creating a cost-efficiency matrix comparing AHDriFT to the accepted survey protocol. We set 15 omni-directional AHDriFT arrays comprising 45 camera traps across 3 counties in northern Ohio where we have 3 years of robust EMR population data. Results and lessons from our first field season using the system will be discussed.

**The necessity of long-term data for quantification of recovery of long-lived species of conservation concern**

Litzgus J<sup>1</sup>, Keevil M<sup>1</sup>, Brooks R<sup>2</sup>

<sup>1</sup>Laurentian University, <sup>2</sup>University of Guelph

We have been studying the population ecology of Snapping Turtles (*Chelydra serpentina*) in Algonquin Park, Canada for over 45 years. Snapping Turtles display life history traits consistent with a bet-hedging strategy, including late maturity, extreme longevity, low nest success, and dependence upon high adult survivorship and iteroparity for population persistence. In the 1980s, the population suffered an acute mortality event when otters predated over 40% of adults during hibernation. A study shortly after the mortality event predicted weak density-dependent compensation and slow recovery. Subsequently, we estimated abundance and survival of nesting females before, during, and 23 years following the mortality event. We found limited evidence of recovery over more than two decades despite high post-catastrophe survivorship and connectivity with other populations. Species with “slow” life histories, like turtles, are expected to recover slowly from such catastrophes because of their long generation times. In Canada, Snapping Turtles are listed as a species of Special Concern both provincially and federally, yet in other jurisdictions of their range (eg, within the USA) they are subjected to largely unmonitored legal harvests. Managers need to recognize that recovery of long-lived species is on a much greater time scale than most ‘game’ species, and long-term data are essential to inform policy decisions.

## Climate extremes, variability and trade shape biogeographical patterns of herpetological fauna in the Anthropocene

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Anthropogenic dispersal of alien species is breaking down traditional, native biogeographical boundaries, allowing species to occupy more potential climatic niches in their invaded ranges. Climate has therefore been suggested as playing an essential role in explaining invasion-driven reorganization of biogeography. However, what remains unclear is which climatic parameters most account for invasion-redefined biogeography, which is crucial for predicting future biogeographical patterns in response to accelerated rates of biological invasions and climate change. Here, based on global distribution data of 361 alien amphibians and reptiles in 271 native and 297 current nations and sub-nations, we explored the effects of climate average, variability and extremes on the change of global biogeographical patterns by controlling for natural and anthropogenic dispersal of alien species. We found that global herpetological diversity has been homogenized, going from 10 to four groups after modern-day invasions. Geographic distance, a proxy for natural dispersal barriers, was the dominant variable contributing to native biogeographical patterns. In contrast, bilateral trade, and extreme high temperature and precipitation seasonality, which are two hallmarks of global climate change, explained more unique variation in biogeographical patterns after invasions. Our results thus imply that human-assisted species introductions may combine with climate change to accelerate the reorganization of global biogeographical patterns in the Anthropocene. Incorporating climate extreme and climate variability data may help predict range shifts of both native and invasive species and associated biogeographical patterns in response to climate change.



## Can tuatara offset a male-biased sex ratio with behavioural changes?

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Sex ratio is a critical parameter in the population dynamics and evolutionary ecology of a species. Tuatara (*Sphenodon punctatus*) are particularly at risk of an extreme male-biased sex ratio under global warming due to their rare pattern of temperature-dependent sex determination (TSD), where eggs develop as males at higher temperatures. We investigated sex ratio in a small isolated population of tuatara on North Brother Island, where there has been an increasing male bias documented from the late 1990s until 2012, leading to greater risk of population extinction. However, the potential for fluctuations in sex ratio over longer time scales is poorly understood, and predictions from nesting surveys in 2002-2005 on a nearby island predicted a female bias in new hatchlings due to earlier nesting after warmer winters, which may counter a male bias in tuatara populations. Recent population surveys on tuatara of North Brother Island in 2016 and 2018 now allow analyses of sex ratio over three decades. Using mark-recapture methods, we found more balanced sex ratios after 2012. Previously they had risen to as high as 3.06M : 1F in 2010, but fell back to 1.71M : 1F and 1.96M : 1F in 2016 and 2018 respectively. New cohorts appearing in the recent capture data suggest that female-biased sex ratios may be occurring in more recent years of hatchlings. Long-term monitoring is needed to understand the potential for sex ratio variation in tuatara to balance over time, and the effect of such fluctuations on population parameters and viability.

## **Large-scale monitoring of breeding phenology in tropical and temperate anurans: insights for climate change research**

**Llusia D<sup>1</sup>**

*<sup>1</sup>Universidad Autónoma de Madrid (UAM), <sup>2</sup>Universidade Federal de Goiás (UFG)*

Most anurans signal during breeding, providing a useful (and “loud”) source of information to study amphibian phenology. Emerging technologies in sound recording and signal processing are today improving our chances to exploit this trait by enabling automated monitoring of breeding at large temporal and spatial scales. In this talk, I will present research that applies passive acoustic monitoring and machine learning to examine phenological responses of tropical and temperate anurans to changing climatic environments. First, factors determining inter- and intraspecific variation in breeding phenology were investigated based on comparative analyses between populations located at the climatic extremes of the species distribution ranges. Second, climatic breadth of calling behaviour was estimated and used in predictive models to forecast shifts in breeding phenology under climate change scenarios. Our findings provide insights into the capacity of anuran species to cope with climate change and the mechanisms underlying species responses. Potential consequences of the observed and predicted shifts in phenology will be discussed in light of our current knowledge about the key role of acoustic communication in amphibian breeding.

## The biology of the Microhylid subfamily Hoplophrynine

### Loader S<sup>1</sup>

<sup>1</sup>*Natural History Museum*

Microhylids are one of the largest family of Anura found across the world and currently divided into thirteen subfamilies. The subfamily Hoplophrynine is a small group restricted to the mountains of Tanzania, currently including two genera and three species. Hoplophrynine is of particular interest as this clade forms a grouping to all other microhylids - providing a potential window on ancestral attributes of microhylids. Opinions of different authors on the classification and phylogenetic position of the subfamily are summarized. I will go onto outline the current knowledge of this group, highlighting several features of note on their diversity, reproductive biology and biogeography. The future priorities of Hoplophrynine research and conservation are outlined.

**The genetic basis of anole dewlap color, a diverse trait in an adaptive radiation.**

**Longo Hollanda de Mello P<sup>1</sup>**, Hime P<sup>1</sup>, Glor R<sup>1</sup>

<sup>1</sup>*University of Kansas*

Pigmentation plays a crucial role in animal communication. In reptiles, for example, pigmentation is used in the identification of potential mates and competitors. However, despite its evolutionary importance, little is known about the genetic architecture of reptilian pigmentation. The goal of this study was to use transcriptomic data from skin samples with different coloration to identify candidate genes for reptilian pigmentation. We first used RNA-seq data to assemble and annotate the skin transcriptome of *Anolis distichus*. *Anolis distichus* dewlap coloration correlates with its surrounding environment: orange dewlaps are found in more mesic, and yellow dewlaps in more xeric environments. Furthermore, liquid chromatography of anole dewlaps has shown that shades of orange and yellow are respectively determined by pteridines and carotenoids. We used RNA expression data to test the hypotheses that : (i) dewlap skin has a set of differentially expressed genes relative to belly skin; and (ii) orange and yellow dewlaps are respectively enriched for pteridine and carotenoid pathway genes. We assembled a skin transcriptome for *A. distichus* that included 93.15% of benchmark Busco genes, and a E90N50 of 2,829 bp. Corroborating our first hypothesis, we identified 203 differentially expressed genes between belly and dewlap tissues. Next, gene set enrichment analyses corroborated the hypothesis that pteridine and carotenoid pathway genes are up-regulated in orange and yellow dewlaps, respectively. Our data updates the list of candidate pigmentation genes in reptiles, and corroborates results that suggested that pteridine and carotenoid pathway genes are responsible for orange and yellow coloration, respectively, in reptiles.

## Roles of isolation, secondary contact, and ecology in diversification and speciation of a temperate frog

Lougheed S<sup>1</sup>

<sup>1</sup>*Queen's University*

The best natural systems for studying speciation consist of replicate lineage pairs of varying ages that form secondary contact zones. These systems allow us to examine the same lineages in geographical isolation and in contact, and thus quantify the timing and importance of selective and genetic forces in the emergence of distinct species. The spring peeper, *Pseudacris crucifer*, fits these criteria well. It is a small, spring-breeding treefrog that occupies a massive range from the US Gulf Coast to James Bay, Canada. We combine phylo- and population genomics, morphometrics, sonographic analysis, and behavioral studies to understand evolution in this species. Our work reveals a deep and dynamic evolutionary history with 6 deeply-diverged mitochondrial lineages, distributed within 3 well-delineated nuclear lineages that originated in ancient refugia, clear zones of secondary contact, with some discordance among genomes. While there is marked diversity in male advertisement calls, controlling for temperature and body size, we find only a Western lineage to be differentiated. We do find divergence in male calling behaviour with southern males calling disproportionately from elevated perches, and northern males from ground level, an observation that we posit is caused by differences in trade-off between call transmission and desiccation pressures. We find varying outcomes of secondary contact with some exhibiting broad zones of introgression and cytonuclear discordance, and others, narrow clines in mtDNA, nuDNA, and call rate. Our work reveals the complex interplay among historical isolation, range expansion and contact, and selection in variable environments in shaping evolutionary trajectories and speciation.

**Effects of larval ultraviolet-B exposure regime on physiology, performance and condition of *Litoria caerulea***

**Lundsgaard N**<sup>1</sup>, Cramp R<sup>1</sup>, Franklin C<sup>1</sup>

<sup>1</sup>*The University Of Queensland*

Laboratory and field studies have demonstrated the lethal effects of ambient solar ultraviolet-B radiation (UVBR) in many amphibian species. However, researchers are still debating how UVBR affects amphibians at the population scale because of the difficulty of estimating UVBR exposures in complex natural systems. In particular, it is unclear whether it is the overall dose, or the intensity of UVBR exposure that determine the extent and magnitude of its physiological effect in amphibians. Furthermore, climatic changes are increasing the frequency and intensity of short-term peak UVBR events in some regions, yet there is little research on how such acute, high intensity UVBR exposures might affect long-term amphibian health. To address these issues, we acutely exposed tadpoles of the Australian green tree frog (*Litoria caerulea*) to a combination of different UVBR intensities and doses in a fully factorial laboratory experiment, and assessed a suite of physiological and performance traits, including carryover effects in resulting metamorphs. Our results show the relative significance of intensity versus cumulative dose of UVBR exposure on amphibian physiology and condition, as well as some of the mechanistic underpinnings of these whole animal responses. Our findings clarify the effects of different characteristics of UVBR exposure on amphibian physiology and performance, helping to lay the foundations for understanding how complex UVBR exposure regimes in the field could contribute to ongoing global amphibian declines.

**Sex or Candy? Neuroendocrine mechanisms of seasonal life-history transitions in garter snakes.**

**Lutterschmidt D<sup>1,2</sup>**, LeMaster M<sup>3</sup>

<sup>1</sup>University of California Irvine, <sup>2</sup>Portland State University, <sup>3</sup>Western Oregon University

Many vertebrates exhibit distinct life-history stages that are associated with specific physiological and behavioral functions, such as reproduction, migration, and foraging. In seasonal organisms, resource availability relegates these life-history stages to a specific time of year, and therefore seasonal transitions between life-history stages are often accompanied by dramatic changes in both physiology and behavior. Red-sided garter snakes (*Thamnophis sirtalis*) are an exceptional model for understanding the mechanisms mediating life-history transitions. We previously found that adrenal glucocorticoid “stress” hormones play a central role in the seasonal transition from reproduction to migration and foraging. For example, males have decreased sensitivity to capture stress during mating. Further, plasma glucocorticoids are elevated during mating in both sexes and decline as snakes begin to migrate away from the breeding grounds in search of food. Experimentally decreasing glucocorticoid signaling with a synthesis inhibitor or glucocorticoid receptor antagonist prematurely induces the behavioral switch to foraging, as males choose to pursue feeding cues over mating opportunities in two-choice Y-maze trials. However, increasing the duration of elevated glucocorticoids with hormone implants does not extend the duration of courtship behavior, nor can the switch to feeding be reversed once the seasonal transition has been made. These results indicate that once glucocorticoids decline to some threshold level, it alters the fundamental nature of how glucocorticoids interact with the brain to mediate behavioral switching. Collectively, these data provide a framework for understanding the role of glucocorticoid “stress” hormones in seasonal life-history transitions and the mechanisms by which hormone signals reconfigure regulatory systems.

## Latest news of environmental DNA metabarcoding approach for monitoring amphibian species in tropical forests.

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Declines and extinctions are increasing globally and challenging conservationists to keep pace with biodiversity monitoring. The analysis of DNA traces left by the organisms in the environment (eDNA) is a highly sensitive method with the potential to rapidly assess local diversity and the status of threatened species. In the last 6 years we have applied the eDNA metabarcoding to: 1) test the feasibility of this approach for surveying water and ground dwelling amphibians in Brazilian forests; 2) compare the performance of traditional and eDNA methods for species inventories; 3) search for DNA traces of 42 amphibian species that are reported to be declined or completely disappeared from the Brazilian Atlantic forest; and 4) identify the possible causes for these disappearances. We amplified the 12S rRNA of amphibians from water (rivers, streams, rocky seeps, puddles, ponds, swamps and bromeliads) and leaf litter samples. Our results underscore the utility of eDNA metabarcoding as an efficient approach for surveying amphibian species associated to tropical waterbodies. However, it was not supported as a powerful approach for surveying ground dwelling amphibians using leaf litter samples. We successfully detected DNA traces of four declined species (*Hylodes ornatus*, *Hylodes regius*, *Crossodactylus timbuhy*, and *Vitreorana eurygnatha*); two locally disappeared (*Phasmahyla exilis* and *Phasmahyla guttata*); and two species that have not been seen since 1982 (*Cycloramphus cedrensis*) and 1968 (*Megaelosia bocainensis*). We confirm the presence of species undetected by traditional methods, underscoring the efficacy of eDNA metabarcoding at low population densities and its potential application in conservation biology.



## Integrating Spatial and Temporal Studies to Model Salamander Demographic Responses to Climate

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In the face of shifting climates, management for biodiversity and associated ecological services requires mechanistic models of species' distributions and abundances. Such models require integration of long-term and spatially extensive demographic studies, and are, therefore, relatively uncommon for animals. Here we demonstrate the integration of a long-term robust capture-recapture study with a spatially extensive "unmarked" study to estimate the sensitivity of salamander vital rates and abundance to weather and climate, and to project salamander population growth across a portion of western North Carolina where rapid development may conflict with the capacity to support salamander population growth under future climate scenarios.

We studied salamanders in the genus *Plethodon*, which are abundant and ecologically influential in forested ecosystems of North America. We found that 28-day survival estimates varied positively through time with mean daily precipitation, and that sensitivity of survival to precipitation declined with increasing body size. Reproductive rate and abundance increased spatially with increasing mean annual precipitation, such that abundant populations in wetter areas had a higher proportion of juveniles compared to less abundant populations in drier areas. Drier habitats had estimates of mean instantaneous population growth  $< 1$ , suggesting those habitats may be population sinks sustained by episodic wet years with high recruitment or subsidized by small source habitats such as coves. Projections of population growth rate indicate two regions of high population growth, one of which is undergoing rapid development. The other region resides within the Nantahala National Forest where it is better protected against future land conversion

**Role of research reintroductions in successful habitat restoration of threatened amphibians.**

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Habitat restoration and reintroductions for threatened amphibians have proven to be challenging. To a great extent that challenge is related to the role of the amphibian disease chytridiomycosis. Our experience is that a basic tenet of reintroduction science, which is that the threat should be identified and mitigated prior to reintroduction, is not poorly understood when chytrid is the cause of population loss. There are exceptions, where the reintroduction and restoration are conducted as an experimental translocation with the objective of more precisely understanding how the threatening process operates in the environment, and with experimental treatments to mitigate its effect. We describe an eight-year program of habitat restoration for the threatened green and golden bell frog *Litoria aurea*. The first four years involved an enclosed (predator free) and open research plot of ten paired wetlands to which we introduced the frog. After assessing survival rates across the life stages and the role of physical habitat variables along with a designed capacity to alter hydrology, and salinity levels to dampen the impact of chytrid, we embarked on constructing a landscape restoration of eleven wetlands in a 60-hectare plot. Specific design for hydrology and salinity have seen a year on year growth of the population at the site, with confirmed breeding in 9 of the wetlands. The construction of a large wetland mosaic (mix of ephemeral to permanent wetlands), exclusion of fish predators, and a salinity influence are identified as the mitigation factors that have enabled success.

## **Novel, cost efficient and minimally destructive methodology for sequencing mitochondrial genomes of historical museum specimens**

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Natural history specimens form the basis of most taxonomic research, which informs a wide variety of other fields in the biological sciences from systematics, ecology and natural history to conservation, biogeography and disease epidemiology. Historical specimens form a large portion of museum collections, but due to the typically highly degraded DNA in old specimens, a lack of ancient DNA laboratory facilities in most museums, and the comparatively high cost of archival DNA sequencing methods currently utilised, historical specimens are often omitted from molecular based analytical research.

Here we describe a novel, minimally destructive method to extract and sequence mitochondrial DNA for a fraction of the cost of methods that have been used previously, and without the need for specialised ancient DNA laboratory facilities. We included 48 historical Asian frog and lizard specimens from London's Natural History Museum (including type specimens) collected between 1845 and 1952 in a single Illumina sequencing run. Our method had a 66% success rate for sequencing mitochondrial DNA ranging from short gene fragments to almost entire mitogenomes. Our results allowed us to fix erroneous type localities, identify new species and significantly extend the geographic distributions of others.

Beyond resolving complicated taxonomic issues, our method could potentially be applied to address many other exciting questions, including resolving systematic associations of recently extinct species, population genetics and (recent) historical biogeography, temporal loss and gain of genetic diversity through population bottlenecks and hybridisation events.

## **Preliminary analysis on network-scale effects of invasive species on spatially-structured amphibian populations**

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Understanding the factors affecting the dynamics of spatially-structured populations (SSP) is a central topic of landscape ecology and conservation. Invasive alien species are increasingly important drivers of the dynamics of native amphibians. Here we used long-term abundance data to test whether the impact of invasive crayfish on sub-populations can also affect the whole SSP dynamics, through their influence on source populations.

From 2010 to 2018, we surveyed a network of 58 ponds in Lombardy (NW Italy). We recorded the number of the Italian agile frog egg clutches, the occurrence of an invasive American crayfish, and multiple environmental features. Using spatially-explicit Bayesian mixed models, we assessed the relationships between frog abundance in ponds and a) environmental features; b) features of both sub- and spatially-structured populations; c) occurrence of invasive species at both the patch- and the SSP-levels.

If spatial relationships between nearby ponds were not considered, we did not detect clear effects of crayfish presence on frog abundance or on their temporal trends. When we jointly considered habitat, sub-populations, and SSP features, we found a strong negative relationship between frog abundance in a given site and crayfish frequency in the nearby wetlands during previous years.

Our multi-scale analyses revealed that considering SSP processes can identify effects that would remain unnoticed when focussing on single patches. Invasive species can affect population dynamics even in not invaded patches, through the degradation of the sub-population network.

## Which shade of yellow? A preliminary study of dorsal pattern determinants in aposematic salamanders

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One of the most interesting features of aposematic European salamandrids is represented by their dorsal pattern. The coloration is due to the different disposition of the chromatophores that characterize the epidermic cells. In fire salamanders the typical yellow and black aposematic pattern is particularly evident. The aim of this study is to evaluate the variability of *S. salamandra*'s dorsal pattern between populations belonging to different altitudes and habitats with different availability of trophic resources. In particular we studied both adults and metamorphosed from 40 populations living in different piedmont, hilly and mountains areas of the Italian Prealps. For each population we both collected dorsal pictures of adults during multiple night surveys and we collected 10 larvae from the main breeding site. Half larvae were reared at "ad libitum" conditions and half were reared at poor nutritional conditions; after 45 days from metamorphosis we collected pictures of their dorsal pattern. For each population we also estimated the maximum density of available earthworms. From the pictures we extracted all the yellow pixels and we measured HSV mean values of each individual. For both juveniles and adults, the results showed a strong correlation between dorsal pattern coloration and trophic resources. On the contrary we did not detect any effect of the population origin in determining HSV yellow features. Our findings suggest that yellow pattern coloration of fire salamander is a relatively plastic and costly feature, strongly affected by the trophic resources available during both adult and larval stages.

**Saving cows, saving frogs: if you love amphibians, you should care for farm animals too**

**Fonte L**

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Frogs and Cows. Toads and Pigs. Salamanders and Chickens. This project aims to raise awareness of the negative impact that animal agriculture has on the survival of amphibians. There is compelling evidence to suggest that meat, dairy and egg production is one of the main drivers of climate change, habitat destruction and pollution of terrestrial and aquatic ecosystems. According to the IUCN Red List, over 40% of amphibian species are threatened with extinction. Of the species assessed, 88% are threatened by the conversion of its natural habitats to animal agricultural use. Around 25% are directly threatened by animal farming and almost 50% by food crops. Animal agriculture is responsible for 18% of greenhouse gas emissions. Worldwide, at least 50% of grains are produced to feed animals, and the use of pesticides poisons water bodies where amphibians live and reproduce. Natural habitats are destroyed to convert land to grow food crops and for animal grazing, with more than 70% of Amazon's destruction being directly attributed to animal agriculture. We can no longer deny that our eating habits are having a profoundly adverse effect on our planet. If we want to save amphibians, we must care about farm animals too. Reducing meat, dairy and egg consumption is something that everyone is capable of doing. People don't have to be scientists or conservationists to contribute to saving wildlife. By doing so, we would give not only amphibians but the natural world the best possible chance of recovering and thriving once more.

## Using frog genomes in evolutionary genomics: Understanding the genetics and evolution of poison-dart frog coloration

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With improvements in sequencing technology and assembly methods, the number of amphibian genome assemblies available continues to increase. This wealth of new assemblies is opening the door for population-level studies of genomic variation. However, given their size and high repetitive content, amphibian genomes pose challenges for such studies. We discuss our successes and failures during ongoing work to understand the genetic basis and evolutionary history of color pattern in *Phylllobates* poison-dart frogs. Aposematic coloration has evolved dynamically in this group's recent history, with three independent lineages exhibiting remarkably similar color patterns, which arise from nearly identical ontogenies. Integrating phylogenetic, population genetic, genetic association, and gene expression analyses we study *Phylllobates*'s historical demography and biogeography, and identify loci associated with color pattern variation both between and within populations. Comparing the histories of coloration-associated loci with genome-wide patterns of divergence and gene flow, we aim to understand the genetic and evolutionary processes driving the evolution of this trait. To this end, we have used a wide variety of sequencing strategies and analytical approaches, from exon enrichment and RNAseq to whole-genome re sequencing. We will focus on the challenges encountered when using these approaches based on a fragmented de-novo assembly similar to other publicly available frog assemblies, and discuss possible ways to circumvent these challenges.

**Crossroads or tipping points: does thermal melanism hypothesis hold across lizards of different body size?**

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Animals living in cold environments require increased heat absorption to maintain optimal body temperature and as low reflectance increases the rate of heat intake and equilibrium temperature, species with lower reflectance are predicted to inhabit colder regions. This thermal melanism hypothesis has been tested several times, but in lizards, just a handful of studies incorporate reflectance for both the near infrared and visible parts of the solar spectrum. Body size also influences heat exchange via thermal inertia and convective effects due to variation in surface-area ratios. Norris (1967) found that in desert lizard species, small lizard reflectance dropped sharply at the end of the visible while larger lizards had higher near-infrared reflectance. In addition, variation in size and skin colour can be driven by mating, competition and background colour matching. Therefore, body size and reflectance may interact due to heat fluxes but also depending on the life style and the environment inhabited by different species. Here, we explore these interactions using Cordylid lizards, a group that is widely distributed in Southern Africa, encompassing a large variety of climates and body sizes. We first tested if the reflectance of the integument is related to several climate variables (solar radiation, microsite temperature mean and variance, humidity and wind speed). Then we tested if these climate-reflectance relationships differ between large and small species and finally, if reflectance in the NIR responds to climate independently from the visible. These results contribute to understanding adaptive responses and phenotypic trade-offs of lizards to climate variation.



## Habitat assessment for the reintroduction of the agile frog in a human-dominated landscape

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Anthropogenic habitat fragmentation and pollution has depleted populations of the agile frog *Rana dalmatina* on the Channel Island of Jersey such that only one breeding site remains. This last remaining site is in the south-west of the island at L'Ouaisné Common; a popular local and tourist bay surrounded by large privately-owned residential properties and estates. An existing corridor links the site to previously-known habitat of the agile frog at nearby Noirmont where reintroduction has been undertaken following a headstarting programme. Habitat suitability assessments were used to evaluate ponds, terrestrial habitat and connectivity to determine suitable locations for agile frog reintroduction at a number of residential properties within the vicinity of Ouaisné. Fifteen ponds at ten properties were assessed, of which ponds at four properties were identified as immediately suitable recipients of frogspawn from the headstarting programme. Owners of properties were generally positive about encouraging agile frogs within their estates. Improvements were proposed to enhance the suitability of all ponds and properties evaluated. Connectivity potential with Noirmont was also assessed with recommendations for terrestrial habitat alteration and the establishment of intermediate populations made. Barriers to migration were identified and recommendations made for mitigation and community involvement. The project also highlighted potential causes of recent hydrological changes at Ouaisné to help guide future planning policy.

## Foraging strategy of *Dinodon semicarinatum* feeding on sea turtle hatchlings and eggs on Okinawa Island

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Foraging strategy of predators includes two major tactics: active searching and ambushing. Generally, snakes utilize a species-specific single foraging tactic, but some species exhibit both tactics. *Dinodon semicarinatum*, a colubrid snake endemic to Ryukyu Archipelago, Japan, has been reported to utilize both foraging tactics alternately when it forages on sea turtle hatchlings and eggs on the sandy beach on Kerama islands. To understand why this snake shows such a unique foraging and how snakes, in general, select foraging tactics, we studied foraging behavior of *D. semicarinatum* on sea turtles on different island of Ryukyu Archipelago. During 2016 to 2018, we recorded a total of 373 bouts of foraging behavior of *D. semicarinatum* by fixed cameras set on the beach on Okinawa Island. In all cases snakes exhibited active foraging, and they never utilized ambush tactic to capture sea turtle hatchlings. The success rate of foraging depended on the developmental stage of sea turtle nests, but the duration when the snakes spent for foraging attempt on the nest were consistently approximately five minutes at any stage when they failed to find turtles. The search time was also approximately five minutes irrespective of snake body size. We presume that the different foraging strategy among the populations is attributed to the different condition of sandy beach, such as the difference in the difficulty to dig into the nest. We speculate that the search time, approximately five minutes, is optimal staying duration for the generalist snake searches various prey besides sea turtles.

### **Crypsis or camaraderie: unlocking signals in the colour pattern polymorphism of an Australian lizard**

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The adaptive function of animal colour is mediated by the interaction between an animal and its environment. These functions are broad in scope, encompassing physiology (thermoregulation), intraspecific (mate recognition, attractiveness and quality), and interspecific (aposematism, crypsis and mimicry) interactions. Often these functions are not mutually exclusive, and colour may evolve as a trade-off between various selection pressures. Colour polymorphisms represent multiple synchronous strategies within a species in response to imposed trade-offs or selection pressures, and therefore can be used to study ecological mechanisms by which such variation is maintained over time and across populations. *Lampropholis delicata*, a small Australian skink, demonstrates a colour pattern polymorphism with a striped and non-striped morph. The striped variant is expressed at higher frequencies at lower latitudes, and in females, suggesting that selection is both sex- and population-specific. We examined the visual properties of this polymorphism in a two-tiered approach. First, we determined the ability of both avian predators and skinks to distinguish each morph type against its natural background, to determine which group might perceive the signal and to what degree. Second, we observed the colour and type of backgrounds that skinks actively choose, to explore how the morphs select their environment based on their colour, both in a field and lab environment. This study draws together ecological aspects of balancing selection and behaviour, and identifies the signalling properties of a rarely-studied colour pattern polymorphism.

**Sex is determined by XX/XY sex chromosomes in Australasian side-necked turtles (Testudines: Chelidae).**

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Turtles demonstrate a remarkable variability in sex determination and constitute an excellent model for the evolution of sex chromosomes. Notably, the cytogenetic traits as well as sex determination of turtles of the family Chelidae are still poorly explored. This family includes more than 50 species of freshwater turtles distributed in South America, Australia, New Guinea and Indonesia in two major lineages: the South American and the Australasian. We prepared chromosome suspensions from representatives of the Australasian genera *Chelodina*, *Emydura* and *Elseya*. We performed both classical and molecular cytogenetic analyses, such as comparative genome hybridization (CGH) and fluorescence in situ hybridization (FISH) with probes specific for rDNA genes, (GATA)8 microsatellite motif and telomeric-like sequences. Such repetitive elements often accumulated on differentiated sex chromosomes of vertebrates. We were able to identify XX/XY sex chromosomes in all studied species. Surprisingly, the turtles from the genera *Chelodina* have different morphology of sex chromosomes than *Emydura* and *Elseya*. In the genus *Chelodina*, the sex chromosomes are represented by cryptic microchromosomes, while in the genera *Emydura* and *Elseya*, the sex chromosomes are medium-sized. In both clades, the Y chromosomes contain a male-specific region characterized by accumulation of repetitive elements, detectable by both FISH and CGH, crucial for the identification of *Chelodina* sex chromosomes. Despite that the differences in morphology might indicate a non-homology of sex chromosomes between these two groups, we can conclude that male heterogamety was likely already present in the common ancestor of the genera *Chelodina*, *Emydura* and *Elseya*.

## Evaluating long-term population age structure changes of a critically-endangered frog

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Understanding the impacts of threats on populations is critical for designing prudent management strategies for declining species. Unravelling the impacts of chytrid fungus (*Batrachochytrium dendrobatidis*; *Bd*) on amphibian populations is particularly important given the fungal pathogen is currently linked to around 500 species declines globally. Recent studies examining the age structure of amphibian populations have provided insight into species short-term population responses to *Bd* infection. Whilst around 90 species extinctions are now linked to *Bd* infection, some species populations persist despite infection and other populations may even be recovering. Unfortunately, additional threats including invasive predators, also influence many amphibian populations. Gaining insight into how populations cope with chytrid and other threats over the long-term is essential for their recovery. A long-term (~27 year) mark-recapture program for the threatened spotted tree frog provided an opportunity to examine longer-term amphibian population age structure changes for two populations exposed to *Bd* and non-native predatory fish. In these populations, the non-native fish limit recruitment by predated upon the tadpoles and *Bd* reduces adult survival by inducing disease. The age of captured frogs was assessed using skeletochronology and population age structure was evaluated using catch curve analysis. We report on the findings of this study that quantified the effects of *Bd* and non-native predatory fish on a critically-endangered frog and discuss how the results inform management actions for this species.

### **Recovery efforts for the critically endangered Southern Corroboree Frog (*Pseudophryne corroboree*)**

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The Southern Corroboree Frog (*Pseudophryne corroboree*) is a small myobatrachid frog restricted to Kosciuszko National Park that has rapidly declined in recent decades largely due to disease, caused by infection with *Batrachochytrium dendrobatidis*. Population numbers are critically low with the species now only occurring at translocation sites. Immediate management objectives for this species include establishing robust insurance colonies, maintaining populations in the wild via translocation, and developing efficient captive breeding and translocation methods to increase the longer term capacity and resilience of the program. Captive breeding colonies have now been established at Taronga Zoo, Zoos Victoria and the Amphibian Research Centre. The captive population has a genetically-diverse founder base established from wild-collected eggs over many years. Despite initial difficulties, reliably high captive breeding results have been achieved in recent years permitting the use of offspring for reintroduction and research.

Experimental translocations currently include the translocation of eggs into artificial and natural pools at wild sites, eggs into artificial habitats at sites with reduced threat of the disease and frogs into large enclosures within their range that restrict vectors for the chytrid fungus.

## Cryptic and Failed Speciation in Sulawesi Flying Lizards (*Draco*): Part 1, Phylogenetics and Species Delimitation

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The flying lizards of the *Draco lineatus* Group comprise nine currently recognized species that are found on the Indonesian island of Sulawesi (3 species) as well as several satellite islands that surround Sulawesi to the north and east (6 species). Sulawesi has never been connected to the Sunda or Sahul continental shelves and has thus been isolated for its entire ~25 million year history, leading to substantial endemism (among the highest in the world). Furthermore, the complex tectonic history of Sulawesi, which involves the amalgamation of several paleo-islands that was only completed in the last 1-2 million years, has resulted in impressive regional endemism and seven or more Areas of Endemism (AOEs) on Sulawesi alone. We show that the three morphologically distinct *Draco* species on Sulawesi actually represent 12 deeply-divergent regionally-cohesive mitochondrial clades. Phylogenomic and population genomic analyses indicate that most of these 12 mitochondrially-divergent clades indeed reflect cryptic or non-cryptic species boundaries. However, two of the mitochondrial “lineages” appear to be in the process of merging, and two deep mitochondrial breaks appear to reflect failed speciation events – instances where deep mitochondrial boundaries (10.5% and 15.6% uncorrected) have been maintained despite any evidence of a barrier to gene flow in the nuclear data. We propose that mito-nuclear Dobzhansky-Muller incompatibilities can explain this pattern.

## Superior start to life? Effect of carotenoids on the growth and development of corroboree frogs

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Dietary carotenoids are expected to improve vertebrate growth and development, though evidence for beneficial effects remains limited. One reason for this might be that few studies have directly compared the effects of carotenoids from different classes (carotenes versus xanthophylls) at more than one dose. We tested the effect of two doses of dietary  $\beta$ -carotene and lutein (representing two different carotenoid classes) on the larval growth and development of critically endangered southern corroboree frogs (*Pseudophryne corroboree*). Individuals were supplemented with either  $\beta$ -carotene or lutein at one of two doses or given a diet without carotenoids (control), and larval survival, larval growth, time to metamorphosis, and metamorphic body size were measured. Larvae receiving a high dose of  $\beta$ -carotene metamorphosed significantly faster than all other dietary treatments, despite no significant differences in growth rate. This result indicates that  $\beta$ -carotene supplementation in *P. corroboree* has positive effects on development independent of growth effects. Our study provides new evidence for differential effects of carotenoid class and dose on vertebrate development. From a conservation perspective, our findings are expected to assist with the recovery of *P. corroboree* by expediting the generation of frogs required for the maintenance of captive insurance colonies, or the provision of frogs for release. More broadly, our study highlights the potential for dietary manipulation to assist with the ex situ management of threatened amphibian species worldwide.



## ***Varanus rosenbergi* use of spatial habitat, adaptation and social structure**

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*Varanus rosenbergi* (Rosenberg's goanna) is an Australian solitary living, cool climate varanid with a life span of forty years. Flexible home ranges are a sub set of their life range. Home range is defined as the area utilized in a set time frame, eg single breeding season, specific time of year, etc. Life range is the area used by an individual throughout its entire life. In some species, home and life range sizes are the same.

Both home and life ranges of female *V. rosenbergi* are smaller than males. Prior to courtship and breeding, females and males travel extensively, utilising different burrows in different locations. Males use a 3 to 4 day circuit, covering between 80-300Ha. This may be pre-courtship scoping for potential mates. Females also travel and use numerous burrows, but their home ranges are 40-70Ha.

Once courtship has commenced females are less mobile and often remain in areas <10Ha. Males on the other hand sometimes travel between females and cover over 100Ha. After egg laying and protecting the mound a female commences moving and foraging again within a 40-70Ha area.

Life range areas for goannas monitored over 10 to 25 years are individualistic. Females range between 80 to 200Ha while males life ranges cover 450 to 800Ha. One individual has a life range over 1000Ha.

Forty nine years of monitoring a known wild population on Kangaroo Island South Australia is providing data on spatial habitat necessities, adaptation and social structure of this top level native predator.

## Associations between the fungal and bacterial microbiomes of frogs, with implications for disease management

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Microbiomes play important roles in host health and ecology, but most research has focused on bacterial microbiomes, while largely ignoring fungal microbiomes. Therefore, we examined the fungal and bacterial microbiomes of four frog species following an outbreak of chytridiomycosis. Our goals were to compare the fungal and bacterial microbiomes, look for associations between them, and look for associations with *Batrachochytrium dendrobatidis* (*Bd*; the primary pathogen that causes chytridiomycosis). In total, we documented 1122 fungal operational taxonomic units (OTUs) and 765 bacterial OTUs; however, the mean number of OTUs per frog (richness) was lower for fungi than for bacteria. Additionally, bacterial communities tended to have low evenness and were similar among frogs within a species. In contrast, fungal communities generally had higher evenness and more variability among individuals. Despite these differences, there were positive correlations between the communities for both community structure and OTU richness, suggesting that either one community was driving the other or both were being affected similarly by other factors. We did not identify any associations between the structure of either community and *Bd* prevalence or infection intensity; however, both communities showed associations between OTU richness and *Bd*. For both communities, the most sensitive frog species (*Litoria dayi*) had the lowest richness. Further, there were negative correlations between OTU richness and *Bd* infection intensity, possibly suggesting a protective effect of richness. These results provide some of the first insights into the fungal microbiomes of wildlife and suggest that fungal microbiomes may have important roles in disease ecology.

## **A Wildlife Vaccine Campaign: Vaccinating amphibians against the Chytrid fungus**

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Emerging fungal pathogens pose an extreme risk to biodiversity globally and have decimated amphibian populations- causing wide spread extirpations and extinctions. *Batrachochytrium dendrobatidis* (*Bd*) is a pathogenic fungus known to infect amphibians, ultimately causing death through Chytridiomycosis, and freshwater invertebrates, ultimately causing death through gill damage. Previously we found we could induce an acquired resistance response to the fungus by exposing adult amphibians to the dead fungus. This is promising, as it could prove to be an effective practice for inducing acquired immunity in *Bd*-naïve amphibians, allowing us to reintroduce amphibians extirpated by the fungus to their wild ranges. Previous work explored the effect of the dead fungus on adult amphibians, but in order for this vaccine campaign to be effective we need to explore whether we can vaccinate and protect other life stages as well. Here, we exposed late stage tadpoles and metamorphic Cuban treefrogs (*Osteopilus septentrionalis*) to the lab developed *Bd* vaccine or a control daily for two weeks. After which we challenged all individuals with live *Bd* infection. We found that both life stages had the ability to develop an acquired response to the fungus given the appropriate vaccine exposure. This is a particularly exciting finding as it means that vaccinating small natural ponds directly might be an effective way of protecting amphibian populations from future extirpation.

## Evolution of terrestrial reproduction in Old World Tree Frogs (Anura: Rhacophoridae)

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Rhacophorids have four disparate reproductive modes - aquatic breeding (AQ - gel covered aquatic eggs and free-swimming tadpoles), gel nesting (GN - gel-covered terrestrial eggs and free-swimming tadpoles), foam nesting (FN - foam-covered terrestrial eggs and free swimming tadpoles) and direct development (DD - terrestrial eggs and fully developed froglet without a free swimming tadpole) - whose evolutionary and sidereal patterns are investigated using a well resolved phylogeny. We use all available species of rhacophorid tree frogs for which genetic data is available to construct a phylogeny to trace the evolution of terrestriality in these frogs, especially in direct developers. We show that DD has evolved in three lineages, FN in two, GN in eight and AQ once. We show AQ is basal and that GN is a segue to DD and FN. We suggest that GN is the most versatile mode, FN promotes wider geographic distributions and DD promote niche utilization. The two most derived terrestrial modes of development DD and FN have evolved 3 and 2 times independently. Furthermore, DD clades are highly speciose followed by FN and GN. The basal clades of *Philautus* (DD) have a transitional reproductive mode (nidicolous) between GN and DD and *Mercurana*, sister taxon of *Raorchestes* (DD) and also has an atypical condition of GN. We also discuss evolution in *Pseudophilautus* (DD) form a GN ancestor. The most derived terrestrial modes, DD and FN, seem to have evolved during Eocene-Oligocene transition (EOT), a period of climatic cooling conducive to terrestrial modes of development.

### Conservatism and plasticity in the ecophysiology of a lizard across a steep environmental gradient

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Ectotherms ranging across steep environmental gradients may exhibit adaptive/plastic responses, conservativeness or both. We analysed three physiological parameters of ecological relevance for lizards: 1) preferred temperature (T<sub>pref</sub>), correlating with physiological optima and displaying phylogenetic signal; 2) evaporative water loss (EWL), depending on size/shape but also on phylogeny; and 3) potential metabolic activity (PMA) a measure of respiratory electron transport showing altitudinal shifts in ectotherms. We examined the variation of these parameters in an outstanding model organism, *Gallotia galloti* (F. Lacertidae), a generalist species widely distributed in Tenerife Island 0-3700 m in <50 km air distance. We sampled eight populations, five on the dry southern and three on the humid northern slope, belonging respectively to two evolutionary lineages with current gene flow. We predicted that T<sub>pref</sub> should decrease with altitude, EWL should be lower in the southern slope and PMA should be higher at high altitudes to compensate activity restrictions. Contrary to our predictions, no variation of T<sub>pref</sub> across altitudes was found within the same slope but northern populations had lower T<sub>pref</sub>; EWL was even more conservative varying only with size; and PMA was higher at mid altitudes (regardless slope). These unpredicted results suggest that evolutionary constraints act at variable timeframes; that lizard thermal and hydric ecophysiology may respond to similar climates in different ways depending on the group; and that better understanding of these systems requires metabolic studies. Findings are crucial to understand the role of physiology of ectotherms and to infer their capacity to respond to future climate changes.

## Cool or not to cool? Intestinal coccidians disrupt the thermoregulatory response to tick infestation

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While fever has always been presented as an important adaptive thermoregulatory strategy to fight infections, the adaptive potential of behavioral hypothermia remains poorly understood. In this contribution we compared the thermoregulatory behavior of tick-infested lizards against tick-infested lizards co-infected with two different species of coccidians (*Lankesterella occidentalis* and *Acroeimeria sceloporis*). Cost of tick infestation was confirmed because lizards housed with basking spot and fed ad libitum lost weight at a constant ratio to initial tick load, independently of other infections. In addition, we found that tick-infested lizards sought cooler temperatures in the laboratory, and this response was independent of co-infection status by *L. occidentalis*. Our findings suggest a conservative strategy to save energy which also suggests, in turn, an adaptive role of behavioral hypothermia against tick infestation in these lizards. The broader implications of these findings are discussed in the context of climate change. This thermoregulatory response was not observed in lizards co-infected with intestinal coccidians, *A. sceloporis*. This implies that co-infection with this intestinal parasite prompts lizards to be active. Interestingly, this infection by *A. sceloporis* was observed in lizards with duller breeding coloration which likely limits their sex-appeal and social status as well. Therefore, the maintenance of activity by infected lizards, when un-infected lizards remain less active, might increase their otherwise low chances to mate.

### Testing a mechanistic hypothesis: how does climate predict the distribution of parasites in lizards?

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Environmental perturbations, such as abrupt climatic shifts, may induce physiological stress which can reduce host immune competence against parasites. This is one of the reasons why parasites have been often used as environmental stress bio-indicators. We tested this mechanistic hypothesis in *Gallotia galloti* (Squamata: Lacertidae) analysing 1) the relationship between bio-climatic variables and mean values of corticosterone (a stress hormone) during the summer, and 2) possible synergies of climate change and parasitization by adjusting an ecological model to the association between climate and levels of blood parasites (haemogregrines). We sampled ~1,000 lizards during six years across 30 localities in Tenerife (Canary Islands), which widely represent the microclimatic variation of the other six major islands in the archipelago. We used models of current climate (extracted from WorldClim, 1970-2000) to infer mean bio-climatic conditions of sampled localities. Using an equation that defines the relationship between climatic variables and parasites only in Tenerife, we modelled projections for local abundances of parasites for the entire archipelago. Then, we validated this model of distribution of parasites based on climate by comparing its predictions to real parasitemia data across the archipelago. Conclusively, our mechanistic hypothesis was supported because aridity was the most important predictor, ahead of other plausible predictors such as vector and host abundances, elevation, or an index of human disturbance, and it was positively associated with both mean abundance of blood parasites and corticosterone concentration.

### The global diversity and distribution of lizard clutch sizes

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Clutch size is a key life history trait, which, in lizards, ranges over two orders of magnitude. Geographic variation in clutch size has been extensively studied in birds, and shown to be related to latitude: larger clutches are laid closer to the poles. While clutch size is intensively studied in lizards, wide-scale tests of its geographic variation are lacking. We used clutch size data for over 3800 lizard species to map such geographic variation and found that larger clutches are laid at higher latitudes, and in more productive, and seasonal environments. Island taxa lay smaller clutches. We hypothesize that the constraint imposed by short seasons of activity and abundant resources are the main drivers of large-clutch evolution away from the tropics. We speculate that such conditions, which are unsuitable for species constrained to laying multiple small clutches may limit the distribution of fix-clutched taxa.



**Phenotypic plasticity and rapid adaption drive response to invasive predators in a threatened frog.**

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Invasive alien predators constitute a major driver of biodiversity loss at the global scale. The lack of common evolutionary history often undermines prey capacity to recognize a non-native predator as a threat and thus to express effective anti-predator responses. Two processes can allow prey response to non-native predators. First, phenotypic plasticity can favour the activation of inducible defences when the presence of a non-native predator is perceived as a risk. Furthermore, when predation pressure is particularly elevated, rapid adaptation might occur. In this study we focused on the response of native amphibians to the North-American crayfish (*Procambarus clarkii*), which is a widespread alien predator posing a serious threat to freshwater environments. We assessed whether frog tadpoles (*Rana latastei*) show plastic morphological and life-history responses to the presence of invasive predators. Furthermore, to assess possible evolutionary responses, we compared populations where crayfish invasion has not occurred yet, with populations invaded 8-15 years ago. In a laboratory experiment, we reared tadpoles from invaded and not invaded populations with and without the non-native crayfish. Tadpoles exposed to crayfish expressed inducible morphological traits typically associated to predator presence (e.g. deeper tail muscle) and metamorphosed 10 days earlier than non-exposed tadpoles. Furthermore, development rate was much faster in tadpoles from invaded populations. We show that native species can express complex combinations of developmental plasticity and fast adaptive shifts in response to threatening invasives. If predation pressure is strong enough, 3-5 generations can be enough to evolve significant life-history changes helping to withstand harmful invasive species.

## **Integrative approach using phylogenomics and high-resolution X-ray computed tomography for species delimitation in cryptic taxa**

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Morphologically cryptic taxa that show strong genomic structuring across the landscape but lack distinct morphological differences pose a conundrum, with traditional morphological analyses of these cryptic lineages struggling to keep up with species delimitation advances. Micro X-ray computed tomography (CT) with geometric morphometric analyses provide a promising avenue for species delimitation of morphologically cryptic taxa. However, this 3D imaging technique has, until now, not been used in combination with genomic data in a comparative analytical framework for species delimitation. We present an integrative approach incorporating genomic and geometric morphometric evidence to assess the species delimitation of grassland earless dragons (*Tympanocryptis* spp.) in north-eastern Australia. Using mitochondrial and nuclear genes (ND2 and RAG1, respectively), along with >8500 SNPs (nuclear single nucleotide polymorphisms), we assess the evolutionary independence of target lineages and several closely related species. We then integrate phylogenomic data with osteological cranial variation between lineages using geometric morphometric analyses of three-dimensional CT models. High levels of genomic differentiation between the three target lineages is also supported by significant osteological differences between lineages. By incorporating multiple lines of evidence we provide strong support that there are three undescribed cryptic lineages of *Tympanocryptis* in north-eastern Australia that warrant taxonomic review. Our approach demonstrates the successful application of CT with integrative taxonomic approaches for cryptic species delimitation, which is broadly applicable across vertebrates containing morphologically similar yet genetically distinct lineages.

## Effect of moon phase on nocturnal snake activity

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The effect of moon-phase on nocturnal activity has been documented in several organisms, but primarily in terrestrial invertebrates and marine organisms. Here we examine how moon-phase affects the nocturnal activities of snakes, opinions on which for the past 60 years have remained divided or contradictory in the literature. We studied the effect of moon-phase on nocturnal snakes' activity in an assemblage in South Carolina, USA. Over a 7-year period, snakes were surveyed on 502 nights, yielding 2097 snakes of 27 species. An intricate relationship between activity and moon-phase and the timing of moonrise was evident. The average number of snakes encountered during large-phase moons was significantly lower than during small-phase moons. However, the data revealed a significant burst of activity in which snakes crossed roads more frequently just prior to the rise of an impending large-phase moon. When the moon was in a large-phase, snake activity remained high during the period of darkness or of low light until the moon reached its halfway position in the sky, at which point activity dropped significantly. This indicates an alertness of the impending large-phase moon, since in terms of illumination, the conditions are similar to that of a new moon. During small-phase moons, when there was little or no illumination, activity levels for the entire night may be high, but on a per-hour basis, are low, thus making surveys less efficient. With better knowledge of the moon's role in influencing activity, targeted surveys can be made much more efficient.

### Using camera traps (NEWTRAP) to produce efficiently high-resolution time series of newt observations

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Methods commonly used to estimate newt population sizes rely on capture-recapture approaches (using dip net or funnel trap) requiring intensive field effort, handling individuals and disturbing habitat. Therefore, population size estimates are difficult to obtain on the long-term and/or at large scale. We developed an underwater camera trap (NEWTRAP; patent LU93388) automating the production of ventral images (colour or infrared) without newt handling. A dedicated web application (NEWTRAP Manager) supports management of metadata and annotation of images and videos. We tested NEWTRAP in Western Europe in the frame of a capture-recapture study targeting *Triturus cristatus*. Automatic photoidentification was performed with AMPHIDENT. This study aims at (1) comparing the efficiency of the NEWTRAP with classical funnel traps at the same location over the same period, (2) defining a protocol to use properly NEWTRAP.

Both methods allow presence detection of newts (*T. cristatus*, *Ichthyosaura alpestris*, *Lissotriton vulgaris* and *L. helveticus*), and individualisation of *T. cristatus*. NEWTRAP observes a higher number of individuals by day and by trap than funnel trap (11.5 and 10 respectively), and decreases by minimum 4 the survey effort with even overall human resources. NEWTRAP produces images with straighter body newts and standardised lighting conditions. NEWTRAP with visible light induces a higher newt activity during night than with infrared, likely due to the attraction of preys. Videos provide several observations of intra- and interspecific relations such as mating or predation. Additional biostatistics, logistic, wellbeing and behaviour results are presented.

The study is cofounded by the Luxembourg National Research Fund.

## Online Instruction of Herpetology for Gifted Students

### Metler A<sup>1</sup>

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Online instruction is a growing industry in education, providing opportunities for students to access courses remotely. However, herpetology instruction has a large focus on providing live animals for students to interact with, which can not be done online. Some online resources for herpetology exist, but very few to none exist below the college level. Because of this, experimentation has been being implemented into creating an online herpetology course for interested students in the gifted elementary and middle school demographic, via the Athena's Advanced Academy online provider. The provider uses a combination of the "classroom" containing forums, website URLs, and embedded files and live classes using audio and Powerpoint slides or images. Webcams are not used. Via these available resources, an attempt was made to produce an 8-week class covering the basics of herpetology, with each week focusing on a different group of reptiles or amphibians, with the first week covering their basic characteristics. The weeks were as follows: Reptile and Amphibian Characteristics, Snakes, Lizards, Chelonians, Crocodilians, Frogs, Other Amphibians, and a final week focusing on student presentations on topics in herpetology that interested them. The live sessions included Powerpoint slides covering characteristics of the group that week focused on with opportunities for student discussion and questions as well as images and information about various particularly notable members of the group and overviews of conservation concerns to the featured group. Overall, students' reviews of the course were overwhelmingly positive.

## Chromosome diversity in Malagasy reptiles: an evolutionary perspective from available data and new chameleon karyotypes

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The exceptional diversity of the Malagasy reptiles includes more than 430 endemic species and this diversity is also reflected by high karyological variability. Although just a fraction of the reptile diversity of the island has been studied with cytogenetic methods, different evolutionary trends have been hypothesised in different taxa, including augmentation and reduction of the chromosome number, and the independent diversification of sex chromosome systems.

Here we review and evaluate the available cytogenetic data from different evolutionary lineages belonging to seven different families (Lamprophiidae, Gekkonidae, Boidae, Opluridae, Gerrhosauridae, Typhlopidae and Chamaeleonidae).

More specifically, we discuss newly generated data from the four genera of Malagasy chameleons (*Brookesia*, *Palleon*, *Furcifer* and *Calumma*), including more than 35 newly described karyotypes and map the available karyological information on the phylogenetic relationships of the whole family Chamaeleonidae. In total, our dataset comprises more than 95 chameleon karyotypes, representing more than 50% of the species (about 180) included in the phylogeny of the family. The high chromosome variability of chameleons is characterized by differences in (i) karyotype formula (from  $2n = 22$  to  $2n = 62$ ), (ii) chromosome morphology, (iii) heterochromatin content and distribution, (iv) location of specific molecular markers (e.g. NORs, repeated sequences) and (v) levels of sex chromosome differentiation. This variation makes chameleons an ideal system for studies of chromosomal evolution, as well as for testing new methods in evolutionary cytogenetics.

## Twofold stealth; a new perspective on anti-predatory repertoires

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Within the swathe of anti-predatory repertoires, detection avoidance is first port of call. This is no more evident than in extreme ambush predators that have evolved impressive visual crypsis through selection-driven systems. But few researchers have considered the effects of these same selective pressures acting on olfactory cues. Field observations of puff adders (*Bitis arietans*) going undetected by several scent-orientated predator and prey species led us to test for chemical crypsis in this ambushing species using four carefully selected, macrosmatic biosensor species, namely dogs (*Canis familiaris*), meerkats (*Suricata suricatta*), snouted cobras (*Naja anulifera*), and African elephants (*Loxodonta africana*). None of these biosensors were able to detect puff adders using olfactory cues, but could in stark contrast, detect selected controls that ranged from five actively foraging snake species to puff adder shed skin with impressive accuracy. Our findings clearly demonstrate that puff adders are chemically cryptic; a phenomenon that was once-thought impossible. Though this is the first demonstrated example of chemical crypsis among terrestrial vertebrates, it is unlikely to be unique. Rather, this previously overlooked modality of crypsis may be commonplace, especially among species who share similar life histories in areas where risk from olfactory-orientated predators is high. Detection of chemical crypsis in other systems may fundamentally change our understanding of chemical-subterfuge among predator and prey species and cast new light onto the landscape of chemical ecology.

### **Assessing wildlife translocation proposals: the Victorian experience**

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Translocations, when applied judiciously and appropriately can be valuable for biodiversity conservation. However, when undertaken incautiously, translocations can disrupt ecosystems, introduce or worsen disease, or foster a false sense of security that enables further habitat destruction and species declines. In 2011, the state government of Victoria, Australia established an expert advisory panel -- the Threatened Fauna Translocation Evaluation Panel (TEP) -- to assess the merit of proposed translocations for all threatened fauna (except fish and aquatic invertebrates) into, out of, or within the state, including taking threatened species into or releasing them from captivity. Where necessary, TEP also works with proponents to elevate proposals to an acceptable standard. A guiding principle for the TEP is that a translocation: 1) should result in a probable significant conservation benefit, 2) is unlikely to pose an unacceptable risk to the source population(s), and 3) be unlikely to have adverse impacts at the release site(s). Implicitly, this means that the causes of decline are removed or effectively managed, and that the project has adequate funding and expertise for an adequate duration.

More than 35 proposals have been assessed, with ~85% being supported. Herpetofauna are overrepresented among unsupported proposals. Most agency-led translocations are supported after changes or clarifications are made, whereas proposed mitigation translocations have not been supported by the TEP to date. We discuss the shortcomings of most mitigation translocations, and compare these to the broader suite of translocation proposals for threatened fauna in Victoria.



## Incubation temperatures and intestinal length, are they telling us something about sea turtle hatchling survival?

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Environmental temperatures are rising at sea turtle hatching sites worldwide. Incubation temperatures during the middle third of incubation are key as that time is important for intestinal growth and development. Anecdotal observations of dead-in-nest turtles and turtles that died shortly after hatching from hot nests, suggested that intestinal tracts were shorter than normal. Therefore, we hypothesized that incubation temperatures had a direct effect on the percentage of gastrointestinal tract composed of intestine. We also hypothesized that the percentage decreases as incubation temperatures increase. To test these hypotheses, we evaluated the effect that incubation temperatures have on the intestinal length and percent available to absorb yolk sac nutrition in the immediate post-hatch period. We necropsied 41 loggerhead sea turtle (*Caretta caretta*) hatchlings from Boca Raton, Florida that incubated in situ, in relocated nests in the beach, or in nests moved to lab incubators. The carapace length, total alimentary length, and intestine length were measured, and the percent intestine was calculated. The mean temperature for the middle third of incubation duration was negatively correlated with intestine length. The mean temperature for the middle third of incubation and the maximum temperature experienced in the initial third of incubation were negatively correlated with percent intestine. Shorter intestinal length fractions at higher incubation temperatures could mean less intestinal surface area and less enteral absorption of the yolk sac contents, leading to negative energy balance and subsequent death. These data are concerning and suggest a possible link to decreased survival in hatchlings incubated at higher temperatures.

### Look, that salamander is being eaten alive!

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*Batrachochytrium salamandrivorans* (*Bsal*) has the potential to cause high mortality in amphibians, especially salamanders, and to be spread world-wide through the pet trade, making it a pathogen of global concern. Therefore, experimental challenges are underway to estimate species susceptibilities in order to understand invasion risk and develop disease mitigation strategies that will minimize the global distribution of *Bsal*. Over 30 species have already been tested and include both salamanders and anurans. For each of these challenges, individuals are exposed to *Bsal* in a water bath at one of five concentrations (0, 5 x 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup>) for 24 hours, and their condition monitored for six weeks, documenting development and progression of disease (chytridiomycosis). In general, severity was correlated with dose. Gross changes progressed from increased skin sloughing to discrete ulcerations and were correlated with histological progression from superficial to full-thickness epidermal invasion. These changes varied by species but were seen in salamanders and anurans, which is an added level of concern. Identifying changes along the disease continuum will allow us to identify key time points for disease mitigation strategies and explore treatment options in susceptible species.

### **A Ten-Year Study of An Urban Turtle Metapopulation**

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Turtles are common residents of urban environments, living in park lagoons, golf course ponds, urban streams and rivers, and other aquatic and terrestrial habitats. Urban areas provide unique habitats and problems for turtles. Nine ponds of various sizes are located on the 740-acre campus of Missouri Western State University, St. Joseph, MO, USA. We began trapping and marking turtles in campus ponds in 2008 as part of a series of undergraduate research projects. We have more than 460 captures of 196 marked turtles consisting of five native and one introduced species in the campus ponds: *Apalone spinifera*, *Chelydra serpentina*, *Chrysemys picta*, *Graptemys pseudogeographica*, *Trachemys scripta elegans*, and *T. scripta scripta* (nonnative). Population size estimates for each pond ranged from 7-49 turtles. These ponds are small (< 1 ha) and vary greatly in depth and hydrology, with several ponds drying and refilling during the study. Pond fidelity was high, with several turtles captured multiple times in the same pond over 10 years (e.g., one slider has been captured 27 times in the same pond since 2009). However, at least 20 turtles moved among the campus ponds, crossing roads and potentially parking lots, traveling straight-line distances from 133-890 m. We have also documented one turtle moving from an off-campus pond to a campus pond, a distance of approximately 1500 m. In addition to the population ecology of these turtles, we will discuss the unique problems faced by urban turtle populations.

## Evolutionary morphology of osteoderms in anguimorph lizards

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Osteoderms - bony structures embedded in the dermis – occur in a diversity of squamate reptiles. It has been hypothesized that these structures not only form a protective layer against predatory attacks or aggressive conspecifics, but might also aid in thermoregulation and in the prevention of water loss through the skin, or even play a role during locomotion. While the presence of osteoderms is a phylogenetically conservative trait in the majority of squamate clades, others are characterized by immense variation in osteoderm expression and morphology. These morphologically diverse clades provide unique opportunities to gain insight into the ecologically and evolutionary drivers of this enigmatic trait. In anguimorph lizards (Squamata: Anguimorpha), particularly, osteoderms show a peculiar difference in expression patterns, with some taxa possessing fully imbricating osteoderms, whereas others show intermediate phenotypes or possess no osteoderms at all. In this study, we examine the morphology and expression of osteoderms in anguimorph lizards using micro-computed tomography ( $\mu$ CT) scans of representatives of all genera within the Anguimorpha. Using phylogenetic comparative methods, we subsequently test whether the observed variation relates to climatic conditions (specifically aridity) as previously shown in cordylid lizards. The ultimate goal of the study is to provide a more profound understanding of the conditions under which osteoderms might have evolved, not only in anguimorph lizards, but also in vertebrates in general.

## High variability in Red Tree Frog advertisement calls may act as buffer to anthropogenic disturbance

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One of the major drivers of global biodiversity declines is habitat loss and modification, often associated with anthropogenic environments. To mitigate biodiversity declines, a comprehensive understanding of how species respond to these novel anthropogenic environments is required. These environments are characteristic of increased noise and light pollution, potentially affecting acoustically communicating species, such as frogs. Human-modified areas may force frogs to modulate or alter their calls to communicate with potential mates, as they compete with anthropogenic noise. Using large-scale citizen science data, coupled with remotely-sensed data, we examined how the advertisement calls of the Australian Red Tree Frog (*Litoria rubella*) changed in response to a gradient consistent with anthropogenic disturbance. We provide the first comprehensive baseline acoustic measurements of *L. rubella* (N=385) and discovered that their advertisement calls showed no response to a gradient of anthropogenic disturbance. Most interestingly, calls across the species range had high variability in all call parameters, suggesting that male *L. rubella* can still be heard despite anthropogenic disturbances as 1) they are a loud species typically heard over background noise and multi-species choruses, and 2) the variation in their calls is wide-ranging – most likely serving as a buffer to any acoustic disturbances. Overall, these results provide evidence that some frog species may be acoustically urban-tolerant and are informative for future conservation decisions for Australian frogs.

### Six sex-chromosomes in a Taiwanese frog

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Sex is genetically determined in amphibians, of which sex chromosomes still stay homomorphy in both sexes in a majority of species. In contrast, very rarely, the sex chromosomes change to heteromorphic state in male or female at a stage of speciation or even of geographic differentiation within a single species. Therefore, amphibians give us an excellent opportunity to study on the mechanisms of primary sex chromosome evolution. Here we report finding of very unique heteromorphic sex chromosomes in a Taiwanese brown frog, *Odorrana swinhoana*. Surprisingly, the three different chromosome pairs are largely heteromorphic in males while completely homomorphic in females. Based on chromosome banding patterns and meiotic chromosome configurations, it was unveiled that triangular translocations, which occurred simultaneously only in males, created the multiple sex chromosomes. We define the six sex-chromosomes  $\sigma X_1Y_1X_2Y_2X_3Y_3$ - $\text{♀}X_1X_1X_2X_2X_3X_3$ . In addition, we identified ancestral populations, of which sex chromosomes still stay homomorphy in both sexes. We will mention the biological importance that the frog holds on for the studies on sex chromosome evolution in anurans and further in vertebrates.

**Nature's pitfall trap: salamanders as rich prey for carnivorous plants in a nutrient-poor bog ecosystem**

**Moldowan P<sup>1</sup>**, Rollinson N<sup>1</sup>

<sup>1</sup>*University of Toronto*

Botanical carnivory is an evolutionary marvel of the plant kingdom that has long fascinated general onlookers and naturalists alike. The Northern Pitcher Plant (*Sarracenia purpurea purpurea* L.) is found across eastern North America and has been subject to a wealth of observational and experimental studies. The documented prey assemblage of *S. purpurea* is diverse and comprised almost exclusively of invertebrates. In this study, we use a collection of observations in *Sarracenia* to illustrate that vertebrate prey can occur with a striking frequency. Visual surveys of pitcher plants were conducted during the metamorphic *Ambystoma* salamander dispersal period at a fishless kettle lake-bog site in Algonquin Provincial Park, Ontario, Canada. We recorded salamanders captured in up to 20% of surveyed plants and estimated that plants are responsible for 4-5% of salamander early life stage mortality in our study population. This presentation will discuss our surprising observations from the investigation of two widely distributed and well-studied species, and how salamanders influence and are in-turn influenced by the environment. The high frequency of salamander captures in carnivorous plants suggests that these plants serve as a non-trivial source of mortality for salamanders and salamanders serve as an appreciable seasonal nutrient pulse for carnivorous plants at our study site.

## Conservation progress for critically endangered lizard taxa in Aotearoa/New Zealand

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<sup>1</sup>NZ Department of Conservation, <sup>2</sup>NZ Department of Conservation

Of the 106 recognised native lizard taxa in Aotearoa/New Zealand, eight are considered 'Nationally Critical' under national threat listing criteria pertaining to size of population and rate of decline. These eight taxa include seven skinks and one gecko. Primary threats to these taxa include predation by introduced mammals, habitat modification and increased storm events associated with climate change. Over the past decade, significant progress has been made in both understanding extant populations of all eight taxa and in implementing adaptive management strategies for several. The establishment of a Lizard Technical Advisory Group (TAG) in 2010 with a national scope was pivotal in focussing research and management attention on the taxa most in need. Regular revision of threat status, a national species prioritisation programme and strong relationships between the Lizard TAG and operational staff have also contributed to progress. However, significant challenges remain for these eight Nationally Critical lizard taxa, and the fate of six of them is uncertain. This is the impetus for a national lizard strategy currently in development, which will synthesise research needs, major threats and priority management actions. An engagement process is currently underway for the strategy whereby tangata whenua (Māori people) are invited by the Lizard TAG as primary partners to create an engagement plan and include other stakeholders in its development and implementation. It is hoped that the national strategy, together with greater investment in biodiversity by the government of Aotearoa/New Zealand, will improve the plight of the country's Nationally Critical and other threatened lizards.



## Winter ambushing site fidelity of individual pit vipers (*Ovophis okinavensis*) over 22 years

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Many predators adopt a sit-and-wait foraging mode to hunt prey animals. When these predators decide a place to sit-and-wait, they usually select a site where the efficiency of encountering prey is high. Such efficient ambushing sites may not be stable and seasonally change. If seasonally available feeding sites are predictable, predators may visit the same ambushing site every year, resulting in high ambushing site fidelity. *Ovophis okinavensis* is a small pit viper endemic to Japan and is a typical sit-and-wait predator. Our long-term ecological study in a mountainous area of Okinawa Island showed that this snake aggregates to feed on two species of frogs that explosively breed in a limited area of mountain streams in winter and disperses into surrounding forest areas during spring to fall. Our study also demonstrated that longevity of *O. okinavensis* is quite long, at least thirty and forty years for males and females, respectively. Here, we focused on three breeding sites of the frogs and recorded occurrence of the snake based on the mark-and-recapture method over 22 years. Although two breeding sites of one frog species are located approximately 200 apart from each other, which are well within a home range size of the snake, most individuals of *O. okinavensis* used only one of them over the study period. In addition, within each breeding site, snakes tended to ambush in a same, particular point in every visit. Our observations demonstrate the high fidelity of annual ambushing site selection by *O. okinavensis* over a long period.

### **Accounting for uncertainty: pathogen detection and management of amphibian disease**

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Infectious diseases are a prominent threat to amphibian populations; *Batrachochytrium dendrobatidis* (*Bd*), *Batrachochytrium salamandrivorans* (*Bsal*), and Ranavirus have caused declines in over 500 species globally. Despite the importance of disease as a threat to amphibian biodiversity, there have been very few examples of successful management actions that reduce negative impacts of disease. Developing new actions that are effective may rely on a solid understanding of pathogen ecology, distribution, spread, and subsequent disease risk for amphibians. However, designing studies to estimate these quantities without bias and with minimal uncertainty is challenged by imperfect detection, variation in molecular assay sensitivity, difficulty capturing host species to sample, and heterogeneity in pathogen occurrence both on the landscape (i.e., spatial occurrence) and within populations (i.e., prevalence). We consider hierarchical landscapes commonly of interest in amphibian research, where quantities of interest may include a pathogen's distribution in wetlands at regional or local scales, as well within host populations. We illustrate optimal sampling strategies for several common questions of interest using a case study on ranavirus and *Bd* at a northeastern wildlife refuge. In addition, we discuss optimal design of surveillance programs for invading pathogens.

**Does size structure in a facultatively paedomorphic predator (*Ambystoma talpoideum*) alter prey density and diversity?**

**Mott C<sup>1</sup>**, Whiteman H<sup>2</sup>

<sup>1</sup>*Department of Biological Sciences, Eastern Kentucky University,* <sup>2</sup>*Watershed Studies Institute and Department of Biological Sciences, Murray State University*

Theoretical ecologists have historically treated all members of a species as functionally equivalent when modelling species interactions and their impacts on community composition. Recent studies, however, have found that intraspecific (within-species) variation in ecological function in some species can be equal to or greater than interspecific (among-species) functional variation. Such species are predicted to exert strong community effects, though this prediction has rarely been tested. In determining how intraspecific variation in apex predators can influence community structure, we are currently quantifying body size variation in facultatively paedomorphic apex predators (*Ambystoma talpoideum*) and obligately metamorphic predators (*Ambystoma maculatum*) in fishless ponds in the Land Between the Lakes Recreation Area, western Kentucky, while estimating density and taxonomic diversity of their macroinvertebrate prey. Based on results to date, size variation among larval mole salamanders of both species combined was not a significant indicator of macroinvertebrate prey diversity. However, we are currently assessing the specific impacts of *A. talpoideum* and addressing confounding variables such as predator density, which likely act together with body size variation in affecting invertebrate diversity. Our current results are still useful, however, because they represent some of the first estimates of temporal trends in apex predator body size variation, through which more accurate models of size-structured community interactions may be modelled.

**CritterPic®: A new tool for passive detection and photography of herpetofauna**

**Muchna K<sup>1</sup>**, Blackie H<sup>1</sup>, Irie K<sup>2</sup>, Hagedorn M<sup>2</sup>, King S<sup>1</sup>, Barrett B<sup>3</sup>, Reutemann P<sup>4</sup>

<sup>1</sup>*Boffa Miskell*, <sup>2</sup>*Red Fern Solutions*, <sup>3</sup>*Boffa Miskell*, <sup>4</sup>*University of Waikato*

Commercially available camera traps are valuable tools for wildlife inventory and monitoring surveys, but are unable to reliably and efficiently detect small animals such as invertebrates and lizards. The CritterPic® animal detection system ('CritterPic') is a long-life image capture device, which automatically captures and close-up high-quality pictures of a full range of small to medium sized animals (invertebrates, lizards and mammals). It features greatly reduced false trigger rates compared to traditional camera traps; is fully automated; and can upload data in real-time to the cloud over the cellular network, providing instant reporting of interactions.

The devices are powered by standard AA batteries and can last for several months. Because of the high quality of images collected it is also suited to Artificial Intelligence (AI), with initial training of an AI network demonstrating 100% success in identifying key species.

This presentation will describe the results of field trials of CritterPic alongside traditional survey methods on predator free islands and across mainland New Zealand. We will also discuss future design developments and further research applications.

## **Savanna survival: how fire regimes, livestock grazing and predation by feral cats affects small-medium reptile survival in north-west Australian savanna**

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The northern savannas have experienced new threats in the past 150 or so years, including changes to fire regimes, grazing by introduced livestock, and predation by feral cats. These threats have caused declines in some guilds, such as small-medium sized mammals, riparian birds and obligately seeding plants. The impacts of these threats on reptiles is poorly described and poorly understood. Some of the mechanisms of decline operating on mammals, such as predation by cats that is amplified by frequent fire and the presence of livestock, could conceivably also affect reptiles. We measured changes in reptile abundance and species richness over 13 years, in a large area (900,000 ha) of the Kimberley region of northwest Australia, by sampling sites annually with varying habitats, livestock management and fire management outcomes. Reptile abundance and richness varied amongst habitats, and generally increased as fire frequencies and extents decreased, and increased as mean vegetation age, and the heterogeneity of vegetation age around the sampling site increased. The presence of an intense fire within the year preceding the sample led to lower abundance and richness. The presence or absence of livestock interacted with fire variables in complex ways. The response of individual species depended partly on when they were active (night vs day) and whether they were surface-dwellers, arboreal or burrowing. The results suggest that parts of the reptile community are sensitive to short-term changes to vegetation caused by fire and grazing, possibly because loss of cover leads to increased predation, but that reptiles recover more quickly post-fire than sympatric mammals. Nevertheless, our results suggest that fire management that increases heterogeneity of vegetation age at a fine-scale and includes some long-unburnt vegetation may be optimal for reptiles in the northwest savannas.

## Evaluating the effectiveness of a 15-year headstarting program for wood turtles (*Glyptemys insculpta*)

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<sup>1</sup>Laurentian University, <sup>2</sup>Stewardship Council, <sup>3</sup>Toronto Zoo, <sup>4</sup>University of Guelph

Two adjacent populations (PopA, PopB) of endangered Wood Turtles were studied extensively beginning in 1988. By the mid-1990s, both populations declined by 70% as the result of a suspected poaching event. A population viability analysis (PVA) determined that extirpation of both populations was inevitable if no intervention was undertaken, and so a headstarting program was initiated in 2003 and the first cohort was released in 2005. Our objective was to quantitatively assess the effectiveness of the 15-year headstarting program by modeling population-specific demographic parameters to evaluate recovery efforts to date, and determine the next phase of recovery. PopA has continued to decline despite the release of 123 headstarts and now has an estimated population size of 18 turtles (11 adults, 7 juveniles). PopB has slowly increased with the release of 330 headstarts and now has a population size of 117 turtles (31 adults, 86 juveniles). PopA and PopB experience relatively high adult annual survivorship (89%, 93%) but low 1-year post-release survivorship of headstarts (36%, 52%). Our PVA projected that PopA will continue to slowly decline whereas PopB will slowly recover, and that both populations would recover if a predator-management strategy was implemented. Six headstarted turtles have reproduced suggesting both populations may become self-sustaining. Since 2015, subsidized predators have killed 11 adult turtles and we have detected three diseases (mycotic shell disease, ranavirus, herpesvirus) in the headstarts. Headstarting alone is not enough to save the populations from local extinction as they face multi-faceted problems for which management is challenging.

## **Understanding probabilities of movement at different scales to inform conservation strategies**

**Muths E<sup>1</sup>**, Bailey L<sup>2</sup>

<sup>1</sup>*U.S. Geological Survey*, <sup>2</sup>*Colorado State University*

Most amphibian movement studies on movement focus on a single level of organization (e.g., local population) and a single life stage (e.g., adults) and many suggest potential conservation actions or imply that the information is useful to conservation, yet these presumptions are rarely clarified or tested. Movement studies to date provide little information to guide conservation decisions directly because they fail to integrate movement across scales with individual or population parameters (i.e., fitness metrics); this is exacerbated by a general failure to set movement studies in a probabilistic context. An integrative approach allows prediction of population or metapopulation responses to environmental changes and different management actions, thus directly informing conservation decisions. To support this perspective we: 1) illustrate the focus on single scales and underscore the importance of movement – at all scales – to conservation; 2) emphasize that movement, breeding, and other demographic probabilities are intertwined and studies executed at different scales can help to understand species' responses to varying environmental and/or management conditions; 3) identify limitations of existing movement-related research to predict conservation action outcomes and inform decision-making; and 4) highlight under-utilized quantitative approaches that facilitate research that either connects movement

**Spatial Ecology of Indochinese Spitting Cobra (*Naja siamensis*) Within Rural Sakaerat Biosphere Reserve, Thailand.**

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<sup>1</sup>Suranaree University of Technology, <sup>2</sup>Sakaerat Environmental Research Station

Monocled Cobra (*Naja kaouthia*) and Indo-Chinese Spitting Cobra (*Naja siamensis*), are together responsible for the third highest number of envenomation's, and second for snakebite mortalities in Thailand. The study aims to identify spatial ecology of *N. siamensis*, at the Sakaerat Biosphere Reserve (SBR) in north east Thailand, and to develop a snake bite prevention programme. Cobras were captured in residential areas using active survey, passive trapping and community notification. Fifty-three *N. siamensis* have been captured between September 2012 to May 2019. Of these we fitted twenty-one individuals with internal radio transmitters. Daily radio tracking has begun to elucidate movement patterns for the 21(11,10) cobras tracked for minimum of forty-seven days, providing large quantities spatial and behavioural data. To May 2019, analysis shows males characterises with bigger minimum convex polygon (MCP) home ranges (with average of 26.4 ha) than females (averaging at 12,3 ha). Mean Kernel density estimation area (50%) for males is 3.1 ha and 17.5 ha for 95%, while 2 ha and 11.1 ha for females respectively. We calculated Duncan Index to investigate habitat preferences for the species. During the period of study, we recorded seven human caused mortalities of radio tracked individuals, indicating a need for public education and cobra conservation in the study area.



### Targeted eDNA Sampling for Cryptic, Arboreal Snakes Based on Microhabitat Selection

**Nafus M<sup>1</sup>**, Yackel Adams A<sup>1</sup>, Barnhart P<sup>1</sup>, Oylar-McCance S<sup>1</sup>, Reed R<sup>1</sup>

<sup>1</sup>*U.S. Geological Survey*

A major challenge to early detection and rapid response programs for cryptic reptiles is their extremely low detection probabilities, combined with the need to detect populations while still at low densities. For cryptic herpetofauna, achieving reasonable confidence that lack of detection equates to absence requires major survey effort, especially for arboreal species that are not attracted to coverboards or other traditional sampling tools. Sampling for environmental DNA (eDNA) has been demonstrated to be an effective early detection and rapid response tool for aquatic species, as well as for establishing presence of semi-aquatic cryptic snakes, but as yet no known protocols exist for using eDNA to document arboreal snake presence. We used broad soil sampling and targeted sampling of habitat resources selected by Brown Treesnakes (*Boiga irregularis*), an ecologically devastating invasive snake on the island of Guam that is also projected to have high transportation risk to other Pacific Islands. We evaluated four factors that could inform development of eDNA protocols for enhanced detection probabilities for low density populations of cryptic and arboreal snakes: 1) using non-targeted soil sampling, 2) targeted collection from water-pooling plants, such as Pandanus, that are preferentially used by Brown Treesnakes, 3) the effects of sampling immediately after rainfall in contrast to a wet wipe of dry plants on detection probabilities, and 4) the duration of time that eDNA persists after repeated cycles of rainfall and drying.

## **Applying species traits that predict invasiveness to inform probability of success for herpetofauna conservation translocations**

**Nafus M**<sup>1</sup>, Reed R<sup>1</sup>

<sup>1</sup>*U.S. Geological Survey*

Wildlife translocations, broadly defined as human-mediated movement and release of wildlife, are one species-specific management action that can be implemented to combat global herpetofauna declines. A persistent problem of conservation or mitigation-based translocations, however, is that most fail. Whether intentionally or accidentally translocated and native or not, small propagules typically fail to establish due to Allee or stochastic effects combined with behavioral responses to translocation. Translocation outcomes for herpetofauna may be improved by forecasting species traits associated with establishment success and using introduction approaches that mitigate for low establishment probability for species that are likely to fare poorly following release. As a field, Invasion Biology has assessed events that occur during the introduction process and species-level traits that positively associate with establishment. Information is available on biotic and abiotic conditions that promote invasion and on species traits that correlate with introduction, establishment, and spread of invasive species, broken down in taxonomically specific evaluations; in contrast, evaluations of conservation translocations is a young field of study. Most published articles on conservation translocations measure isolated case studies of successful outcomes. The field is comparatively depauperate in studies forecasting when conservation translocations are likely to be a successful investment of resources. We present species traits or introduction methods that inform establishment probability, as well as similarities and differences between outcomes for invasive species relative to native species to inform risk assessment, with the goal of informing and improving the likelihood of successful conservation translocations for herpetofauna.

## The use of social media to educate and increase awareness of snakes

Naik H<sup>1</sup>, Starkey M<sup>1</sup>

<sup>1</sup>*Save The Snakes*, <sup>2</sup>*Save The Snakes*

Millions of people use social media on a regular basis, especially Facebook. Social media marketing is a tool that organisations are implementing to tell the story of the work their organisation does and educate others about their cause. It understands the target audience to better convey the necessary messages in wildlife conservation. Snakes are often classed as unpopular species and ignite fear in many people. However, they are gravely misunderstood, and their value needs to be communicated to people. To increase worldwide snake conservation efforts, USA-based nonprofit organization, Save The Snakes, was founded in 2017 to protect threatened snake populations and reduce direct human-snake conflicts by implementing mitigation strategies which will lead to peaceful coexistence. Our goal has been to use social media as a platform to communicate, educate and engage with people about snakes and snake conservation. We used Facebook, Instagram and Twitter to increase our engagement and this has had a valuable impact. We have grown our audience on all three platforms with our posts reaching over 1000 people daily on Facebook. Since May 2018 to May 2019 our followers have increased on Facebook from 2000 to 4000, Instagram from 30 to 1200 and Twitter from 100 to 590. Our posts on awareness of snakes and education about snakebite performed the best. Through our increased engagement we have shown that social media has the power to create awareness and educate people about snakes. We hope to continue this outreach and education work through more engaging and informative posts.

## Using high throughput sequencing to elucidate the evolutionary history of sea snakes

**Nankivell J**<sup>1</sup>, Sanders K<sup>1</sup>

<sup>1</sup>*University Of Adelaide*

Understanding adaptive radiations and the processes that drive them are an important objective in evolutionary biology, but to do this, robust phylogenetic hypotheses are required. Rapid adaptive radiations are inherently challenging to resolve due to factors such as incomplete lineage sorting and introgression. Sea snakes represent the most successful extant marine reptile radiation, despite being of recent (>15 Mya) origin. However sea snakes did not initially undergo a rapid radiation (e.g. the *Aipysurus* group) but rather it occurred later within the *Hydrophis* group, with estimated origin between 3 and 7 Mya. Within the core *Hydrophis* radiation morphological diversity is far greater than that of other marine snakes despite their much younger age and convergent evolution is rampant. This has historically hampered attempts to reconstruct a well resolved phylogeny of the group using either morphological characters or <10 mitochondrial/nuclear genes. Sequence capture using a custom probe set for marine hydrophiines shows promise to 1) Reconstruct a better resolved phylogeny 2) Understand the genetic basis of why some groups undergo extremely rapid morphological change and others do not 3) and understand how these processes have led to functionally similar assemblages of 'ecomorphs' in separate ocean basins. Additionally a number of species complexes in *Hydrophis* remain poorly understood and a high level of unrecognised diversity is present.

## Social Structure of the Spiny-Tailed Iguana, *Ctenosaura similis*: Network, Personality, and Genetic Relatedness

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<sup>1</sup>University of Northern Colorado

Social environments of most reptile groups are poorly understood, yet social interactions may influence mate choice, foraging, nesting sites, predator avoidance, and disease transmission. To address this scarcity of knowledge, I examined personality, network position, and genetic relatedness of a group of free-ranging Spiny-Tailed Iguanas (*Ctenosaura similis*). These non-cooperative animals form stable aggregations (>6 years), developing relationships through repeated, non-random interactions. During four field seasons, ~28 lizards wore physical proximity tags, recording associations when lizards were  $\leq 6$  meters apart. Networks were analyzed for individual and group measures. To evaluate personality, lizards were assayed via flight initiation distance, arena tests, and conspecific agonistic encounters. There is strong support for distinct personalities and a boldness/aggression behavioral syndrome. Social network positions fluctuate by season; female associations remain constant during but differ between seasons, perhaps indicating less need to remain near a preferred mate. Network analysis demonstrates high group density, yet animal disappearances were not rapidly filled, possibly weakening group cohesion. Targeted individual removal may therefore deteriorate the social structure, and this offers the possibility of control where *C. similis* is invasive. This is the first large-scale study to look at personality and social network structure in subfamily Iguaninae, an oviparous, Neotropical lizard that exhibits facultative group formation. Results to date demonstrate a level of complexity hypothesized as an important step in the evolution of more multifaceted sociality. As an IUCN “species of least concern”, this proxy may reveal social structure characteristics needed to support self-perpetuating populations of endangered iguanas.

## **Anuran thermal preferences in health and disease: considerations on a complex problem**

**Navas C<sup>1</sup>**

<sup>1</sup>*University of São Paulo*

The notion of Thermal Preferences permeates the literature on thermal relationships of herpetological models. The concept has a long-standing history in the literature; indeed it is possible to track it to Warden's treatise series on Comparative Psychology in the 1930's, and to authors such as Doudoroff in the same decade. This early literature would not associate lightly the concept of "preference" to a "taxis", and even less to an "optimum". Warden et al. (1936) was a reference for Cowles and Bogert's seminal paper published in 1944, which presented a case in which a "preference" was likely to coincide with a "taxis". This coincidence, however, was a particular case, and cannot be seen as generalization applicable to all ectothermic tetrapods. I defend that thermal preferences and thermal taxis must be treated as independent concepts, and that this distinction is particularly useful to understand the thermal relationships of anuran amphibians. In this talk I aim to discuss this, and to explain the relevance of these considerations to understand the ecological relevance of behavioral fever in anurans.

**Bioacoustics reveal the calling activity of an endangered frog (*Phyloria kundagungan*) in response to climate.**

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The Mountain frog, *Phyloria kundagungan*, is an endangered species endemic to the upland rainforests of subtropical eastern Australia. This species is considered to be at risk from climate change however, population monitoring of *P. kundagungan* has been hampered by their cryptic calling behaviour and subterranean habits.

The influence of environmental variables on calling activity was investigated at six sites across the species' geographic and altitudinal range. Audio recording equipment (Wildlife Acoustics, Songmeter II) was deployed at each site and programmed to record sound once every hour for ten minutes, between July 2016 and March 2018. Soil temperature, along with a range of meteorological conditions were recorded every hour at each site.

Kaleidoscope Pro 4 (Wildlife Acoustics, Inc.), was used to automatically detect *P. kundagungan* calls in the 8760 hours of sound recordings and our analysis detected >2 million *P. kundagungan* calls. The true positive rate for the presence of *P. kundagungan* calls in 5000 randomly selected recordings was >98%. The results of this study indicate that calling activity is driven primarily by soil temperature and time of day. This study will allow future targeted surveys of this cryptic species to be conducted under conditions that induce calling activity thus increasing detection probabilities.

**ZZ/ZW sex chromosomes in the granite night lizard (Scincoidea: Xantusiidae)**

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Squamate reptiles (lizards and snakes) are potentially an ideal clade for studying sex chromosome evolution—they are old, speciose, geographically widespread, and exhibit myriad sex determining modes. Unlike other ‘model’ clades, however, the vast majority of squamate species lack heteromorphic sex chromosomes. Cataloguing the sex chromosome systems of species lacking easily identifiable, heteromorphic sex chromosomes is essential before we are to fully understand the evolution of vertebrate sex chromosomes. We here use restriction-site associated DNA sequencing (RADseq) to classify the sex chromosome system of the granite night lizard, *Xantusia henshawi*. RADseq is an effective alternative to traditional cytogenetic methods for determining a species’ sex chromosome system (i.e. XX/XY or ZZ/ZW), particularly in taxa with non-differentiated sex chromosomes. Although many Xantusiid lineages have been karyotyped, none possess heteromorphic sex chromosomes. We here identify a ZZ/ZW sex chromosome system in *X. henshawi*—the first such data for this family. Furthermore, we report that the *X. henshawi* sex chromosome contains fragments of genes found on *Gallus* chromosomes 7, 12, and 18 (which is homologous to *Anolis* chromosome 2p), the first such combination.



## Superfrogs in the city – impact of urbanity and agriculture on the morphology of frogs

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Despite the growing pressure on biodiversity deriving from increasing anthropogenic disturbances some species persisted. To understand the environmental drivers and thresholds behind that process we collected long-term data based on museum specimens and recent measurements of the widespread European Common Frog, *Rana temporaria*. We hypothesized that the morphological traits body size, fluctuating asymmetry and nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) (i) have changed over the last 150 years (ii) that changes in these traits can be attributed to increasing urbanization and agricultural intensity. Our results show that after the Second World War individuals were larger than before in Berlin but not in the rural Brandenburg. Fluctuating asymmetry analysis revealed a similar tendency with lower levels in Berlin after the Second World War and higher levels in Brandenburg. Enrichment of  $\delta^{15}\text{N}$  decreased over time in both regions but was higher and less variable in sites with agricultural land use. The proportion of sealed surfaces around the frog sites could not be identified as the main driving factor. Largest individuals originated from areas with moderate sealing degree ( $> 35\% < 70\%$ ), not from the extremes. We detected a positive effect of greenspace. Body sizes increased, fluctuating asymmetry tended to decrease and  $\delta^{15}\text{N}$  values decreased with increasing amount of greenspace. Our results exemplify that urbanization must not necessarily worsen environmental conditions. Public parks, cemeteries and private gardens can serve as suitable refuges. These findings underline the urgency of maintaining, constructing and connecting such urban habitats and considering their importance for future urban planning.

## Comparative study on antioxidant defense responses to ammonia stress in three fresh water turtle species

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Turtles have received increasing interest for their unique stress tolerance ability. Ammonia is a common stressor in water, especially in aquaculture ponds. This work compared antioxidant responses to ammonia stress in three popularly cultured fresh water turtle species, the three-keeled pond turtle *Chinemys reevesii*, the common snapping turtle *Chelydra serpentina* and the soft-shelled turtle *Pelodiscus sinensis*. Each species was randomly divided into three groups (n=10/group): Control group C0, exposure group C1 (189.52mg/l TAN, 96h exposure) and recovery group C2 (96h recovery after exposure). Results showed that blood ammonia in all three turtles increased significantly in C1, and then decreased to C0 levels after recovery. Hb and MetHb levels in *C. reevesii* and *P. sinensis* increased significantly in C1 and C2, while Hct did not change much. In contrast, Hct of *C. serpentina* decreased over time, while Hb and MetHb levels kept constant. ROS and TAOC levels in *C. reevesii* kept constant; while in *C. serpentina*, hepatic and renal ROS levels increased significantly in C1 and C2; in *P. sinensis*, both ROS and TAOC in liver increased during exposure and then recovered after recovery. Tissue MDA concentrations did not change much in all three species. The patterns of antioxidant defense system responding to ammonia exposure in three species are distinct. In *C. reevesii*, the variation of GSH/GSSG in brain was most significant. In *C. serpentina*, GSH showed strong antioxidant effects in all tissues. As for *P. sinensis*, GSH was largely consumed in brain, while SOD was activated clearly in liver and kidney.

## Thermal plasticity in metabolic physiology in a widespread Australian lizard

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Phenotypic plasticity enables individuals to modify their phenotype in response to changes in the environment. Typically, plasticity is represented as a reaction norm where a phenotypic trait is measured as a function of the environment with the slope viewed as 'plasticity' (function-valued perspective). However, phenotypic variation in different environments can also be considered as separate traits (character-state perspective). Depending on how evolutionary biologists choose to view plasticity, selection can either act on the entire reaction norm or parts of the reaction. Here, we compare and contrast both function-valued and character-state approaches in exploring individual variation in metabolic responses to temperature in delicate skinks (*Lampropholis delicata*). We show that individual slopes were moderately repeatable and that repeatability of MR increased with temperature. We also found that metabolic rate at adjacent temperatures more strongly correlated than with temperature extremes, suggesting that the shape of the reaction norm can only change under certain constraints. We advocate the use of both approaches as they bring different insights in understanding how selection can act on reaction norms which is integral to understanding the evolution of plasticity.

## **Community efforts to re-establish grand and Otago skinks, and jewelled geckos, in modified shrub/grassland ecosystems**

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<sup>1</sup>*Manaaki Whenua - Landcare Research*, <sup>2</sup>*Wildland Consultants*

The Central Otago Ecological Trust is a community group restoring Central Otago's lost lizard communities in a shrub/grassland ecosystem modified by agriculture and introduced mammalian pests. To test whether a translocation of locally-extinct lizards in this ecosystem would work, the Trust conducted a pilot translocation of captive-bred Otago skinks (derived from an extant population in the east of their former range) into a 0.3-ha mammal-proof fenced area in a public conservation reserve retired from agriculture. This was successful until mice breached the fence and were observed attacking skinks and reducing their survival. After removing mice and the remaining skinks, and improving the fence design, the Trust translocated genetically-distinct, higher conservation value grand and Otago skinks collected from the west of their former range. This essentially failed, perhaps due to co-location of the two species within the small confines of the fence, an overshoot of the carrying capacity, or something peculiar to western skinks. The Trust has since built a 14-ha mammal-proof fence, and in 2018 translocated 33 grand and 36 Otago skinks from the west into separate parts of the fenced area. Also, 86 Jewelled geckos were translocated from the Lammermoor Range into a thick vegetated gully. Based on unique natural markings of individual lizards, we report photo/re-sight data collected to date. Our advice to community conservation groups restoring fauna communities is to use a methodical and scientific approach, expect setbacks, be incredibly patient, and enjoy the rewards of seeing a vision transpire and the joy it brings to others.

## Polyploidy and adaptation in Australian burrowing frogs *Neobatrachus*

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Polyploidy is rare in animals, and most polyploid animals reproduce asexually. Amphibians represent a dramatic vertebrate exception, with multiple independent sexually reproducing polyploid lineages. The Australian burrowing frog genus *Neobatrachus* is comprised of 6 diploid and 3 polyploid species and offers a powerful model animal polyploid system. We generated exome-capture sequence data from 87 individuals representing all 9 species of *Neobatrachus* to investigate population genomic effects of polyploidy on genus-wide demography. We document widespread gene flow between the tetraploids, asymmetric inter-ploidy gene flow directed from sympatric diploids to tetraploids, and current isolation of diploid species from each other. Changes in ecologically suitable areas corresponded to estimates of demographic histories and suggested that diploids may be suffering the early impacts of climate-induced habitat loss, while tetraploids appear to be avoiding this fate.

At the same time, polyploids have to adapt their cellular machinery to ensure proper segregation of chromosomes during meiosis. We have assembled a draft genome of *N. pictus* using 10xGenomics technology and conducted a first selection scan between the *N. pictus* (2n) and the *N. sudellae* (4n). Our preliminary results show that selected genes in the tetraploids are enriched for microtubule motor activity function. This suggests modifications of the homologous pairing process during meiosis. Continuing this work we hope to provide the first description of adaptation mechanism(s) to autotetraploidy in animals. Overall, we demonstrate that *Neobatrachus* is an attractive model to study the effects of ploidy on evolution of adaptation in animals.

### **Green Infrastructure from urban sinks to prestigious pads for frogs**

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Urbanisation has been identified as a major threat to biodiversity worldwide. As well as consuming land, urban expansion perturbs natural processes such as flooding and nutrient cycling, and fragments habitats. Flooding is of particular concern in many countries as extreme weather events appear to be increasing in frequency. In compliance with the EU Water Framework Directive, constructed wetlands known as Sustainable Drainage Systems (SuDS) have been installed in all Scottish cities to reduce flood and pollution risk. Amphibians, with their limited dispersal abilities, sensitivity to pollution and, in many cases, reliance on waterbodies for breeding, may seem particularly vulnerable to these threats, and potential benefactors of well-designed green infrastructure, including SuDS. Our study surveyed SuDS in 12 Scottish towns and cities, and considered their value as habitats for amphibians and other species, as facilitators of gene flow, and as places where urban people can come into contact with nature. We found that SuDS can offer new habitats for amphibians, there was no reduction in genetic diversity amongst common frog *Rana temporaria*, when compared with rural ponds, and that there is strong gene flow between populations. Biodiversity value varied greatly between SuDS, with poorer neighbourhoods tending to have wetlands of lower ecological quality, however this can be avoided through appropriate design and management. Our findings are being used in SuDS design in three Scottish cities, including designing a habitat network for the European protected species great crested newt *Triturus cristatus*.

## The unexpected genetic mating system of a terrestrial breeding toadlet (*Pseudophryne coriacea*)

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Molecular technologies have revolutionized our classification of animal mating systems, yet we still know very little about the genetic mating systems of many vertebrate groups. It is widely believed that anuran amphibians have the highest reproductive diversity of all vertebrates, yet genetic mating systems have been studied in <1% of all described species. Here, we use single nucleotide polymorphisms to quantify the genetic mating system of the terrestrial breeding red-backed toadlet *Pseudophryne coriacea*. In this species, breeding is prolonged (approximately 5 months), and males construct subterranean nests in which females deposit eggs. We predicted that females would display extreme sequential polyandry because this mating system has been reported in a closely related species (*P. bibronii*). Parentage analysis revealed that mating success was heavily skewed towards a subset of males (30.6% of potential sires) and that nearly all females (92.6%) mated with one male. In a high percentage of occupied nests (37.1%), the resident male was not the genetic sire, and very few nests (4.3%) contained clutches with multiple paternity. Unexpectedly, these results show that sequential polyandry is rare. They also show that there is a high frequency of nest takeover and extreme competition between males for nest sites, but that males rarely sneak matings. Genetic analysis also revealed introgressive hybridization between *P. coriacea* and the red-crowned toadlet (*Pseudophryne australis*). Our study demonstrates a high level of mating system complexity, and it shows that closely related anurans can vary dramatically in their genetic mating system.

## Identifying climatic drivers of breeding and emigration phenology in an imperiled salamander

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<sup>1</sup>*United States Geological Survey*

For many amphibians, temperature and rainfall are key drivers of life history events like breeding and metamorphosis, and changes in these variables could shift amphibian phenology over time. A better understanding of the climatic drivers of adult breeding and juvenile emigration phenology would help inform the timing of habitat management and restoration, especially for imperiled species. In 2016, I initiated a mark-recapture study to examine the breeding phenology of federally-threatened frosted flatwoods salamanders (*Ambystoma cingulatum*). I used funnel traps along drift fences to monitor salamander movements at three known breeding wetlands at St. Marks National Wildlife Refuge (Florida, USA), marked and measured all salamanders. Peaks of breeding activity in fall (October-December) typically coincided with cold fronts moving through the region, even if minimal rainfall accompanied the cold front. Metamorph emergence in spring (March-May) was less tied to discrete weather events. Differences in metamorph emigration phenology among ponds were largely explained by hydroperiod variation. In both adult breeding and juvenile emigration, phenological patterns varied greatly among years, illustrating the dependence of this species on both weather events and hydrology. By studying the phenological distribution of breeding and emigration in this species, we can better predict how phenology may change in the future. These predictions will inform both management (e.g., when to conduct prescribed burns) and conservation (e.g., population viability) actions for this imperiled species.



### Impacts of the developmental environment on amphibian disease susceptibility

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Changing environmental conditions may leave ectotherms with complex life cycles more vulnerable to disease via sublethal effects on physiology and immune function. In a rapidly changing climate, amphibian larvae are facing the dual threat of variable hydroperiods and increased water temperatures, and while developmental plasticity may allow some species to escape a drying pond, this plasticity might result in trade-offs with physiology and performance, including investment in immune function. Using two species of North American leopard frogs impacted by disease, we examined the effects of pond drying and elevated temperatures on development, growth, physiology, and immune function. Larvae were raised in mesocosms in Pennsylvania (*Rana pipiens*) and Louisiana (*R. sphenocephala*) under varying drying and heating regimes. We predicted that amphibians experiencing rapid pond drying and elevated pond temperatures would be smaller at metamorphosis and demonstrate lower survival and long-term alterations in physiology and immune function. In both Pennsylvania and Louisiana, rapid pond drying resulted in smaller metamorphic animals, but only *R. sphenocephala* in Louisiana responded to pond drying with an increase in developmental rate. Furthermore, drying and the impacts of drying (e.g. reduced size and a shortened larval period) resulted in reduced markers of innate and adaptive immune function in both species. Drying during development also reduced survival in *R. pipiens* after a pathogen challenge with *Batrachochytrium dendrobatidis*, but not in *R. sphenocephala*. Thus, a variable developmental environment can result in complex trade-offs between amphibian growth and immune function, which might increase the cost of pathogen resistance in some species.

**Parental care by male Japanese giant salamanders – a note on a partially small den-master found in an upstream section.**

**Okada S<sup>1</sup>**

<sup>1</sup>Hanzaki (*Giant Salamander*) Research Institute of Japan

Paternal care in salamanders is known in only two families, Cryptobranchidae and Sirenidae, and this mode of parental care is associated with aquatic life and external fertilization. Our recent studies show that large males of *Andrias japonicus* that occupy nesting sites (called den-masters) care for offspring from hatching to dispersal of larvae. It is also known that *A. japonicus* form a communal nest in which multiple males and females contribute to the resulting offspring that is subsequently cared by a single den-master. Whereas den-masters are typically large males, there is noticeable variation in their body size. We compared body sizes of den-masters in two rivers. In one river where the slope is gentle and there are relatively fewer barriers to migration such as low-head dams and falls, the variation in body size is small with their size being consistently large (total length [TL] = 90-100 cm). In the other river where the slope is steep with many migration barriers, the variation in body size of den-masters is remarkably large; the body size in the downstream section is 90 to 100 cm TL while that in the upstream section is 50 to 70 cm TL. We monitored a small den-master with 55 cm TL and confirmed that even this small male provides parental care in the same fashion as in typical large males and it lasted over 7-month long. We report the details of the parental care by the small den-master and discuss possible reasons for the existence of small den-masters.

## Nonlinear body size cline in a salamander: patterns due to latitudinal compensation and its prevention

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<sup>1</sup>*Tokyo Metropolitan University*

In ectotherms, lower temperatures in high-latitude environments would reduce the growth rates of individuals. Because slower growth and resultant smaller body size has a negative impact on fitness through increased predation risk and decreased egg production. Therefore, individuals in higher latitudes may evolve compensatory responses as maintaining body size by accelerating growth rates or/and developing cold tolerance. It's called latitudinal compensation. If latitudinal compensation is achieved, there will be no latitudinal variation in body size. However, if temperature adaptation is prevented at the range margin, partial body size cline may be observed, but there are few cases verified in natural settings. The salamander, *Hynobius tokyoensis*, is endemic mainly to Kanto region in Japan. Here, we examined the geographic variation in genetic structure and body size in *H. tokyoensis* along a latitudinal gradient in distribution range. In addition, we conducted rearing experiments to compare compensatory adaptation on 17 local populations. Genetic analyses found loss of genetic diversity near the northern limit. Although body size did not change from southern limit to mid latitude, decreasing rapidly near the northern limit. In addition, strengthening of compensatory adaptation was observed among populations from south to mid latitude, but it was stagnant near the northern limit. Therefore, our findings suggested that the lack of genetic variation prevents latitudinal compensation, and therefore the body size cline was formed at the northern margin of geographic distribution.

## Stream-riparian forest management for herpetofauna: Beyond Best Management Practices

Olson D<sup>1</sup>

<sup>1</sup>*Us Forest Service, Pacific Northwest Research Station*

There is a growing list of stream-riparian ecosystem services for management in today's forests. These include historical Best Management Practices (BMPs) addressing water quality issues including chemical contamination, erosion and sedimentation, and water temperature changes. Beyond traditional BMPs, concerns for declining aquatic-riparian species have ballooned over the last quarter century; globally, freshwater-associated herpetofauna and fishes are the most-endangered vertebrates. Retention of multiple species' breeding, foraging, and dispersal habitat in stream-riparian ecosystems warrant incorporation into contemporary forest and plantation planning to retain ecological integrity. These concepts are especially important for aquatic-riparian amphibians and reptiles which often have restricted distributions and are of conservation concern due to multiple additional stressors (e.g., diseases, invasive species, overuse) which interact with habitat management. Key considerations for stream-riparian forest biodiversity in managed forests in the current millennium include a recipe of: 1) retaining a mix of streamside buffer widths in space and time for habitat protection and restoration aims; 2) extending some stream buffers over ridgelines to reserve patches and adjacent watersheds for promoting stable populations and enhancing connectivity; 3) growing large riparian trees for future down wood and complex habitat structures; 4) promoting minority tree species and plant-and-invertebrate biodiversity in riparian areas; 5) retaining unique aquatic habitats such as ephemeral wetlands; 6) working across jurisdictions to accomplish multiple resource objectives at broader watershed-to-landscape scales; and 7) monitoring sensitive populations for specific risk-management approaches. United States Pacific Northwest examples highlight adaptive forest management approaches for endemic amphibians.

## World Wide Web portals for herpetofaunal pathogen data

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With burgeoning knowledge of amphibian disease threats over the last three decades, a challenge emerged to keep abreast of current information. Development of globally accessible online pathogen databases has been a boon to both science and management. In 2007, we compiled *Batrachochytrium dendrobatidis* (*Bd*) data for the Global *Bd* Mapping Project, which then became the founding database of Bd-maps.net, the first amphibian pathogen web portal. Bd-maps data is now merging into AmphibiaWeb's updated Amphibian Disease Portal: <https://amphibiandisease.org>. As an interim update, as of May 2019, the database documents *Bd* detections from: 1) 3706 of 9503 (39%) field sites sampled; and 2) 1015 of 1854 (54%) species sampled. *Bd* sampling spans >100 countries, 20% of world amphibian species, and 93% of world amphibian families. The new portal also accommodates *B. salamandrivorans* data, captive animal data, surveillance plans, and pdf uploads of supplemental information such as study details or *Bd*-strain information. In 2015, the Global Ranavirus Reporting System (<https://mantle.io/grrs>) was created by EcoHealth Alliance in cooperation with the Global Ranavirus Consortium and the US Forest Service. Including studies from over 25 countries to date, this is a growing database documenting a relatively little-known pathogen tied to mass mortality events in amphibians, reptiles, and fishes. These portals have become critical tools informing management decisions for surveillance and biosecurity priorities. They have aided scientist's project development and provided novel opportunities for pattern assessment such as understanding pathogen niche space and pathogen-infection associations with host phylogeny, traits, habitat use, community composition, and declining populations.

### Shining the light on a career full of sex, conflicts, and transgender tactics (via video)

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Charles Darwin was intrigued by how sexual ornamentation, that ought to attract predation and increase mortality, could be favored by selection and lead to the evolution of extreme differences in appearance between males and females. Darwin's insight was that covariation between male traits and reproductive success result in variance in male lifetime production of offspring. What Darwin overlooked was some other key processes that contribute to sexual dimorphism and tactics, such as sexual differences in ecological niches. Rick's deep insight into these processes fundamentally influenced evolutionary and ecological research into sexual selection and tactics at a critical time of the subject's development. Later, in a series of Nature publications, Rick *et al.* derived fitness consequences for males and females, both from a proximate perspective (how sex-specific temperature optima affect developmental 'quality' of the offspring), and how temperature-dependence influence seasonal timing of hatching and ultimately, sex-specific consequences for lifetime offspring production. In garter snakes, Rick *et al.* analyzed the evolutionary disagreement over male and female optima in reproductive tactics (sexual conflict), and how "she-males" produce female-like skin pheromones that tactically attract courtship from other males, inflating 'she-male's' mating opportunities. My link into Rick's world was when we (and others) researched the final steps in sexual selection, how the female reproductive tract constitutes an arena for sperm competition, the biasing of paternity towards 'best' fathers, and the benefits of polyandry during a climatic cline of temperatures over a decade. I review this plethora of Shining contributions to the field of sexual selection.

### **Conservation of the Galapagos land iguana (*Conolophus subcritatus*)**

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The Galapagos Land Iguana (*Conolophus subcritatus*) is a vulnerable species (IUCN) threatened by habitat loss and introduced predators. Historically, the reintroductions of land iguanas to predator-free sites or to areas where these are managed, have played an important role in the conservation of the species. Since the 1970s there have been suggestions to reintroduce land iguanas to Santiago Island, which historically had one of the largest populations of land iguanas in the archipelago up until the early 1900s. At the start of the 2000s, introduced predators were eradicated from Santiago Island, and the possibility of reintroducing the land iguana there has resurfaced with greater intensity. While the habitat on Santiago Island is considered adequate for land iguanas, and the expertise to handle and transfer these animals exist, there are significant gaps in knowledge. We evaluated the impact of the presence of feral cats and the population structure of land iguanas; and estimated the carrying capacity for this species on Santiago Island. In populations where iguanas and cats coexist we confirmed a different population structure and also significant differences in morphology relative to cat-free populations. Specifically, there is a lower proportion of juveniles resulting from depredation. On Fernandina Island (642 km<sup>2</sup>), where there are no feral cats we estimated a population of land iguanas of approximately 220,000 individuals. Further we estimate that Santiago Island (585 km<sup>2</sup>) has the capacity to sustain 830000 iguanas considering the actual distribution of vegetation on the island.

## Conservation status of the Floreana racer (*Pseudalsophis biserialis biserialis*) in the Galapagos Islands

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The Floreana Racer (*Pseudalsophis biserialis biserialis*) is a medium-sized colubrid endemic to the islets of Gardner-by-Floreana and Champion off the coast of Floreana Island, in the South of the Galápagos Archipelago. The subspecies was historically abundant on Floreana Island, as indicated by sub-fossil remains, however it has since been extinct there since the late 1800's as a result of the effects of large-scale habitat modification, fires and the introduction of rats and cats. We conducted the first field study on the population size, inter-island morphological variability and the diet of the Floreana racer on the two islets where the species occurs as a first step towards the translocation of Floreana racers to Floreana Island. We conducted daylight searches for Floreana racers yielding a total of 123 individuals, 20 on Champion and 103 on Gardner-by-Floreana. Of these 119 individuals were marked with unique PITTags. We also conducted a mark-recapture estimate of population size on both islets. The resulting estimate of the global population of the subspecies is approximately 3088 individuals (Champion 209 snakes; Gardner-by-Floreana: 2879 snakes). We also investigated the diet of Floreana racers via analysis of faecal samples from 29 individuals. The most common prey items are lava lizards (*Microllophus grayii*), followed by the Floreana gecko (*Phyllodactylus baueri*).



**A review of the conservation status of the Galapagos Islands Terrestrial snakes (*Pseudalsophis* spp).**

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The Galapagos terrestrial snakes, or 'Galapagos racers' (*Pseudalsophis* spp.) are a monophyletic group of nine species within Dipsadidae, exclusively found in the Galapagos Islands. A single continental species, *P. elegans*, is the closest relative of these island species, and is found from Ecuador to Chile. The group has been the subject of various taxonomic reviews since the early 1900s. Although there have been significant advances in understanding the evolutionary history of the group and their phylogenetic affinities, basic aspects of their biology have remained largely undocumented. From 2015 to 2019, we conducted the largest survey of populations of Galapagos terrestrial snakes on 13 main islands of the Galapagos Archipelago to document the presence/absence of terrestrial snakes and their current demographic status. In total, we evaluated 25 populations for nine species. Galapagos terrestrial snakes occurred from sea level to 1470 m asl. They reached higher densities on small islets free of introduced predators (13.6 individuals per hectare), and on coastal areas (20.3 individuals per hectare) of large islands also free of introduced predators. They feed on a variety of vertebrate prey that includes bird eggs and chicks, geckos, lava lizards and iguana hatchlings. Our observations indicate that targeted introduced predator control across large areas of Isabela, San Cristobal and Santa Cruz Islands are necessary to increase local density and to restore 9 populations. For two species (*P. biserialis* and *P. dorsalis*) translocation to predator-free sites will be necessary to reduce local extinction risk.

## Macroevolution and taxonomy of Oriental Brown Frogs (*Rana* spp.) across the East-paleartic

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The reconstruction of sympatric speciation in Oriental Brown Frogs is vital to resolve their large-scale taxonomy. Here, to retrace the macroevolution patterns of the genus *Rana* across the East-paleartic mainland, we sampled 222 *Rana* individuals from fifteen species: *R. uenoi*, *R. coreana*, *R. amurensis*, *R. chensinensis*, *R. zhenhaiensis*, *R. dybowskii*, *R. pirica*, *R. asiatica*, *R. arvalis*, *R. chensinensis*, *R. kukunoris*, *R. sauteri*, *R. longicrus*, *R. tsushimensis* and *R. huanrenensis*. Analyses of concatenated 16S and 12S (770 bp) and concatenated LIG-4 and RAG-1 (1511 bp) provided evidence that geographical barriers only had a weak impact on genetic divergence. We demonstrated the monophyly of species distributed around the Yellow Sea, such as *R. coreana* from the Chinese and Korean clades. We also showed that the Baekdu Mountain range along the eastern mainland coast was not a barrier to gene flow in *R. uenoi*, and we provided significant support to the monophyly of *R. uenoi* across South Korea. We found *R. dybowskii* to be segregated within at least five subclades, thus suggesting local intraspecific adaptation despite its limited range on the Asian mainland. In addition, *R. amurensis* sampled in Mongolia fell into a different clade than that in China, implying two separated lineages. Our study also confirms the sister-clade relationship between *R. coreana* and *R. amurensis*. Finally, we suspect the presence of cryptic diversity in *R. chensinensis* across latitudinal gradients. In conclusion, our study highlights regional taxonomic diversity, likely related to habitat and climatic niche.

## Evolution of antigen processing genes in salamanders

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At the time when amphibian biodiversity is severely threatened by emerging diseases, understanding the mechanisms driving the evolution of the adaptive immune response in amphibians is urgent. Coevolution between Antigen Processing Genes (APG) and Major Histocompatibility Complex class I (MHC I) has been proposed as the ancestral gnathostome condition what might limit flexibility of the adaptive immunity. The hypothesis of coevolution predicts that a widespread signal of positive selection in APG should be detectable at deep and intermediate phylogenetic scales, as these genes should keep pace with the rapid adaptive evolution of MHC class I genes. Using sequences of APG identified in transcriptome assemblies of over 40 salamander species from six different families, we did not find pervasive signal of positive selection, although all genes show some codons under episodic positive selection. Regarding the evolution of two APG that are components of the immunoproteasome, we identified two highly diverged allelic lineages in PSMB8 gene, as observed in several other organisms, but also in PSMB9 gene, reported here for the first time. Evolution of both genes is presumably driven by strong overdominant balancing selection. Considering APG duplications, divergent lineages, apparent gene loss and lack of pervasive positive selection found in this study, APG seem to have evolved dynamically across salamander phylogeny and their evolutionary dynamics does not support the coevolution hypothesis.

## Differential Introgression Across Newt Hybrid Zones – Evidence From Replicated Transects

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Examination of genome-wide patterns of introgression is crucial to understand the genomic architecture of speciation. Hybrid zones allow for direct quantification of introgression under natural conditions. Because the genetic basis of reproductive isolation may differ across species range and introgression can be affected by environmental factors, the genomic landscape of introgression should be assessed in multiple transects throughout the hybrid zone. However, such studies remain rare. Here, we analyse introgression in replicated transects through two hybrid zones between the Carpathian newt (*Lissotriton montandoni*) and two evolutionary lineages of the smooth newt (*L. vulgaris ampelensis* and *L. v. vulgaris*). More than 1000 protein coding genes located in a linkage map were examined in 154 populations from 5 transects to test for differences in genome-wide landscape of introgression between the hybrid zones. Narrow allele frequency clines and bimodally distributed genotypes in mixed populations indicate strong reproductive isolation in all transects. However we detected marked differences in the extent of hybridization and introgression between the two hybrid zones. These differences result probably from different ages of the two zones, as suggested by the differences in the length of genomic tracts of introgression. The genomic landscape of introgression did not differ between zones more than between transects within zones, but was heterogeneous - genes showing increased introgression were overrepresented on two out of 12 linkage groups. Our results demonstrate that the genomic landscape of introgression can differ in various regions of the contact zone even at late stages of speciation.

**Use and traditional knowledge of endemic chelonians of the Mapimi Bolson in northern Mexico.**

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In Mexico, chelonians have been an important source of food, clothing, traditional medicine and other uses. Over-harvesting of wildlife poses a threat for biodiversity conservation and local livelihoods. We researched the local beliefs on the protection status of the endemic turtle and tortoise species such as the Bolson tortoise (*Gopherus flavomarginatus*) and the Durango mud turtle (*Kinosternon flavescens durangoense*) in different localities of their current range. We conducted 91 semi-structured interviews during three periods: from May to September 2015, from June to July 2016 and from July to November 2017 throughout the Mapimi Bolson in northern Mexico. Our aim is to 1) examine the local traditional knowledge, 2) document the use and historical trade of the species and 3) understand the level of awareness and openness to protection of these reptiles. Our results indicate that men and women are equally able to identify the local turtle species and no significant difference was found among the age groups of 46-60 and 61-79 ( $F = -0.156, P = 1.00$ ). Despite conservation efforts for the Bolson tortoise by the Federal Government agencies, poaching still occurs in a small degree. Tortoises and turtles are being used as pets, and on a lower scale for food and medicine. Harvesting practices are unsustainable, and the local communities believe there are less tortoises now than the last 15 years, and that it is important to protect tortoises and international participation is welcome.

## How to (un)make a sexy snake: suppression of female pheromone production via steroid action

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Chemical communication is the principle mechanism by which squamate reptiles (snakes, lizards) locate and assess potential mates. In snakes, the pheromones enabling mate choice are modified skin lipids, and pheromone composition tracks with sex-specific traits regulated by steroid hormones: female quality (length, condition) and male sexual behavior (trailing, courtship). Ergo, sex hormones (androgens, estrogens) have long been implicated as the primary drivers of chemical signal production. Our research has focused on one species of snake, the red-sided garter snake (*Thamnophis sirtalis parietalis*), to parse the physiological relationship between sex hormones and pheromone expression. We have primarily manipulated males to discover that estrogens induce female pheromone production and attractivity but, surprisingly, that testosterone dominantly suppresses pheromone expression. Recently, my lab has manipulated female garter snakes to understand these inhibitory androgen effects. By either blocking the synthesis of estrogens in females or implanting them with testosterone, we have reduced female attractivity. Females subjected to inhibition produce less of and a lower quality pheromone than controls, and these females receive less courtship from males in the wild. With further corroboration from gene expression studies, we conclude that divergence in pheromone production resulted from two sexually-dimorphic processes. Males fail to produce appreciable amounts of female sex pheromone due to testosterone-driven inhibition coupled with a reduced ability to naturally synthesize estrogens. Females, however, escape inhibition because they have low circulating testosterone aromatized (cleared/metabolized) into activational levels of estrogens, primarily by the ovaries. Pheromone production in snakes thus results from reciprocal mechanisms of steroid hormone action.

### Road avoidance and its energetic consequences for reptiles

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Roads are one of the most widespread human-caused modifications to habitats that can increase wildlife mortality rates and alter behaviour. For animals living near roads, how do roads affect movement patterns and fitness? Roads can act as barriers with variable permeability to movement and can increase distances wildlife travel to access habitats. Movement is energetically costly, and avoidance of roads could impact an animal's energy budget. We tested whether reptiles avoid roads or road crossings, and explored whether the energetic consequences of road avoidance may decrease individual fitness. Using telemetry data from Blanding's turtles (*Emydoidea blandingii*; 286 turtles, 15 sites) and eastern massasaugas (*Sistrurus catenatus*; 49 snakes, 3 sites), we compared frequency of observed road crossings and use of road-adjacent habitat by reptiles to simulations. Turtles and snakes did not avoid habitats near roads, but both species avoided road crossings. Compared to simulations, turtles made fewer crossings of paved roads with low speed limits, and snakes made fewer crossings of all road types. Turtles travelled longer daily distances when their home range contained roads, but the predicted energetic cost was negligible: substantially less than the cost of producing one egg. Snakes with roads in their home range did not travel further per day than snakes without roads in their home range. We found that reptiles avoided crossing roads but road avoidance is unlikely to impact fitness of turtles and snakes through energetic consequences. Therefore, mortality from vehicle strikes remains the most significant impact of roads on reptile populations.

### **Tuatara conservation through Māori involvement in policy**

**Paul L<sup>1</sup>**, Adams L<sup>2</sup>, Eyre S<sup>3</sup>, Nelson N<sup>4</sup>

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Conservation of reptiles can be extremely difficult for cryptic species particularly when people are unaware of their importance. This is not the case for the internationally well-renowned tuatara (*Sphenodon punctatus*). Conservation action has been targeted and effective through two Recovery Plans, and national and international involvement in wild and captive management and research action. Conservation of tuatara has been successful over the past three decades, and can be demonstrated by more wild populations, increased restoration of their distribution and no extinctions of populations. However, future challenges include climate warming resulting in male-biased sex ratios, disease risk potential, and lack of strategy around management due to higher priorities for species needing more urgent conservation action. We developed a captive management policy that brings together representation from local iwi (Māori), Ngāti Koata, with direct linkages to the source of the captive colony and the most populous wild population on Takapourewa (Stephens Island), the Captive Manager of tuatara, Department of Conservation staff and researchers. The captive management plan was a catalyst to cement conversations with iwi about management of the captive colony nationally, but also how the interface of captive and wild populations could be improved to provide further restoration opportunities, and risks and opportunities for international captive colonies. The plan creates opportunities for iwi to enact their increasing capacity to manage natural resources as they choose, as well as new ways forward for conservation of tuatara.



**Aggressive behaviors and their effect on resource use by female Little Brown Skinks, *Scincella lateralis***

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Studies of aggression and space use are essential to understanding resource use by lizards. Knowledge of this area, however, is biased in that the seminal work has been done on (1) males; and (2) species that are highly visible in their habitats (e.g. iguanians). Studies of females and of secretive species are less common. Here, we present results of a lab study of dyadic encounters of adult female Little Brown Skinks (*Scincella lateralis*; Scincidae) and compare them to results obtained from an earlier study of adult males. Two female Little Brown Skinks were confined on either side of a partitioned observation tank for 48 hours. Then the partition was removed, a single retreat was placed in the center of the tank, and the behavioural interactions of the two lizards were recorded for 60 minutes. Female Little Brown Skinks never interacted unless they were within one body length of each other. By far, the most common behaviour exhibited was flight of one lizard (the subordinate) away from the other lizard (the dominant). This resulted in the two lizards spending more time apart in the tank than close together and they rarely shared the retreat. The larger of the two females was dominant in 9 of 10 trials. Compared to adult males, adult females showed fewer aggressive behaviours such as lunging or chasing, and never bit each other. Nevertheless, patterns of space use and retreat use were nearly the same between females and males.

**Time of year affects study of gut microbiota of the lizard *Sceloporus consobrinus* (Phrynosomatidae)**

**Paulissen M<sup>1</sup>**, Cisar C<sup>2</sup>

<sup>1</sup>*Northeastern State University*, <sup>2</sup>*Northeastern State University*

In temperate climates, lizard gut microbiota must deal with long periods when their host is hibernating and therefore not eating. As a first step toward understanding how lizard gut microbiota deal with seasonality of their hosts, we studied the gut microbiota of a common forest-dwelling lizard, *Sceloporus consobrinus* (Phrynosomatidae) from northeastern Oklahoma, USA. We captured live lizards, obtained fecal samples the following day, and attempted to extract DNA from the fecal samples using fecal/soil DNA miniprep kits (Zymo Research or Qiagen). Samples with sufficient DNA were then sent off for 16S rRNA gene sequencing and bacterial identification. Preliminary analyses showed tremendous variation between lizards with regard to their gut bacteria. Of interest here, we were significantly more successful at obtaining DNA from fecal samples of lizards caught in the spring than from lizards caught in the fall. Some hypotheses for why this might be included: seasonal changes in the lizard diet, changes in lizard gut physiology from spring to fall, and effects of seasonal differences in temperature and precipitation (which may affect both the lizards and their food sources).

## Using Citizen Science to Reduce Detection Times for Invasive Reptiles and Amphibians

Pauly G<sup>1</sup>

<sup>1</sup>*Los Angeles*

Urbanization presents one of the world's greatest biodiversity threats. Not only is urbanization responsible for habitat modification, but the number of people and goods moving through urbanized areas also increases the chance of nonnative species being introduced. Because of the increased availability of water and thermal retreats, urban areas can also allow nonnative species to get established in regions that otherwise would prove inhospitable. As a further complication, urbanized areas largely consist of private property (e.g., backyards) that is difficult for biologists to access. Fortunately, citizen science can be an effective method for documenting urban biodiversity. By partnering with citizen scientists through the Reptiles and Amphibians of Southern California (RASCals) project, we have documented five new state records and over 20 new county records of nonnative species in California over the past five years. These efforts are dramatically reducing detection times for nonnative species. Some of these observations have resulted in citizen scientists and museum researchers co-authoring peer-reviewed, scientific publications. These efforts also identify the nursery trade as a common pathway for introductions. However, the success of citizen science projects depends in large part on outreach efforts. Thus, we also review several outreach strategies that have proven effective at increasing participation in studies of urban biodiversity. Given the increasing availability of smartphones and internet access, and the increasing use of iNaturalist and similar citizen science platforms, citizen science should become a standard tool for conservation biologists around the world.

## Evidence for ancient hybridization between the Komodo dragon and Australian monitor lizards

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The increase in the number of phylogenomic datasets has revealed that hybridization is relatively common in animals. Coupled with recent methodological advances, it has allowed to detect unsuspected instances of ancient hybridization, even within well-studied groups such as hominids and felids. Based on a phylogenomic dataset, we found evidence supporting the past presence of gene flow between the Komodo dragon (*Varanus komodoensis*) and Australian monitor lizards in the *Varanus* subgenus. Hybridization likely occurred in northern Australia during the Neogene. This supports previous studies reporting the presence of *V. komodoensis* in mainland Australia as late as the Pliocene.

**Peacocks of the lizard world: phylogeny and taxonomy of the spectacular African flat lizards (*Platysaurus*)**

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The African flat lizards (*Platysaurus*) are some of the most spectacularly coloured reptiles in the world. They are dorso-ventrally flattened habitat specialists, morphologically adapted to life in narrow rock fissures. Their tight association with rocky outcrops across southern Africa has led to the evolution of multiple species that primarily have been diagnosed based on striking variations in male colour. We present the first comprehensive genetic assessment of *Platysaurus* based on 172 individuals representing all 14 described taxa, as well as recognised subspecies. We also obtained temporal divergence estimates within the genus using StarBEAST2 and fossil dates from closely related taxa. Combined with a thorough morphological assessment by the late prolific African herpetologists Don Broadley and Bill Branch, we present a full revision of the genus, elevating subspecies, sinking subspecies, describing new species and redescribing some existing species to accommodate the description of new clades. The resulting 28 *Platysaurus* taxa can be divided into four species groups; the “Western Cape”, “Southern African”, “Zimbabwe” and “Mozambique” *Platysaurus*. The differentiation of species within these clades is thought to have begun around the mid-Miocene in line with the onset of widespread aridification of continental Africa. A burst of divergence events around the Plio/Pleistocene was likely fuelled by the repeated submergence of rocky outcrops as the Kalahari sands shifted eastward.

## Population genetic analyses of the rediscovered Hula painted frog uncover unexpected structure

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Since its unexpected rediscovery in 2011, the rare and critically endangered Hula painted frog (*Latonia nigriventer*) has remained a puzzling animal. While our recent investigation of the only known viable population of this species confirmed the expected low estimates of both effective population size (~16.6–35.8) and potentially reproducing adults (~236–244), we found the population to have maintained a surprisingly high genetic diversity. A subsequent population structural analysis revealed that the individuals in this only ~600 m long shallow ditch belong to two genetically different clusters ( $F_{ST} = 0.075$ ;  $P > 0.001$ ). Astonishingly, we found expected and observed heterozygosities (mean  $\pm$  SD) to be relatively high for each sub-cluster:  $0.669 \pm 0.113$  and  $0.727 \pm 0.147$  for sub-cluster 1, and  $0.784 \pm 0.073$  and  $0.796 \pm 0.084$  for sub-cluster 2. Furthermore, allele frequencies differed substantially between the two sub-clusters that were both relatively large (sub-cluster 1:  $N = 45$ ; sub-cluster 2:  $N = 89$ ). These perplexing findings could perhaps be explained by the hypotheses of either genetic rescue from yet undiscovered, neighbouring Hula painted frog populations or by recent admixture of genetically divergent subpopulations. We applied the environmental DNA (eDNA) approach to detect additional populations and found traces of the Hula painted frog at 20 other locations within the Hula Valley, suggesting that both hypotheses may plausibly explain the observed structure. In any case, the high genetic diversity of the investigated population might have been promoted by the past drainage of the Hula marshes by facilitating contact among previously isolated subpopulations.

**Using Crowdsourced Data for Conservation: The Idaho Amphibian and Reptile iNaturalist Project.**

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Data on the occurrence and distribution of amphibians and reptiles are needed to identify and address conservation problems. Observations from crowdsourced and citizen science projects can be an important source of data for conservation and management. The goal of this project is to improve available data for Idaho amphibians and reptiles by collecting observations using iNaturalist, a mobile application that allows people to contribute observations of organisms using their mobile devices. Observations can also be contributed and viewed via the iNaturalist website ([inaturalist.org](http://inaturalist.org)). Observation records include photographs, time, date, geographic coordinates, and any other comments the observer wishes to make (e.g., life stage, habitat, and behavior). The Idaho Amphibian and Reptile iNaturalist Project was initiated in June of 2016 by the ISU Herpetology Laboratory. As part of the project, we created on-line and downloadable field guides for all of the species of Idaho amphibians and reptiles. As of May 2019, the project has over 2400 observations of 37 of the 38 species of Idaho amphibians and reptiles from over 438 participants, including students, teachers, agency personnel, and interested citizens. We evaluate and verify all observations which are then shared with the Idaho Department of Fish and Game Species Diversity Database. These observations will be used to help evaluate the status and trends for all Idaho amphibians and reptiles for the State Wildlife Action Plan which is used to establish conservation priorities.

### The long-term impact of cane toads on Australia's large varanids

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The introduction of toxic cane toads (*Rhinella marina*) has massively impacted Australian ecosystems, and their spread across the landscape has caused rapid population declines in large varanid lizards ("goannas"). But what are the long-term impacts? Do goannas recover, go extinct, or just 'hang on'? To clarify these long-term impacts, we conducted surveys with 51 goanna populations across two continental-scale transects, each spanning the full 80-year toad invasion chronosequence. We gathered data on population demography and goanna behaviour in two species; *Varanus varius* (east coast) and *Varanus panoptes* (northern Australia). Our sampling was stratified to incorporate 'time since toad invasion' as short, mid, long-term and uninvaded populations.

Encouragingly, goannas continue to persist throughout the entire invasion chronosequence, though the trajectories of population recovery are not consistent across species. *Varanus panoptes* populations declined immediately following toad arrival, and most continue to remain heavily depressed regardless of the time since toad invasion. *Varanus varius* populations decline gradually following invasion, but they too lack evidence of population recovery over time.

We discuss the factors driving these patterns: a combination of seasonal timing and quality of resource availability, compounded by toad invasion speed and landscape heterogeneity.



### **Indigenous Culture and Folk Taxonomy as Communication and Engagement Tools in Herpetology (via video)**

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From praise-songs with conservation messages, to animal totems and given names that have animal references, South Africa's indigenous culture is filled with hints of a close relationship with nature. The scientific community has viewed this relationship as one that is mostly detrimental for the environment but recent developments are changing this outlook. Thanks to transdisciplinary research, the outlook has progressed so much that some ecological knowledge is being drawn from indigenous culture. Here we share methods inspired by a pilot study investigating similarities between amphibian folk and scientific taxonomy in one province as a communication tool and later progressed into a nationwide project to study the relationship between South African herpetofauna and people's culture in order to understand the relationship's state and relevance as a science-based policy tool for conservation and social inclusion. The project builds on the pilot's results which include initiating a comprehensive list of indigenous species names for South Africa's amphibians and the country's first bilingual frog handbook written in English and an indigenous language (isiZulu).

### The influence of species assemblages on thermoregulation and habitat use in novel environments

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In lizards, behavioral thermoregulation is critical to maintain their body temperature within an optimal thermal range that allows physiological processes and behavioral mechanisms to take place. However, challenges associated with thermoregulation (i.e. interactions with other organisms) may impinge on this mechanism affecting the individual performance and eventually fitness, with consequences at population level. Depending on individual characteristics, organisms will be more or less capable or stimulated to cope with factors that prevent optimal thermoregulation. We explored individual behavior to understand the influence of social interaction on thermoregulation and habitat use of two species of anoles (*Anolis sagrei* and *A. carolinensis*) and a gecko (*Phelsuma laticauda*) introduced to and commonly co-occurring in the Hawaiian Islands. Data on habitat use and thermal availability were recorded through daily observations and thermographs of lizards within semi-natural enclosures with different combinations of community assemblages in a controlled environment. Thermal gradient trials showed large individual variation in thermal preferences which resulted in a broad overlap in this trait among the three species. In the enclosures, species shifted their thermal use in response to the different species assemblages: *Anolis sagrei*'s thermoregulatory behavior was slightly affected by the presence of the other two species whereas *A. carolinensis* and *P. laticauda* were decreasingly effective at thermoregulating when sharing the habitat with other species. Future data on the individual variation of the exploratory behavior and thermoregulation will provide new insights on whether these shifts in the use of thermal micro-environments explain the change in the species distribution through the islands.

**Using stable isotopes to investigate trophic transfer of mercury and radiocesium in *Nerodia floridana***

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Stable isotope techniques provide an approach for evaluating trophic transfer of contaminants. Typically, carbon isotopes ( $\delta^{13}\text{C}$ ) are indicative of consumer diet, while nitrogen isotopes ( $\delta^{15}\text{N}$ ) reflect trophic position. A positive relationship between  $\delta^{15}\text{N}$  and contaminant concentrations is viewed as evidence for biomagnification in a system. In this study, we quantified the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of scale samples taken from 74 *Nerodia floridana* (Florida Green Watersnakes) collected from three former nuclear cooling reservoirs (i.e., Par Pond, Pond B and Pond 2) to examine bioaccumulation and biomagnification of two contaminants - mercury (Hg) and radiocesium ( $^{137}\text{Cs}$ ). We observed significant among-site differences in average  $\delta^{13}\text{C}$  ( $F=80.38$ ;  $df=2, 70$ ;  $p<0.0001$ ) and average  $\delta^{15}\text{N}$  ( $F=13.36$ ;  $df=2, 70$ ;  $p<0.0001$ ). Among-site variation in average delta values was likely due to differences in isotope baselines and prey diversity at each site. We found a significant positive relationship between  $\delta^{15}\text{N}$  and Hg concentrations ( $r^2=0.29$ ,  $df=73$ ,  $p<0.001$ ). We found a significant positive relationship between  $\delta^{15}\text{N}$  and  $^{137}\text{Cs}$  for individuals collected at Par Pond ( $r^2=0.85$ ,  $df=9$ ,  $p<0.001$ ) but not for individuals collected at Pond B or Pond 2. Additionally, snake  $\delta^{15}\text{N}$  values ranged from 5.6‰ to 9.3‰, which is typically interpreted as a span of two trophic levels. Collectively, our results support that biomagnification of contaminants is occurring in our study systems. We are working towards quantifying contaminant concentrations and isotopic composition of prey occurring at each site. Analysis of these additional food web components will allow us to better characterize Hg and  $^{137}\text{Cs}$  biomagnification within our study sites.

## Documenting Frog Distributions Using Mobile Phones and Citizen Scientists

**Platenberg R<sup>1</sup>**

<sup>1</sup>*University Of The Virgin Islands*

Biodiversity monitoring is important for species and habitat management, although it can be costly in terms of personnel time and expertise. Many species of frogs can be effectively monitored aurally, and Citizen Science projects that engage community participants in conducting surveys using frog calls can provide high volumes of data at little cost. These data, however, are often associated with unknown observer error. Projects that remove decision-making (i.e., species identification) not only improve accuracy but also allow for higher participation by eliminating expertise requirements. A project using Citizen Science principles to solve logistical challenges was developed for amphibian survey across an island array in the United States Virgin Islands by inviting the community to collect recordings of frogs using cell phones. Nearly 300 recordings and location data were submitted by community participants during a two-week survey window. These were processed manually using aural and visual methods for species identification. Species detections were comparable to previous methods of survey although new distribution records were substantially increased. An additional benefit was that participants engaged in discussion about frogs through social media. Subsequent efforts to improve data submission, mapping, and results dissemination using mobile apps has proved challenging due to operating system and connection issues across participants, although these improvement efforts are continuing. This method offers potential for significantly expanding survey effort for predictably vocalizing species as well as gaining community understanding and support for amphibian protection.

## The Snake That Made It Back On Its Own: recolonization by the Puerto Rican Racer

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Caribbean snakes have been besieged by severe threats over the past several centuries. Non-native predators coupled with habitat loss have driven many endemic snakes to localized extinctions, particularly the diurnal ground dwellers. Efforts to reverse these trends can be culturally, financially, and ecologically challenging and do not always result in desired population restorations. The Puerto Rican Racer (*Borikenophis portoricensis*) is an apparent exception. The racer is native across the Puerto Rican Bank in the northeastern Caribbean, but has been extirpated from the human-inhabited islands of St. Thomas and St. John in the United States Virgin Islands, primarily due to high numbers of non-native mongoose (*Herpestes auropunctatus*). The snakes do, however, persist in abundance on nearby mongoose-free islands. At some point in the 1990s the mongoose populations on the eastern end of St. Thomas crashed, and there has been a slow recolonization of that part of the island by racers who have been arriving by sea, on their own, from nearby islands. Which islands are providing the source population is unknown. This offers an interesting study into metapopulation dynamics, species colonizations, and over-water dispersal. The results can provide valuable direction for island species conservation and insight into species invasions.

## Preparing for reintroduction: behavioral and morphological fitness of captive-bred amphibians produced from cryopreserved sperm

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With increasing rates of global biodiversity decline, strategies such as reintroduction programs, have become increasingly important in species conservation. To sustain reintroduction programs, insurance colonies are established and bred in captivity. Captive-bred offspring are then released to augment wild populations or establish new populations. A key issue determining the success of reintroduction programs, therefore, is the fitness of captive-bred individuals and their ability to survive once released. Unfortunately, thus far, little is known about the fitness of captive-bred offspring produced using assisted reproductive technologies, such as gamete cryopreservation. To fill this gap in scientific knowledge and conservation practice, we examined differences in tadpole morphology, tadpole behavior, metamorph morphology, and duration of larval stage between Fowler's toad (*Anaxyrus fowleri*) offspring produced using cryopreserved sperm (experimental, cryo-derived individuals) and offspring produced by amplexic adults (control individuals). Results indicated cryo-derived individuals were smaller as tadpoles, emerged as smaller metamorphs. However, predator avoidance behavior was not significantly different between the two treatment groups. Smaller body size in cryo-derived individuals can negatively affect their post-release survivorship, thus limiting the potential success of reintroduction programs. This pioneering study provides insights into the comparative morphological and behavioral fitness of individuals produced using cryopreserved sperm across two distinct life-stages. We show that although cryopreservation has often been proposed as a promising way of contributing to wildlife conservation, more detailed examinations are needed to assess the quality of offspring produced for it to be an effective conservation tool.

## Examining Spotted Marsh Frog *Limnodynastes tasmaniensis* call races using large-scale citizen science data

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Call races, representing significant intraspecific variation in advertisement calls associated with geographic regions, have the potential to offer insights into the phylogeographic history of a species. In the case of species which have call races occurring in contiguous regions, there is also an opportunity to advance our understanding of hybridisation and speciation. The Spotted Marsh Frog (*Limnodynastes tasmaniensis*) is widely distributed throughout eastern Australia. It has three documented call races, southern, northern and western, all of which are most readily distinguished by the number of notes per call. Overlap zones occur along geographic points of contact between the call races and are characterised by hybridisation of the calls. Broad boundaries of these overlap zones were assessed over 50 years ago. We now take advantage of citizen science data, collected by FrogID, to examine the current extent and interaction of the call races on an unprecedented scale. The large volume of data analysed, over 2000 recordings from across the range of *L. tasmaniensis*, has further allowed us to consider call variation in this species as a whole. FrogID is a nationwide citizen science project aimed at capturing validated biodiversity data on frogs. Call recordings were collected with smartphones by users of the FrogID app. We then allocated calls to call races and produced maps to show the relative stability of the call race boundaries and overlap zones over time. This project demonstrates the potential importance of citizen science data for improving our understanding of speciation and frog distribution.

**There and back again: invasions of *Microhyla* (Amphibia: Microhylidae) frogs from Southeast Asia to India**

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The narrow mouthed frogs of the genus *Microhyla* (Amphibia, Microhylidae) inhabit various altitudes and habitats from primary tropical forests to paddy fields in South, Southeast and East Asia. Possibly due to small size and superficial morphological similarity the taxonomy of the genus was long neglected and currently is in a state of a flux complicated with findings of lineages that cannot be related to any of described species. In this report we present the most complete phylogenetic analysis and shed light on historical biogeography and body size evolution of the genus *Microhyla*. Our work offers the most complete taxon sampling to date by including more than 90% of the diversity of the genus (43 out of 46 species). We used fragments of mtDNA genes (2598 b.p. of 12S —16S rRNA) and nuDNA gene (720 b.p. of BDNF) to analyze 225 samples of Microhylidae. Analyses provide the most comprehensive taxonomic and gene sampling for *Microhyla* to date and reconstruct biogeographic scenario for the genus. Our analyses indicate presence of at least 6 undescribed species-level lineages of *Microhyla*. Extreme miniaturization appears in different lineages of *Microhyla* independently and we tend to associate it with ecological specialization. Our results contribute to the interpretation of diversification patterns of Southeast Asia batrachofauna and provides insights into the historical biogeography of this region. The research was supported by the Russian Science Foundation RSF, grant No. 19-14-00050.



## Recent progress in studies of Asian narrow-mouthed frogs (Anura: Microhylidae)

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The collision of the Indian subcontinent with Eurasia is widely recognized as one of the main factors in the formation of its biodiversity. Ancestors of three endemic Microhylidae subfamilies known to occur in Asia have supposedly reached Eurasian landmass on the Indian tectonic plate: Microhylinae, Kalophryinae and Melanobatrachinae. The fourth subfamily, Asterophryinae, which is the most speciose group among all microhylids, is distributed mostly in northern Australia, New Guinea, and adjacent Australasian islands with very few species occurring west from the Wallace line. The “Antarctic route scenario” was proposed for asterophryines, suggesting that their ancestor colonized Australia via the Antarctic land bridge and subsequently dispersed to Australasia. However, the discovery of *Siamophryne* and *Vietnamophryne* – two new asterophryine genera from Indochina in 2018, as well as recognition of *Gastrophrynoides* from Sundaland as an asterophryine, suggests that the basal split of the subfamily may have occurred on the Eurasian mainland, from where it dispersed to Australasia, thus supporting the alternative “out of Indo-Eurasia” biogeographic scenario for asterophryines. At the same time, the growing amount of evidence suggests a more complicated history of Cenozoic biotic exchange events between India and Eurasia than thought before. Dispersals from India to Eurasia likely took place in early microhylid history prior to the hard collision of the two landmasses. Multiple independent colonizations of India from Eurasian ancestors are suggested for several groups of microhylines starting from the late Eocene. This study was carried out with financial support from the Russian Science Foundation (RSF grant № 19-14-00050).

## Aspects of the ecology of two freshwater turtle species in KwaZulu-Natal, South Africa

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South Africa's freshwater systems are under increasing anthropogenic pressures due to a growing population, land-use change and climate change. It is important to understand how organisms reliant on wetland systems are coping with pressures to assess their ability to persist. There is a paucity of general knowledge of freshwater turtle ecology in South Africa, despite their importance in freshwater ecosystems. Measures of movements, regularity, and activity regimes can provide valuable insights into an animal's behaviour and general condition, and what is required to ensure populations remain stable. Consequently, we investigated aspects of freshwater turtle (*Pelomedusa galeata*, and *Pelusios sinuatus*) ecology, using several different techniques. Ten individuals of each species had UHF tags attached to monitor movements and activity patterns at two different field sites in KwaZulu-Natal, South Africa. This showed trends in individual behaviour/movement in relation to environmental variables, in particular temperature and drought conditions. As well as the two telemetry sites throughout the province non-telemetered animals were permanently marked on their marginal scutes using a standardised numbering system in order to easily recognise recaptured individuals, recaptured individuals had their location and all their metrics recorded again, all specimens had bio-morphometric data collected to evaluate general condition, and a tissue sample was collected for genetic analysis. We present our findings on both species' ecology from throughout KwaZulu-Natal. Our findings can aid in the conservation and management of both species.

## The effect of constant egg incubation temperatures across life stages in the brown anole

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The environment dramatically influences phenotypic variation either by acting as a selective force on existing phenotypes, or via phenotypic plasticity. Plastic responses to the environment can be observed across taxa and life stages, but developmental stages are particularly sensitive to the environment. Furthermore, plastic responses during these stages have the potential to carry fitness consequences into later life stages. Developmental plasticity has been studied in many taxa, but oviparous reptiles have served as excellent models. The brown anole (*Anolis sagrei*) has well-documented plastic responses to developmental environments, such as moisture and temperatures conditions during egg incubation. These factors affect incubation duration, survival, and fitness related traits. However, many studies use few treatment groups and lack the capacity to provide well defined reaction norms. Furthermore, many studies end at hatching and do not examine the effects of incubation conditions on later life stages. To determine the effects of incubation temperature across life stages, we incubated eggs of the brown anole under eight constant temperature regimes and conducted a field-based mark-recapture study on the hatchlings. We found that egg hatching success had a quadratic relationship with temperature such that it increased to a peak and decreased sharply at warmer temperatures. This effect of incubation temperature on survival differed at the juvenile stage, suggesting that different stages have different optimal incubation temperatures.

## The use of community science to study the ecology of herpetofauna in cities

**Putman B<sup>1</sup>**

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Community science (also known as citizen science) is a powerful tool that can generate large datasets across large geographic scales and in relatively short time periods. These data have been harnessed by researchers to evaluate changes in phenology and species distributions, and we expand on the uses of these data to examine fitness-relevant traits in association with urbanization. We used photographs of Southern Alligator Lizards (*Elgaria multicarinata*) submitted to the community science platform, iNaturalist to test the hypotheses that predation risk increases with urbanization and parasitism decreases with urbanization. We quantified predation risk by measuring tail loss rates and we quantified parasitism by counting tick loads on lizards within the photographs. We found that tail loss rates increased along a rural to urban gradient, but declined in highly urban areas (e.g., commercial districts). Conversely, ectoparasitism loads decreased with urbanization likely due to a loss of intermediate hosts (small mammals) and anti-tick medications used on domesticated animals. In all, we show that a community science-dataset provides larger sample sizes than those obtained from studies using traditional data collection techniques, and that the geographic extent of the community science-dataset is larger than those of traditional datasets such that quantitative measures of urbanization (e.g. percent impervious surface cover) can be associated with phenotypic traits as opposed to qualitative measures (e.g., urban vs rural).

## Social complexity drives the evolution of display complexity in agamid lizard *Phrynocephalus przewalskii* from China

Qi Y<sup>1</sup>

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Movement-based signals are quite common and have been documented in diverse taxonomic groups, ranging from mammals to invertebrates, and are used in a variety of contexts including opponent assessment, mate choice, and for deterring predators. Lizards from the Iguanid and Agamid families have been widely studied in the context of movement-based signalling. Recent analyses have suggested that signal complexity in lizard displays relates to habitat structure, with differences in signal complexity between species influenced by ecological factors such as home range size and diet. In China, the toad-headed agamas from the genus *Phrynocephalus* are known for their diversity of tail signalling, yet the driving forces behind the evolution of display complexity is not well understood. Consequently, we quantified the structure and complexity of tail displays used for signalling in toad-headed agamid *Phrynocephalus przewalskii* from 12 populations across the whole distribution area of this species. We also estimated the social complexity using the sex ratio and population density. We subsequently related measured signals with social complexity and examined the association between display complexity and social complexity. The results shown that there is significant association between display complexity and social complexity, the higher the social complexity, the higher the display complexity. Our study provides the first, as we known, through and direct evidence on the effect of social complexity to display complexity within species.

**Breeding behavior of Indochinese Spitting Cobra (*Naja siamensis*) in Sakaerat Biosphere Reserve, Nakhon Ratchasima, Thailand**

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Though Thailand is thought to have over 200 species of snake, very few have been documented as exhibiting nesting attendance. Of those 200 only 5 species have been documented attending a clutch. Nest attendance is thought only to occur in King Cobras (*Ophiophagus hannah*) in the family Elapidae; however, we report evidence of Indochinese Spitting Cobra *Naja siamensis* staying with their clutch until neonate arrival. From January 2015 through May 2019 we radio tracked 21 (11 males, 10 females) *Naja siamensis* in the Sakaerat Biosphere Reserve, North East Thailand. Through radio tracking and utilizing field cameras, we recorded two mating pairs and a total of 5 nests attended by 4 different radio tracked females. One female was killed by local villagers during nesting period. Using camera trap data and in-field observations, we recorded potential nest predators in the nest area. One of the clutches was unsuccessful due to predation caused by Small Banded Kukri *Oligodon fasciolatus*. In total, we documented 35 neonate cobras. The information gathered here has wide reaching implications for understanding the behavior and natural history of the medically important, yet misunderstood species.

## **Urban Herpetofauna and Evaluation of Public Attitude towards Significance and Conservation of Amphibians and Reptiles**

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Amphibians and reptiles do not get attention in research and conservation initiatives in Pakistan. These are considered as unhygienic, filthy and dangerous due to various myths and negative social perception. We aimed to record common herpetofauna of urban areas of Islamabad Capital Territory and Rawalpindi City. We conducted questionnaire survey to evaluate attitude and understanding of local people about herpetofauna. We recorded amphibians such as Indus Valley Toad, South-east Asian Toad, Common Skittering Frog and reptiles such as Monitor Lizard, Wall Lizard, Striped Grass Skink, Garden Lizard, Leopard Gecko, Black Cobra, Indian Krait, Brown River Turtle and Indian Flapshell Turtle. The results from the survey showed that out of 251 respondents, 47% respondents were interested in wildlife conservation. Only 32% respondents were aware about wildlife species diversity in Islamabad/ Rawalpindi district. About 76% of the respondents showed their preferences towards birds while 20% showed interest in mammals. However, only 1% respondents showed interest for amphibians and reptiles. About 30% of the respondents had some knowledge about significance of amphibian and reptiles. However, most of them were unable to differentiate between a frog and a toad. Majority (92%) of the respondents showed negative attitude about reptiles and hold the opinions that all lizards and snakes are poisonous/venomous should be killed. The conversion of natural habitats into urban settings have favored spread of tolerant and invasive species. We suggest to carry out conservation awareness campaigns to make inhabitants of the twin cities aware about significance of amphibians and reptiles.

**A primer of amphibian research and conservation in Pakistan: Current situation and the way forward (via video)**

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<sup>1</sup>*PMAS Arid Agriculture University Rawalpindi*

Despite concerns about global decline in the populations, amphibians do not find any place in wildlife conservation and management decisions, policy making and research agendas in Pakistan. We are conducting detailed studies and conservation awareness program on amphibians of Pakistan for the first time. During the first phase of our projects, we recorded eight anuran species from sub-tropical scrub and pine forest (District Rawalpindi and Islamabad Capital Territory). Our results showed that pine forest featuring high altitude and natural freshwater streams with low water temperature (<13 °C) had a relatively higher anuran endemism. Space use data suggested high site fidelity by endemic anurans (Hazara Torrent Frog, Murree Hills Frog). Increase in the atmospheric/ water temperature or water withdrawal from the streams by local community could seriously impact populations of these species. The species might be forced to perform over land migration through the forest to occupy nearest streams which are situated at a distance difficult to travel by amphibians or upstream migration that would require considerable energy reserves and may cause stress in the individuals. The data on impacts of climate change on amphibian species inhabiting northern parts of the country are lacking. However, it is feared that these species might face similar consequences as that of amphibians found elsewhere in the world. We believe a few anuran genera require detailed taxonomic studies, and that molecular taxonomy would add more species to known anuran diversity. We have suggested options for education, research, conservation, management and stewardship of amphibians of Pakistan.



### **How can mitigation guidelines be applied effectively?**

#### **Randall L<sup>1</sup>**

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Mitigation translocation involves moving animals that would otherwise be destroyed or negatively affected by development activities to an alternative release site. The use of translocation as a mitigation tool for threatened amphibians is increasing, but it is not without risk—the practice can result in injury, mortality, stress, exposure to pathogens and genetic risks for the translocated animals or animals at the release site. Little is known about the success of these types of translocations, but success rates are presumed to be low, mainly due to dispersal from the release site or homing to the collection site, or because release sites lack suitable habitat to meet the needs of all life-stages. Many jurisdictions in Canada currently allow translocations without providing clear guidance on how to plan, implement, and monitor translocations. We developed amphibian mitigation guidelines in collaboration with government agencies in a 4-year process. We discuss the use of guidance documents and adoption of guidelines into policy in Canada's Prairie Provinces and in the wider context of Canada.

## The species status of the viviparous sea snakes

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Globally we have around 65 species of viviparous sea snakes recognized depending on the species concept used. The group is distributed in the Indian and Pacific Ocean; however, most species occur in the Indo-Malayan Archipelago, the China Sea, Indonesia and the Australian region. The viviparous sea snakes are not at all easy to identify to species level and the group include some taxonomic problematic species that need a more throughout morphological and molecular systematic investigation. At the moment the group is separated into two distinct clades: 1) The *Aipysurus* group, including only few recognized problematic species as *Aipysurus pooleorum* and the *Emydocephalus*-complex (including three accepted species for the moment) and 2) The *Hydrophis* group which have many more recognized problematic species as *Hydrophis cyanocinctus/pacificus*, *H. curtus*, *H. fasciatus/atriceps*, *H. inornatus*, *H. melanosoma*, *H. torquatus*, and *H. vorisi* just to mention the most obvious. The talk will be concentrated about some of the above mentioned taxonomic problematic species and how we can solve the problem in getting more morphological as well as molecular data gathered.

**Climate change impacts on amphibians in Madagascar: shifts to higher elevation and increased risks of extinction**

**Raxworthy C<sup>1</sup>**

<sup>1</sup>*American Museum of Natural History*

Madagascar's biodiversity is world-renowned for its island endemism, exceptional species richness, and conservation concern. The amphibians of Madagascar exhibit all these qualities: 99% endemic, 365+ species, and are often highly restricted in their distributions, confined to small regions, single mountain systems, and/or specific habitat types. As natural forest has been cleared or degraded in Madagascar, this has further fragmented and isolated populations. A consequence of all these features is that the amphibians of Madagascar are exceptionally vulnerable to climate change, where documented warming temperatures are shifting species to higher elevations. Although some species may be able to rapidly change their climatic niches, most are expected to shift distributions upslope, with population extinctions on the 'warm' lower elevation edge. However, montane endemic species living close to mountain summits, and other species lacking migration access to higher elevation habitat (for example due to habitat loss and fragmentation) will lose distribution and become more vulnerable to extinction. Amphibian survey results for the highest massif in Madagascar: Tsaratanana, will be used to assess these climate change impacts for montane species. And data from the Global Amphibian Assessment for Madagascar will be presented for all montane species, to describe extinction threats from upslope shifts under future climate warming scenarios. The ongoing degradation of forest elevational transects, and the discovery of new cryptic amphibian diversity, suggests that we may still be underestimating the true extinction threat of climate change to amphibians.

## Research priorities for lizard conservation in New Zealand

Reardon J<sup>1</sup>

<sup>1</sup>Doc

New Zealand has more than 106 spp. of extant terrestrial reptiles. Ninety-six are classified as in some form of conservation need and 37 are at imminent risk of extinction, according to the New Zealand threat Classification System. Only ten species are classified as Not Threatened. To avert the looming extinction event, it is essential that conservation research priorities are carefully examined and clearly stated. Threatening processes include habitat loss through continued anthropogenic development and ecological processes some of which may be exacerbated by climate change, but invasive predator impacts are by far the most ubiquitous and serious threatening process. Over the past 15 years there have been several research by management studies that have limited those impacts to achieve recovery in lizard populations, but they are often extremely expensive and intensive, generally focus on apex and larger meso-predators but have also often failed to demonstrate a strong response in lizard populations. The impacts and mitigation of invasive meso-predators are much less well understood for lizard conservation. Noteworthy amongst these is the role of house mice, *Mus musculus*. Robust evidence describes mouse impacts on lizards yet there are currently no tools or strategies for their sustainable control other than eradicated off-shore islands or mammal-proof fenced areas that face their own suite of risks and cannot cater for most threatened lizards. Thus, the priority for lizard conservation in NZ is to test new rodenticides, tools and strategies within existing top-tier predator-controlled areas to develop successful, sustainable predator control that accounts for mice.

## **The conservation management of grand and Otago skinks as a case study**

**Reardon J<sup>1</sup>**

<sup>1</sup>DOC

The grand and Otago skink are two of NZs largest lizards, occurring in disjunct and relictual areas of their former range. Monitoring populations under predator management during the early 2000s suggested a precipitous decline towards extinction. Agents of decline were unclear with invasive predatory mammals, disease, habitat degradation and Allee effects all potential contributors. A recovery programme review led to the testing of mammal proof fences and landscape scale predator control with captive management as security. The life history and low population sizes of these species meant there was a small window to interpret the outcomes of the management trials before it became impossible to implement management prior to functional extinction. Both mammal proof fenced enclosures and landscape scale predator control yielded strong responses for both species over three seasons, with increases of 30% and 66% in mammal proof fenced populations of grand and Otago skinks and in the core of landscape predator control, recovery of 46% and 94% for grand and Otago skinks. At non-treatment control sites Otago skinks showed no change whilst grand skinks declined by 85%. Stochastic predation events appear to be a significant factor in decline. The cost of this work was sizable, whilst the outcomes for the managed populations were positive resulting in down-listing from Nationally Critical to Nationally Endangered, methods have not been expanded to manage a more ecologically meaningful area, nor have they been employed to prevent extinction in distinct western populations of these species or applied to other similarly threatened lizard populations.

**Rapid eradication tool testing for invasive amphibians: *Duttaphrynus melanostictus* in Madagascar**

**Reardon J<sup>1</sup>**

<sup>1</sup>Doc

Anuran invasions in Australia, North America, and Hawaii have provided evidence of ecological impacts that have elicited management responses attempting to control, contain or eradicate the invasive populations. In 2014, the Asian or black-spined toad (*Duttaphrynus melanostictus*), native to southern Asia, was recorded in Madagascar. Biological similarities to the cane toad raised concerns for Madagascar's globally significant biota, which lacks toads and their toxins. Effective response required coordination between the international community, industry, and government. A feasibility study identified the need to test potential eradication tools whilst gathering resources to employ tools at a trial scale. We measured toad densities across habitat types in the infested zone. We also tested the efficiency of toad removal using (1) manual removal, (2) pitfall trapping with drift fencing, (3) tadpole traps, and (4) citric-acid sprays. Results suggest that manual collection of toads can remove large numbers of individuals, but considerable effort is required to see catch rates decline. Drift fencing with high-density pitfall trapping is effective but is only applicable to a limited extent, and maintenance is challenging in agricultural environments. Tadpole trapping was ineffective. Citric-acid spray (w/w25%) consistently resulted in 100% mortality for small toads (SVL<35mm) and high mortality rates (30-100%) in adult toads, even in complex habitats. Our results suggest viable eradication tools may exist for this and similar species, but strategies and leadership to progress eradication or management in Madagascar are extremely weak. Eradicating future incursions can build on these technical advances, but response protocols and coordination require rapid development.

### Cellular and whole-organism effects of prolonged vs. acute heat stress in a montane, desert lizard

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Climate change includes both prolonged periods of higher-than-normal temperatures, and more frequent and severe heat waves. Both types of temperature increases are likely to be detrimental to ectotherms, and even if they do not cause mortality directly, compensating for such temperature increases will likely entail tradeoffs. We tested the effects of prolonged periods of higher temperatures and short-term, acute heat stress in wild populations of desert short-horned lizards (*Phrynosoma hernandesi*), a temperate, montane lizard of the Colorado Plateau in the USA. We transplanted one group of lizards from a high- to a low-elevation site, exposing them to a prolonged period of warmer temperatures. We exposed a second group of lizards to acute heat stress by heat-shocking them with temperatures near their critical thermal maximum for 4 hours. Lizards exposed to prolonged periods of higher-than-average temperatures experienced no change in sprint speed, endurance, or heat shock protein expression after treatment compared to baseline levels; however, they had lower water content and lower body condition after the transplant to a warmer climate compared to before the transplant. In contrast, lizards exposed to a short period of acute heat stress had no change in endurance, water content, or heat shock protein expression following heat shock; however, heat-shocked lizards had slower sprint speeds following heat shock than before heat shock. Our results demonstrate that prolonged temperature increases and acute heat stress, both of which are predicted to occur with climate change, have substantially different effects on lizards at both the cellular and whole-organism levels.

**DNA-based methods for monitoring pond-breeding amphibians: the case of the Natterjack toad in Ireland**

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Knowledge of species distribution and abundance plays a central role in population management and species protection. Recent advances in genetics have enabled the development of new methods with broad applications in conservation projects. We present two non-invasive molecular approaches for monitoring the rarest amphibian species in Ireland: the Natterjack toad. Genetic capture-mark-recapture (CMR) was used for estimating the population size and breeding sex ratio. We compared the results from CMR to the estimates derived from the commonly used method of counting egg masses and argued against the practice of translating egg counts into total population estimates. The second approach used environmental DNA (eDNA) for detecting species presence and estimating relative abundance. eDNA capture and extraction protocols vary across studies but are often focused on sampling clean waters. Application of eDNA methods for detection of pond-breeding amphibians may often require sampling of stagnant and murky water bodies where purification and successful amplification of target regions can be difficult. Our main objectives were to determine the best practices for eDNA detection in turbid water samples and to experimentally test the sensitivity of the developed method and eDNA persistence in water. We took advantage of new technologies in the area of qPCR and developed assays on two portable platforms. Estimates of pond occupancy and eDNA concentration in samples were compared with species presence and abundance from field studies. The results helped target areas where more intensive surveys were needed and evaluated the success of species restoration practises.



## Evolution of skin hydrophobicity in Australian geckos (Diplodactylidae and Carphodactylidae)

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Some animals are hydrophobic (their skin repels water). Gecko skin is hydrophobic because of specialized microstructures (spinules). Spinules provide strong self-cleaning and bactericidal properties, which are enhanced if spinules are long. We hypothesised that higher exposure to dirt and pathogens, in conjunction with terrestriality would select for longer spinules, while arboreal species have shorter spinules. We tested this hypothesis using the advancing contact angle measurement of hydrophobicity (ACA), and measured 24 Australian gecko species to assess the hydrophobicity of species occupying in different microhabitats. We reconstructed the evolution of the ACA values, in relation to the microhabitat use by the geckos, using a published supertree and modelling using phylogenetic generalized least squares (PGLS), and model averaging based on AICc values to estimate the best fit. All species had highly hydrophobic skin (ACA > 132.72°), but longer spinules and small scales were correlated with high hydrophobicity (ACA values). Terrestrial species had significantly higher ACA values than arboreal or saxicoline ones. These results suggest that hydrophobicity has co-evolved with terrestrial microhabitat use in Australian geckos, through a combination of long spinules and small granule scales, ostensibly to resist diseases and fouling.

## Scaly Sociality: Testing for the Presence of Kin-Based Sociality in Australian and African Lizards

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Animals exhibit tremendous diversity in sociality; from organisms that live their life almost exclusively alone to those that live in large groups. Yet, our understanding of sociality has a taxonomic bias. Research is mainly focused on birds, eusocial insects, and primates, which confines our understanding of the diversity and evolution of sociality. In the last few decades, lizards have shown to exhibit a range of social and mating systems. It is thought that, for particular animals, specific ecological and life history characteristics act in concert to select for the evolution of family-living and parental care. For example, live bearing and longevity favour natal philopatry, and a species' reliance on limited resources, like rock crevices for shelter, promote grouping. I will present research investigating the social systems of 5 lizards that exhibit life history and ecological characteristics that, combined, may select for the evolution of family living. First, the Australian Tree Skink (*Egernia striolata*), which belongs to the social *Egernia*-group of lizards, as well as preliminary data on a potentially novel lineage of family-living squamates – 4 species of cordylid lizards from South Africa. We have collected field data on lizard space use and social associations, and are using molecular analyses to estimate relatedness between individuals. Group size and composition will be reported for each species. For Tree Skinks I will also present correlations between home range overlap, social associations, and relatedness data to assess the kin-basis of their social system. This research expands our knowledge of reptile and vertebrate sociality.

***Varanus rosenbergi* and *Nasutitermes exitiosus*: commensalism symbiosis?**

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There are only 2 Varanid species in Australia that exclusively utilize termite mounds as incubation chambers for their eggs. The breeding ecology of *V. rosenbergi* and its relationship to *N. exitiosus* mounds is only one that has been thoroughly examined in the field. Seven to ten days prior to egg laying, *V. rosenbergi* females travel after dark up to 500m from the nuptial burrow and scratch the surface of termite mounds in the area. One of these mounds is then 'chosen' as the incubation chamber. After two to three days of digging the female enters the excavation at dusk and lays her clutch, usually consisting of 12 eggs. In active mounds, termites seal off the entrance before daylight. Temperature monitored in the mounds shows seasonal trends with far less variation than ambient air or ground temperatures. Humidity remains at high levels throughout the year (92 – 98%). Seasonal temperature fluctuations vary between termite mounds and affect the duration of egg incubation. Hatchlings emerged 215 to 259 days after egg deposition depending on mound temperatures.

No true symbiotic relationship has been identified between goanna and termites. Termites encase the semipermeable shell surrounding each egg as it takes on moisture and expands. Young feed on termites after hatching (determined from fecal samples). After emerging, young may continue using the mound as shelter for up to three months. *N. exitiosus* only benefit when eggs are infertile. In this instance, the eggs are consumed entirely.

**Genetic diversity and mito-nuclear discordance in Neotropical toads (*Rhinella*)**

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Toads are known to exhibit complex genetic patterns, including introgression and hybridization, across populations. Species from the genus *Rhinella* in Central and South America provide a useful system to investigate how prevalent these phenomena are in widespread natural toad populations across a large geographic region spanning heterogeneous climates and landscapes. Here we explore genomic diversity and structure of different species belonging to the genus *Rhinella* occurring across complex ecosystems in the Neotropics. We used phylogeographic methods, incorporating both mitochondrial and ddRADseq data, to find mito-nuclear discordance across populations. We also comment on integrative taxonomy for these species. Results from this study aim to improve knowledge about genomic patterns of hybridization and structure in natural widespread toad populations.

## **Artificial retreats increase the abundance of the endemic and endangered lizard *Liolaemus lutzae* from Brazil**

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Use of artificial retreats (ARs) has been advocated as a valuable conservation tool for threatened species, especially during habitat recovery and translocation programs. However, few studies have demonstrated the benefits of these structures for lizards and none of these in tropical climates. The development of relatively simple and inexpensive methods and tools for conservation of biodiversity can improve the efficacy of a conservation program. In this sense, our role as conservation scientists is to provide decision-makers options that enable the investment of the already scarce public money in strategies that may achieve significant results with best cost-benefit ratio. We evaluated the effectiveness of inexpensive artificial retreats and of a non-invasive, ethically acceptable and time-saving method of monitoring lizard populations. Our study model was a well-studied sand lizard (*Liolaemus lutzae*) endemic to the Rio de Janeiro coast. We performed an experiment with 10 control and 10 experimental plots divided into an initial baseline period and a subsequent test period, where 20 artificial retreats were installed in experimental plots. We demonstrated that the number of lizards increased only in plots with ARs, showing clear benefits for the local population, which indicated that ARs could be successfully used in conservation programs also with lizards adapted to warmer climates. These retreats could be a useful and inexpensive tool to monitoring and facilitating the establishment of reintroduced lizards and for the recovery of declining populations, offering additional refuge from predators and from overheating in a world of increasingly high environmental temperatures.

**Climatic preferences of a sand lizard population (*Lacerta agilis* Linnaeus 1758) in western Germany**

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<sup>1</sup>Zoologisches Forschungsmuseum Alexander Koenig

Fast and efficient data collection has long been a concern in applied ecology and land planning. Our study focuses on developing a method for thorough and efficient spatial explicit population assessment of small vertebrate species on landscape level. We exemplify our approach using *Lacerta agilis* population close to Cologne. In a previous study we have performed species distribution models and population connectivity analyses of *Lacerta agilis* populations based on field surveys and remote sensing data. The resulting maps showing potential habitats and gene flow paths are used by an environmental management agency for conservation planning. In our follow up project we expand the data sets and develop much more fine scale predictions for habitat suitability and potential population sizes. One major part of this study is the use of very high resolution remote sensing techniques using a drone, which allow us to quantify not only habitat structure but also microclimatic features. In concert with capture-mark-recapture analyses and radio telemetry, this allows for fine scale assessment of the spatial properties of home ranges and corresponding habitat preference. The study combines conventional field work with novel approaches and has increasing importance nowadays, as detailed small scale ecological assessments are necessary in land use, construction planning and conservation.

## Ecophysiology predicts the fundamental niche of native and invasive populations of the African Clawed Frog

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One main threat promoting the worldwide amphibian decline is the introduction of non-indigenous amphibians, like the African Clawed Frog *Xenopus laevis*, which is now one of the widest distributed amphibians occurring on four continents with ongoing expansion including large parts of Europe. Species Distribution Models (SDM's) and the concept of ecological niche are essential to predict the invasive risk of those species. Previous efforts to predict distributions of invasive amphibians have mainly focussed on correlative approaches but these can be vulnerable to extrapolation errors when projecting species' distributions in non-native ranges. Recently, more robust process-based models, which use physiological data like critical thermal limits, or hybrid models of both approaches were used for this purpose. Previous correlative SDM's for Europe predict different patterns in the potential distribution but it is likely that these models do not access the full invasive potential. Based on physiological performance trials we calculated size and temperature depending response surfaces, which were scaled to the species' range matching the critical thermal limits. These ecophysiological performance layers were used in a standard correlative SDM framework to predict the potential distribution in South Africa and Europe. We found thermal performance differed significantly among native and invasive populations indicating some degree of fundamental niche change, which lead to different potential distribution patterns for the native and invasive populations in the respective ranges. Our hybrid-SDMs revealed that *X. laevis* has a much higher invasive potential than previous correlative models predicted for Europe.

**It's getting hot in here: predicting how diving ectotherms will be impacted by climate warming**

**Rodgers E<sup>1</sup>**

<sup>1</sup>*The Australian National University*

Air-breathing, diving ectotherms are a crucial component of aquatic ecosystems, but the threat of climate warming is particularly salient to this group. Dive durations are inversely related to water temperature and time available for obligate underwater activities- like foraging and predator avoidance- may be cut short as temperatures rise. A meta-analytical approach was used to synthesise data on the effects of climate warming on dive durations in air-breathing ectotherms. Effect sizes were extracted from published literature on a wide range of species, from crocodylians to newts. The buffering role of potential safeguards, including physiological remodelling, behavioural adjustments and geographic range shifts were also assessed. Together, these data shed light on a previously overlooked threat to diving ectotherms, identify knowledge gaps and offer predictions of how this group will fare in a warmer world.



## Snakebite envenoming and rattlesnake conservation vulnerability zones in the Baja California Peninsula, Mexico

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Mexico is considered the diversification center of the genus *Crotalus*, with 36 species present in its territory of the 42 species currently known. Rattlesnakes are very important in the Mexican ecosystems and culture. However, in spite of their huge importance, they are severely threatened by human development. This development has increased human presence in rattlesnake habitat for different purposes ranging from tourism, agriculture, housing to mining and communication roads.

In the Baja California Peninsula, there are, depending on the author, from 8 to 15 species of the genus *Crotalus*; 8 endemic to islands and 2 endemic to the peninsula. All of which are threatened by the same antropic factors. On the other hand, this species has caused over 500 snakebite envenomings from 2003 to 2018, mainly in men, due to their field related jobs.

In order to be able to prevent snakebite envenoming or the decline in rattlesnake numbers, this study delimits vulnerability zones for both scenarios.

With the use of ecological niche modeling (ENM), we obtained the site suitability range for 6 *Crotalus* species in the Peninsula region. Then, we overlaped those ENM with antropic factors, health services, conservation polygons and land use in order to establish zones where humans are more vulnerable to snakebites, and zones where rattlesnakes are vulnerable to decline.

The results where: 1 high vulnerability to snakebite zone, and 3 high vulnerability for rattlesnake decline zones.

Policy aiming those 2 issues should be made based on the available information.

## **Amphibians and reptiles in two geothermal localities in the state of Puebla, México**

**Rodríguez-Canseco J<sup>1</sup>**, Romero-Figueroa G<sup>1</sup>, Raymundo-González I<sup>1</sup>, González-Estupiñán K<sup>1</sup>

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The use of fossil fuels has proven to be an unsustainable form of energy due to high levels of atmospheric carbon dioxide, among other pollutants. For this reason, Mexican government is transitioning to cleaner energies, such as eolic, solar and geothermal. Regarding geothermal energy, Mexico installed its first plant in the 1950's and dismantled it in 1973, since then four more functioning plants have been installed in Baja California, Baja California Sur, Michoacan and Puebla. However, in spite of the importance of transitioning to cleaner energies, there have been no studies on how this "new" way of harvesting energy impacts on the surrounding wildlife and even more troubling, the diversity studies are incomplete and outdated.

This study focuses on the herpetofaunal diversity of two geothermal sites in the state of Puebla, Mexico. Los Humeros, which has over 20 years producing energy and Chignahuapan, which is still in the exploration phase. Both sites have similar characteristics regarding vegetation, antropic activities and altitude. We made 2 samplings in june and november of 2018 with a total of 20 sampling units and per site.

We found a total of 23 species between both sites: 14 species for Los Humeros locality and 17 species for the Chignahuapan locality, 8 of which where found in both sites. 20 species are endemic to Mexico, 8 are under protection by mexican laws and 4 are listed by the IUCN.

This study should serve as baseline for making geothermal plants an even more sustainable way of harvesting energy.

**Do early phenological shifts affect growth and development across life stages in a widespread amphibian?**

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<sup>1</sup>*Case Western Reserve University*

Climate change has shifted breeding phenology which could alter the timing and circumstances of subsequent life history events for offspring. While offspring may be able to compensate for earlier breeding by their parents, compensation may be costly. To examine the effects of phenological shifts early in developmental on subsequent life stages, we manipulated the incubation temperature of wood frog (*Rana sylvatica*) egg masses such that they hatched early or late. We raised the resulting larvae in mesocosms where we manipulated food availability to test for the effects of resources on compensatory growth and development. Midway through the larval stage, larvae from the early egg phenology treatment were developmentally more advanced, and larger. However, larvae from our late egg phenology treatment accelerated growth and development during the second half of the larval stage to metamorphose within one day of the larvae from the early egg phenology treatment. The increase in development came with no cost in terms of mass, regardless of food availability. At metamorphosis, we transferred the wood frogs to terrestrial enclosures where we manipulated density to test for the effects of intraspecific competition on the carryover of the aquatic treatments. Growth in the terrestrial environment was increased by added larval food and decreased by increased terrestrial density but not affected by egg phenology. Our egg phenology treatment had no biological effect at metamorphosis and may indicate that there is potential for resiliency to the effects of climate change on hatching plasticity.

## **A new explanation for the evolution of TSD provides broad insight into thermal limits of reptiles**

**Rollinson N<sup>1</sup>**

<sup>1</sup>*University of Toronto*

No explanation that can broadly account for the evolution and maintenance of temperature dependent sex determination (TSD) in reptiles. The most promising hypothesis stipulates that the ratio of male to female fitness must vary with incubation temperature, but why this ratio might vary across broad species is unknown. Here I point out that the ratio of female to male fitness varies naturally along the axis of condition (general health, rigour, fat reserves, etc). This is because male fitness is limited by mating opportunities, and high condition males secure the majority of mates. Female fitness (fecundity) is limited by gamete production, and is less sensitive to variation in condition. I suggest that incubation temperature affects the general health and rigour of individuals, and it does so in the same way for males and females. TSD evolved so that males are produced in better incubation environments than females, as high-condition males provide greater fitness return than high-condition females. I test and uphold a main prediction of this hypothesis, which is that pivotal temperatures mark the transition from high-quality thermal environments to thermally-stressful environments. I suggest that pivotal temperatures provide a species-specific benchmark for thermal stress, thereby providing new and broad insight into the study of thermal limits in TSD species.

**Amphibian immunity, temperature, and disease: A balancing act between host and pathogen**

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In an effort to understand the seasonality of skin defenses of Southern Leopard Frogs, *Rana [Lithobates] sphenoccephala*, we examined their capacity to secrete antimicrobial peptides (AMPs) as we surveyed for the presence of the pathogenic chytrid fungi, *Batrachochytrium dendrobatidis* (*Bd*) and *Batrachochytrium salamandrivorans* (*Bsal*). We found a predicted seasonality of *Bd* prevalence and intensity that was greatest in the cold seasons and the least in the summer and no evidence for the presence of *Bsal*. Because Southern Leopard Frogs are active for most of the year in Tennessee, we predicted that the capacity for an AMP defense would be constant throughout the year. Instead, we observed a distinct seasonality, with greater amounts of inducible peptides in fall, winter, and spring and the least available peptides in the summer. These findings suggest that this species may be adapted to produce and use more AMPs in the winter and spring during the most active breeding period when more frog-to-frog encounters would occur and skin defenses would be needed to deal with injuries. In summer, when adults are more solitary, such defenses might be of less importance, and other immune defenses might then be of greater importance for protection of the skin from potentially invasive skin pathogens.

### Emerging or enzootic? Disruption of long-term host-pathogen dynamics

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Emerging infectious diseases (EIDs) present one of the most pressing issues in our century. The unprecedented increase of EIDs is now recognized as a major driver of biodiversity loss worldwide. Nevertheless, sudden pathogen emergence and disease outbreak does not necessarily reflect a recent introduction in a natural system but can result from a disruption of an established equilibrium. We tested the hypothesis that introduced pumpkinseed fish may have been the vector of a highly lethal virus leading to disease outbreaks in the late 1990s in a remote area in northern Portugal, and assessed the post-epidemic response of the multi-host amphibian assemblage, two decades after the first recorded outbreaks.

There were steep declines in the abundance of several species since the outbreaks of ranavirosis. Advanced molecular analyses allowed the detection of different strains of *Ranavirus* infecting several hosts from the two ectothermic vertebrate classes (amphibians and fish). The results also revealed that these strains had been present in the area since the 1980s, a decade before the observed disease outbreaks and have been infecting the same amphibian hosts ever since.

Our data suggest that pumpkinseed introduction was not followed by the emergence of new viruses. Instead, this event may have caused a disruption by increasing amphibians' susceptibility to the pathogen already present.

**Braiding data streams: building an Integrated Population Model for the Foothill Yellow-Legged Frog, *Rana boylei***

**Rose J<sup>1</sup>**, Kleeman P<sup>1</sup>, Halstead B<sup>1</sup>

<sup>1</sup>*U.S. Geological Survey, Western Ecological Research Center*

Data on demographic rates for threatened and endangered species are often piecemeal and scattered among a few focal study populations. For threatened amphibians, estimating the recruitment rate of recently metamorphosed juveniles to the adult breeding population is vital for quantifying population viability, but presents a serious challenge given low recapture rates of marked juveniles. Recent advances in Integrated Population Models (IPMs) empower researchers to leverage multiple data types from different populations to precisely estimate demographic rates, including those that are difficult to measure directly. The Foothill Yellow-Legged Frog, *Rana boylei*, is a stream-breeding species that has been extirpated from much of its former distribution in the western USA because of altered hydrological regimes caused by dams, competition and predation by non-native species, and agricultural pesticides. Although the larval ecology of *R. boylei* is well-studied, much remains unknown about the survival of juvenile and adult frogs. We compiled data from several populations throughout the current range of *R. boylei* and constructed an IPM that integrates capture-mark-recapture data with egg mass counts and fecundity data. By integrating multiple demographic data sources, we indirectly estimated the survival of juvenile frogs, which were otherwise a “lost” life stage. We also used data on the effects of flow regimes on the survival of eggs and larval *R. boylei* to perform Population Viability Analyses under different scenarios of flow regulation. Our work not only provides valuable information for conserving *R. boylei*, but also demonstrates the utility of IPMs for estimating otherwise intractable demographic parameters.

## Testing for the Presence of Kin-Based Sociality in Midland Painted Turtles (*Chrysemys picta marginata*)

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Social systems are common and variable across the animal kingdom, and in many species, social systems involve associations between kin. Hamilton's Rule suggests that social associations between kin increase inclusive fitness of participants relative to non-kin. Kin-based sociality is present in the aggregative behaviours of a number of reptiles, but research to date has largely focused on squamates. The other reptilian orders are less studied, and in many cases have gained reputations as solitary, asocial animals. In particular, evidence for turtle sociality has not been reported, owing to the cryptic nature of turtles. The purpose of our study is to investigate the presence of kin-based sociality during nesting and basking aggregations of the Midland Painted Turtle (*Chrysemys picta marginata*). A genotypic analysis will be conducted on our focal population in Northern Ontario, Canada to determine relatedness among individuals. The nesting and basking habits of the population will be monitored throughout two active seasons, with particular attention paid to interactions between individuals. We will report on correlations between social associations and relatedness data that will be preliminarily tested with our first season of data collection. Reptiles are underrepresented in the animal sociality literature, and our study will contribute a description of social organization and structure in a novel reptilian taxon, freshwater turtles. The mating systems of many turtle species are well-studied, but sociality beyond mating has not been examined to the same extent. Understanding the sociality of turtles will benefit research on the evolution of reptile, and, more broadly, vertebrate sociality.



### Shared ancient sex chromosomes in varanids, beaded lizards and alligator lizards

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Sex determination in varanids, Gila monsters, beaded lizards, and other anguimorphan lizards is still poorly understood. Sex chromosomes were reported only in a few species based solely on cytogenetics, which precluded assessment of their homology. We uncovered Z-chromosome-specific genes in varanids from their transcriptomes. Comparison of differences in gene copy numbers between sexes across anguimorphan lizards and outgroups revealed that homologous differentiated ZZ/ZW sex chromosomes are present in Gila monsters, beaded lizards, alligator lizards, and a wide phylogenetic spectrum of varanids. However, these sex chromosomes are not homologous to those known in other amniotes. We conclude that differentiated sex chromosomes were already present in the common ancestor of Anguimorpha living in the early Cretaceous or even in the Jurassic Period, 115–180 Ma, placing anguimorphan sex chromosomes among the oldest known in vertebrates. The analysis of transcriptomes of Komodo dragon (*Varanus komodoensis*) showed that the expression levels of genes linked to anguimorphan sex chromosomes are not balanced between sexes. Besides expanding our knowledge on vertebrate sex chromosome evolution, our study has important practical relevance for breeding and ecological studies. We introduce the first, widely applicable technique of molecular sexing in varanids, Gila monsters, and beaded lizards, where reliable determination of sex based on external morphology is dubious even in adults.

**Distribution, occupancy, and habitat associations of grassland-associated reptile and amphibian species in Northwest Arkansas.**

**Royal E<sup>1</sup>**, Kross C<sup>1</sup>, Willson J<sup>1</sup>

<sup>1</sup>*University Of Arkansas*

Grasslands historically covered much of inland North America, and many species have adapted to the unique conditions found in grassland ecosystems, including fourteen reptile and amphibian species of conservation concern in Arkansas. Less than 1% of grassland habitat remains in Arkansas, with much historic grassland having been converted for urban and agricultural development, resulting in steep population declines for many grassland-associated species. Many reptile and amphibian species are difficult to detect, resulting in sparse, unreliable distribution and abundance estimates for many understudied species. Thus, we assessed the state of herpetofaunal communities in intact grassland habitat, as well in degraded and developed historic grassland habitat throughout Northwest Arkansas. We performed repeated, low intensity herpetofauna surveys, vegetation surveys, and quantified landscape characteristics to determine the influence of land use, hydrology, and vegetative communities on herpetofaunal community composition using hierarchical Bayesian occupancy modeling. We selected 34 sites ranging from fire-maintained intact grassland to active agricultural areas and performed repeated visual encounter surveys at each site over two field seasons. Preliminary results suggest intact grassland and restored grassland habitats can support diverse herpetofaunal communities, including grassland obligate species and species of conservation concern, while degraded and active agricultural historic grassland sites primarily supported less diverse communities composed of generalist species. Herpetofaunal community composition appears to be dictated largely by a few key landscape and habitat variables affected by land use. Our results provide insight into the effects of habitat degradation and restoration on grassland-associated herpetofauna and will help guide management planning.

## Use of Photorecognition and Habitat Manipulation in Reptile Mitigation and Post-development Monitoring

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Translocation of reptiles is a common practice for mitigating development impacts in the UK, but is often carried out with little oversight and limited post-development monitoring. Fences are often used to exclude reptiles from development sites, but there is an increasing trend towards the use of habitat manipulation to prevent access, with little evidence of its efficacy.

In 2015 we undertook a translocation of sand lizards *Lacerta agilis* from an area used for film production where they were subject to disturbance and habitat damage. Prior to the translocation, habitat creation and enhancement were undertaken including felling trees to create new habitat and remove shading, scrub control on existing habitat, control of invasive plants, creation of sand strips for egg-laying, and measures to improve connectivity.

A total of 90 sand lizards was translocated to habitat just outside the development area. We used photorecognition to monitor animals returning to the donor site and at least seven recaptures were made, comprising three different individuals.

Annual post-development monitoring shows signs of success, with good numbers of sand lizards remaining on the site, including several translocated individuals. Gravid females (including lizards that had been translocated) were also observed, as well as recently excavated burrows and hatchlings.

This project has shown that excluding reptiles without the use of fencing is possible but requires high levels of effort, as well as monitoring to ensure animals are not returning.

Photorecognition is an extremely useful tool for sand lizard mitigation projects, and is likely to be for other species.

## Impacts of Agriculture and Roads on Amphibians in the Prairie Potholes of Manitoba, Canada

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<sup>1</sup>*Brandon University*

Following the end of the last ice age, glacial melting across the Prairies of North America created a landscape dotted with depressional wetlands. Wetlands in the Prairie Pothole Region (PPR) typically contain water following snowmelt for some period of time, although they may be temporary, semi-permanent, or permanent. To date, more than 40% of the original wetlands in PPR have been drained (primarily for agriculture and road construction) and thousands of acres are lost every year. Wetlands in the PPR are important breeding grounds for waterfowl and migratory bird species, including many species of amphibians and reptiles. The objectives of our research are to investigate the impacts of agriculture and road construction on species inhabiting Prairie Pothole wetlands in southeastern and southwestern Manitoba, and to recommend mitigation measures for road construction and agricultural activities. We surveyed wetlands from spring to fall of 2016-18 and recorded the following: water chemistry, breeding activity, species richness and morphology, and physical characteristics of the wetland. We selected study wetlands that differed in two main parameters: 1) proximity to type of road (gravel versus paved) and 2) proximity to livestock (full access by livestock, restricted access by livestock or natural). We found that breeding activity and species richness varied from year to year, differed among the wetlands, and was negatively affected by water chemistry (particularly salinity and conductivity), presence of fish and access by livestock.

**Endocrine disruption alters developmental body condition, energy allocation, and juvenile performance in *Rana temporaria***

**Ruthsatz K<sup>1</sup>**, Dausmann K<sup>1</sup>, Peck M<sup>1</sup>, Glos J<sup>1</sup>

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Global change exposes wildlife to an array of environmental stressors with the ability to disrupt endocrine function of the hypothalamus-pituitary-thyroid axis. We investigated how the alteration of thyroid hormone (TH) levels due to exposure to exogenous L-thyroxin (T4; stimulatory) and to the environmentally relevant endocrine disruptor sodium perchlorate (SP; inhibitory) affects body condition, metabolic costs and energy allocation during and after metamorphosis in *R. temporaria*. We further tested for carry-over effects of endocrine disruption during larval stage on juvenile locomotor performance. Energy allocated to development was negatively related to metabolic rate and thus, tadpoles exposed to T4 could allocate 24 % less energy to development during metamorphic climax than control animals. Therefore, the energy available for metamorphosis was reduced in tadpoles with increased TH level by exposure to T4. We suggest that differences in metabolic rate caused by altered TH levels during metamorphic climax and energy allocation to maintenance costs might have contributed to a reduced energetic efficiency in tadpoles with high TH levels. Differences in size and energetics persisted beyond the metamorphic boundary and impacted on juvenile performance. Energetic efficiency varied between treatments due to differences in size allocation of internal macronutrient stores. Altered TH levels as caused by several environmental stressors lead to persisting effects on metamorphic traits and energetics and, thus, caused carry-over effects on performance of froglets. We demonstrate the mechanisms through which alterations in abiotic and biotic environmental factors can alter phenotypes at metamorphosis and reduce lifetime fitness in these and likely other amphibians.

**Bushmaster Conservation Project: uncovering the well-kept secrets of *Lachesis stenophrys* ecology.**

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The bushmaster's ecology is one of the most well-kept secrets in neotropical herpetology. Its secretive lifestyle and over-all rarity has caused many research efforts towards this species' ecology to be unsuccessful. Encounters with this species are scarce, making basic ecological studies challenging. The Bushmaster Conservation Effort, a project founded and funded by the Herpetological Education and Research Project, is the first in-situ conservation initiative on the Central-American Bushmaster (*Lachesis stenophrys*) in Costa Rica. In cooperation with local herpetologists and native inhabitants, a small research station was established in the heart of indigenous Bri Bri primary rainforest. The goals of the project are to set up a fully equipped research station that monitors and studies the ecology of the local population of Central-American bushmasters. This research station will be responsible for the conduction of several basic ecological studies on bushmasters, such as population size assessments, telemetry studies, habitat use monitoring, home range delimitations, ethological studies, and rehabilitation and relocation assessments. Next to these efforts there is a pressing need for education and sensibilization of the local residents towards the presence and vulnerability of the species, as well as the advantages of having these large predators around. The species could also prove very helpful as a flagship species in order to preserve the primary rainforest and indigenous lands it inhabits.

**Plasticity allows colonization, and adaptation leads to incipient speciation in a fire salamander population**

**Sabino Pinto J<sup>1</sup>**

<sup>1</sup>*Technische Universität Braunschweig, Zoologisches Institute*

In the Kottenforst, central Germany, two subpopulations of *Salamandra salamandra* co-occur. These subpopulations present differences in their physiology, genetics and mate preferences and can be recognized by their habitat preference; while one population deposit their larvae in primary streams, as most other populations of this species do, the other deposits their larvae in temporary ponds. In this study we aimed answer to two questions: 1) how well adapted are individuals to their preferred environment; and 2) can individuals adapt to the non-preferred environment. To test these questions we performed a reciprocal transfer experiment of pond- and stream-dwelling salamander larvae, and analyzed changes in the individual morphology and transcriptomic profile, using species-specific microarrays. Pond- and stream-dwelling individuals in their own natural habitats diverged in their gene expression, with pond-dwelling individuals having increased expression of genes related to the phosphorelay signal transduction system and DNA recombination. Pond-dwelling individuals were found to adapt to the stream environment (reversion); however the same was not found for stream-dwelling individuals when transferred to ponds, which increased the expression of genes related to reduction-oxidation processes, possibly due to environmental stress. Our results support the hypothesis that stream-dwelling is the ancestral state of this population, and provides evidence towards the role of plasticity on the divergence of populations.

**Survival strategies of the chytrid *Batrachochytrium dendrobatidis* within and outside its amphibian host**

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<sup>1</sup>*Department of Biological Sciences, Texas Tech University,* <sup>2</sup>*Center for Biotechnology and Genomics*

As an opportunistic pathogen, *Batrachochytrium dendrobatidis* has evolved mechanisms to ensure reproductive success in the presence or absence of its host. We have biochemical, molecular genetic and microscopic evidence to support the hypothesis that this fungus can transition from a motile zoospore to zoosporangia that then aggregate into microcolonies in high- and low-nutrient environments. Individual zoosporangia are associated with one another using a network of carbohydrates and form a film at the air-water interface or on solid surfaces. Carbohydrates identified by gas chromatography from the cell-free monolayers showed the presence of sugars similar to those found in other fungal biofilms. Genes up-regulated in the monolayer-associated cells were distinct from those observed in zoospores and individual sporangia.

Within the host, the pathogen has been shown to penetrate the epidermis with a germ tube and develop a reproductive thallus. Several secondary metabolites have been identified in *B. dendrobatidis* culture filtrates. We show that this chytrid possesses the genetic potential to synthesize these molecules. Using HPLC and mass spectrometry we have identified additional secondary metabolites that may serve different roles to impair host response and function. We have also developed a novel bioassay system to study pathogenesis of the fungus.



### Diversification dynamics in viviparous sea snakes

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<sup>1</sup>*School of Biological Sciences, University of Adelaide*, <sup>2</sup>*Royal Danish Academy of Fine Arts*

This talk will synthesise macro- and micro-evolutionary and developmental studies of sea snake diversification. Relative to terrestrial elapids and other sea snakes, *Hydrophis* are inferred to have three-fold higher rates of speciation and are characterised by wide geographic distributions, with strong geographic genetic structure among-regions but high population connectivity at a finer (regional) scale. Morphological shifts linked to dietary specialisations might promote divergence at the population level, and manifest as elevated rates of skull and body shape change at the macroevolutionary level. Especially prolific, and unique to *Hydrophis*-*Microcephalophis*, are convergent ecomorphs that specialise on burrowing eel prey, and have tiny heads and narrow fore-bodies that develop via changes in segmentation during embryogenesis and modifications to postnatal growth.

**Habitat choice of hatchling Murray River Turtles (*Emydura macquarii*): informing headstarting programmes**

**Santori C<sup>1</sup>**, Van Dyke J<sup>2</sup>, Spencer R<sup>3</sup>, Whittington C<sup>1</sup>, Thompson M<sup>1</sup>

<sup>1</sup>University Of Sydney, <sup>2</sup>La Trobe University, <sup>3</sup>Western Sydney University

The Murray River turtle (*Emydura macquarii*) was once very abundant in the Australian Murray-Darling Basin, but it has suffered a 70% decline in the last 40 years. Loss of recruitment is thought to be a major driver of this decline. Identifying what habitats hatchlings use during the most vulnerable period of their lives provides crucial information for their management. We conducted two experiments to assess whether aquatic vegetation is a preferred habitat for hatchling *E. macquarii*. Firstly, we introduced 36 hatchlings to individual aquaria that were divided in two treatments. Half of the aquaria had 50% of their area covered in vegetation, and half of them were 100% bare. The turtles were rotated to experience both treatments for 3 days. When available, hatchling *E. macquarii* spent significantly more time in vegetation than in bare habitat. We further assessed hatchlings' preference of aquatic vegetation, and investigated if captivity has any influence on this, with a radio-telemetry project in South Australia. We split 36 hatchling *E. macquarii* in two groups (18 to be released right after hatching, and 18 after one month in captivity), fit them with radio-transmitters and tracked their movements daily for two weeks. After an initial long-distance dispersion, hatchlings of the first group settled individually in protected, vegetated areas. However, the captive hatchlings did not select vegetated habitat within the wetland. This information will allow us to design conservation and management plans including headstarting, release practices, and the choice of reintroduction sites.

## Effects of urbanisation and hydrological change on a freshwater turtle

**Santoro A<sup>1</sup>**

<sup>1</sup>*Murdoch University*

Land use modification, climate drying, and invasive species threaten the south-western snake-necked turtle (*Chelodina colliei*) in Perth, Western Australia. The extent of impact of these stressors on *C. colliei* is not known but likely includes increased wildlife-vehicle incidents and predation, and reduced availability and quality of nesting and aquatic habitat. To investigate how these stressors may be altering the turtle's ecology, I used radio-telemetry over two annual cycles to track 50 female turtles in urban wetlands experiencing a continuum of water regimes and a variety of surrounding terrestrial land use modification. Nest predation and turtle corpses were also recorded. It was hypothesised that: the species would move seasonally among the wetlands to maximise access to inundated habitats; nesting ecology would vary based on available habitat; and predation and vehicle related mortality would increase in modified habitats. The study revealed limited movement between wetlands with turtles aestivating rather than migrating, suggesting that continued climate drying will have implications for population viabilities if individuals do not migrate to more suitable wetlands as hydrology changes. Land use modification is placing pressure on the populations through considerable mortality due to vehicle strikes, mostly of nesting females. Feral animal predation was also a major issue affecting both adults and embryos, significantly reducing the populations reproductive potential. The presence of the European red fox (*Vulpes vulpes*) was confirmed using wildlife cameras. These results indicate that urgent management of turtle populations based on a detailed understanding of their ecology and interactions is needed for these urban ecosystems.

## One hundred years of urbanisation makes an endangered species of Canberra's Grassland Earless Dragon

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The Canberra Grassland Earless Dragon (*Tympanocryptis lineata*) is a habitat specialist being restricted to natural temperate grasslands in south eastern Australia. These grasslands were once widespread but are now one of Australia's most threatened ecological communities. The pre-European distribution of *T. lineata* is unknown but it is likely to have been centred on Canberra (the Australian capital) where this small, cryptic, agamid was once common but is now restricted to a few small patches of grassland in a largely urban landscape. Genetic analyses and capture-mark-recapture monitoring have shown these remnant populations to be fragmented and in low abundance and therefore extremely vulnerable to extinction. Two closely related species have also been affected by urban development and have not been seen for decades. In contrast, a third close species, *T. osbornei*, has retained a genetically continuous and apparently stable distribution in an area largely free of urbanisation and vegetation clearance. While habitat destruction has undoubtedly placed *T. lineata* at risk, we have also detected population collapse associated with drought and have measured an impact of increasing environmental temperatures on activity levels. These stressors are likely to be exacerbated by the proximity of *T. lineata* to urban development. The way forward appears to lie with habitat management through an emphasis on grassland restoration that re-connects existing populations and whose biomass is managed to buffer against temperature increases. As one of the few reptiles that inhabit natural temperate grassland, *T. lineata* has a role as a flagship species in the conservation of this important ecological community.

**A meta-analysis reveals temperature, dose, life stage, and taxonomy influence host susceptibility to *Batrachochytrium dendrobatidis***

**Sauer E<sup>1</sup>**, Cohen J<sup>1</sup>, Lajeunesse M<sup>2</sup>, McMahon T<sup>3</sup>, Civitello D<sup>4</sup>, Knutie S<sup>5</sup>, Nguyen K<sup>2</sup>, Roznik E<sup>6</sup>, Sears B<sup>7</sup>, Bessler S<sup>2</sup>, Delius B<sup>2</sup>, Halstead N<sup>8</sup>, Ortega N<sup>2</sup>, Venesky M<sup>9</sup>, Young S<sup>10</sup>, Rohr J<sup>11</sup>

<sup>1</sup>University Of Wisconsin, <sup>2</sup>University of South Florida, <sup>3</sup>University of Tampa, <sup>4</sup>Emory University, <sup>5</sup>University of Connecticut, <sup>6</sup>Memphis Zoo, <sup>7</sup>BioScience Writers, <sup>8</sup>Wildlands Conservation, <sup>9</sup>Allegheny College, <sup>10</sup>Ecole Polytechnique Federale de Lausanne, <sup>11</sup>University of Notre Dame

Complex ecological relationships, such as host-parasite interactions, are often modeled with laboratory experiments. However, some experimental laboratory conditions, such as temperature or infection dose, are regularly chosen based on convenience or convention and it is unclear how these decisions systematically affect experimental outcomes. Here, we conducted a meta-analysis of 58 laboratory studies that exposed amphibians to the pathogenic fungus *Batrachochytrium dendrobatidis* (*Bd*) to better understand how laboratory temperature, host life stage, infection dose, and host species affect host mortality. We found that host mortality was driven by thermal mismatches; hosts native to cooler environments experienced greater *Bd*-induced mortality at relatively warm experimental temperatures and vice versa. We also found that *Bd* dose positively predicted *Bd*-induced host mortality and that the superfamilies Bufonoidea and Hyloidea were especially susceptible to *Bd*. Finally, the effect of *Bd* on host mortality varied across host life stages, with larval amphibians experiencing lower risk of *Bd*-induced mortality than adults or metamorphs. Metamorphs were especially susceptible and experienced mortality when inoculated with much smaller *Bd* doses than the average dose used by researchers. Our results suggest that when designing experiments on species interactions, researchers should carefully consider the experimental temperature, and inoculum dose, and life stage and taxonomy of the host species.

### Recent Studies of Adrenal Enlargement in Toad-eating Snakes

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Enlargement of the adrenal glands in the toad-eating snake *Heterodon platirhinos* was first reported almost 65 years ago. That observation prompted speculation that adrenal enlargement reflects the importance of catecholamines (epinephrine and norepinephrine) in reducing the effects of the bufadienolide toxins produced by toads. Like other cardiotonic steroids, bufadienolides exert their effect by binding to the sodium-potassium pump, Na<sup>+</sup>/K<sup>+</sup>-ATPase, thereby disrupting the ionic balance within cells, including cardiac myocytes. Subsequent research demonstrated that adrenal enlargement occurs in several other toad-eating snakes and that the degree of enlargement is greater in males than females. The adrenal glands of amniotes are comprised of both chromaffin tissue, which produces catecholamines, and interrenal tissue, which produces corticosteroid hormones. Recent studies indicate that the observed difference in adrenal gland size between toad-eating and nontoad-eating snakes reflects enlargement of the interrenal tissue, casting doubt on the presumed role of catecholamines in defending toad-eating snakes against bufadienolide toxins. In contrast, studies of corticosteroid responses to cardiotonic steroids reveal an increased release of aldosterone, which is known to signal upregulation of Na<sup>+</sup>/K<sup>+</sup>-ATPase in mammals. The significance of sexual dimorphism in the size of the adrenal glands in toad-eating snakes remains unexplained. However, the observation that maternally ingested bufadienolides are transferred to the embryos of *Rhabdophis tigrinus* suggests a possible epigenetic influence of the toxins on the developing adrenals, with the further possibility that such an influence differs between the sexes.

## Family specific herbivory in West-African Sabre-toothed Frogs

**Schäfer M<sup>1</sup>**

<sup>1</sup>*Museum for Natural History Berlin*

The regular feeding of plant parts by adult frogs is currently only known from two taxa and plant matter in frog stomach analyses is usually attributed to be swallowed accidentally. Here we present the case of family specific herbivory on a single plant, leaflets of the riparian tree *Parkia bicolor*, by members of the Odontobatrachidae.

The five frog species occur along torrent streams in the West African Upper Guinea biodiversity hotspot. We stomach flushed and analyzed food items of more than 600 individuals from all species, collected during rainy and dry seasons. Generally we detected a dietary composition typical for opportunistically feeding frogs, comprising taxa present in the frogs' habitats, including e.g. aquatic insect larvae, shrimps, as well as snails, and even vertebrates such as geckos and frogs. More surprisingly, one third of female and one fourth of the male stomachs of all species contained large quantities of small leaflets of the pinnate *Parkia bicolor* leaves, some frogs having swallowed more than 100 leaflets. Leaflet numbers in stomachs peaked during the dry season, when annual senescence of leaves made them widely available on the ground. Leaflets were excreted along other food residues within 2 to 3 days. Similar sized leaves of other plants in the very leaf litter were absent in the frogs' stomachs. This absence and the large number of swallowed leaflets make an accidental uptake unlikely. However, leaflets showed no obvious digestion traces after intestinal passage. We discuss some potential reasons for this much unexpected feeding behavior.

## Global impacts of chytridiomycosis on amphibians

**Scheele B<sup>1</sup>**

<sup>1</sup>*The Australian National University*

Amphibians are the most threatened vertebrate class, with approximately one third of all species at risk of extinction. Like other vertebrate classes, habitat loss and degradation are key threats to amphibians. However, on top of habitat loss, uniquely, many amphibian species are also threatened by infectious diseases, whose emergence has been facilitated by anthropogenic trade and the associated breakdown of dispersal barriers. The global spread of the fungal pathogens *Batrachochytrium dendrobatidis* and *B. salamandrivorans*, which cause the disease chytridiomycosis in amphibians, has been associated with widespread amphibian declines. Here we present a spatio-temporal assessment of the global impacts of amphibian chytridiomycosis. We estimate that chytridiomycosis has contributed to the decline of 501 amphibian species over the past half-century, including 90 presumed extinctions. The number of declining species increased rapidly during the 1970s and peaked in the 1980s. The effects of chytridiomycosis have been greatest in large-bodied, range-restricted anurans in wet climates. Chytridiomycosis has caused declines across a broad range of amphibian families, with declines clustered in the Americas and Australia, but also scattered across Europe and Africa. Encouragingly, 60 species have shown signs of recovery, with increases in abundance and distribution reported. However, chytridiomycosis continues to be an ongoing threat for 197 species. Both *B. dendrobatidis* and *B. salamandrivorans* remain absent from some regions with high amphibian biodiversity, and limiting ongoing spread is a conservation priority. Preventing further chytridiomycosis-driven amphibian declines is dependent on developing effective mitigation actions to conserve species at high risk of extinction.



## The past, present, and future of research on Madagascar's microhylids

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Madagascar, Earth's fourth largest island, hosts three subfamilies of Microhylidae with two separate origins. The subfamily Cophylinae is one of the most diverse in the family with over 100 described species. It contains a wide array of ecomorphological guilds, many of which have been shown to have repeated origins. This makes these frogs a fascinating clade for evolutionary study. Their diversity mirrors other centres of microhylid diversity, such as New Guinea, making it an interesting system for comparative work. Research on Madagascar's microhylids has been largely focussed on their taxonomy until recently, but aspects of their ecology and behaviour were also scattered through the literature. Today, my colleagues and I are working on numerous aspects of their evolution and systematics. A great deal about them remains wholly unknown, and the future prospects for the group promise fascinating revelations and further discoveries. I will highlight directions that this research is likely to take, and synergies that will hopefully arise through future collaborations on the local and global scale.

### The weird skull of *Xenotyphlops* and a reinterpretation of blindsnake origins

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Blindsnake osteology has historically been challenging to study because of their tiny size. Hypotheses on their evolution rested strongly on the skulls of a few species that were described in detail based on dissections and slices. Conclusions from these studies were that the Scolecophidia (Leptotyphlopidae, Typhlopoidea, and Anomalepididae) are monophyletic, but these relationships have been strongly rejected by subsequent genetic results. Reconciling genetic relationships with our evolutionary understanding requires re-interpretation of our morphological data. Micro-CT enables us to do that at a hitherto impossible scale. We described the detailed skull osteology of the bizarre typhlopoid *Xenotyphlops grandidieri* from Madagascar, showing that its jaw mechanism in particular strongly resembles those of other typhlopoids. The three main clades in the 'Scolecophidia' in fact have highly different jaw mechanisms consistent with an independent convergence on miniaturisation, fossoriality, and a diet of mostly eusocial insects. The scolecophidian bauplan is therefore unlikely to be indicative of the ancestral anatomy or ecology of snakes.

## The axolotl genome

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Salamanders serve as important tetrapod models for developmental, regeneration and evolutionary studies. An extensive molecular toolkit makes the Mexican axolotl (*Ambystoma mexicanum*) a key representative salamander for molecular investigations. Combining the development of a genome assembler, long-read sequencing and Hi-C based scaffolding we achieved a chromosome-scale assembly of the axolotl genome. We observed a size expansion of introns and intergenic regions, largely attributable to multiplication of long terminal repeat retroelements. Furthermore, topologically associated domains, loops and other organizational properties of the genome, have been scaled accordingly, to accommodate the expansion of the inter- and intragenic space. The axolotl genome provides a rich biological resource for developmental and evolutionary studies and a model for the organisation of large genomes.

## Extreme temperatures and developmental instability across reptiles

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Embryogenesis is a critical period for morphological and physiological development. Most reptiles spend this stage of their life exposed to ambient environmental temperatures, and this has a dramatic influence on developmental rate, survival, and phenotype. Under the range of temperatures typically experienced by a species, we expect stabilizing selection and developmental canalization to reduce phenotypic variation to a single optimum or a phenotypic reaction norm. However, extreme conditions are less often exposed to selection, thus may reveal cryptic genetic variation and induce developmental instability. Here, we use the RepDevo database of reptile incubation experiments to test the prediction that phenotypic variance increases at extreme developmental temperatures. We find that phenotypic variation does indeed increase as incubation temperatures move away from the optimum, but that the most extreme temperatures lead to plummeting survival and a resultant loss of phenotypic variation.

**Repeated evolution of insular dwarfism across three reptile species with population and functional genomic insights.**

**Schwartz T<sup>1</sup>**, Clark A<sup>1</sup>, Sparkman A<sup>2</sup>

<sup>1</sup>Auburn University, <sup>2</sup>Westmont College

The repeated evolution of multiple traits across species found the same habitat provides opportunities to identify the selective forces in the environment, and to identify constraints across biological levels of organization. The “island rule” is the worldwide phenomenon of rapid evolution of dwarfism and gigantism on islands. It is unknown to what degree convergence in insular body size is accompanied by convergence at the level of genetics, physiology, and/or life-history traits. We have identified three reptile species—two snakes and one lizard—on the California Channel Islands that have independently evolved insular dwarfism relative to mainland southern California. We present data from two island and two mainland locations and multiple years of study, demonstrating island populations of all three reptiles have smaller body size, smaller relative head size, and lower blood glucose relative to mainland populations, though with some variation by sex and year. These findings suggest these three species have independently evolved convergent physiological changes corresponding to convergent changes in morphology consistent with a scenario of reduced resource availability and/or changes in prey size on the island. We further this investigation at the genomic level, presenting a chromosome-level genome assembly for the gopher snake (*Pituophis catenifer*) and whole genome population sequencing across mainland and island locations to characterize the genomic divergence across the landscape and specifically in candidate functional genetic pathways. The results are discussed in the context of our ultimate goal—to understand the mechanisms of convergence across the ecology, life-history, physiology, and genetic networks.

**Sticky fingers: microhabitat choice, adhesive performance and toe-pad morphology in velvet geckos (Genus: *Oedura*)**

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In animals, effective locomotion is critical to fitness, so we expect them to perform best in certain environments, and to select microhabitats that enhance performance. Variation in the morphology and performance of the adhesive system among gecko species, in response to habitat structure, is poorly known. We measured substrate choice, substrate characteristics, and shear forces exerted by three different species of velvet geckos (genus *Oedura*), with different preferred microhabitats (one arboreal, one saxicolous, and one generalist). Additionally, we examined the setal dimensions (length and density) and overall pad area of the adhesive toe pads of the three species to investigate the relationship between ability and morphology. To enhance clinging ability, we expected individuals to select appropriate substrates. Consistent with this, all three had better clinging ability on rough substrates, and preferred rough substrates over smooth substrates. The average roughness of the natural substrates used by *Oedura* geckos were not significantly different among species or microhabitats, indicating that all three species faced similar ecological challenges. In spite of these similarities, toe pad morphology differed strongly among species. Behavioural preferences and performance of *Oedura* geckos were consistent with the ecomorphology paradigm, but they use different morphological strategies to overcome similar challenges.

## Controlling cane toads with traps: what have we learned?

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<sup>1</sup>James Cook University, <sup>2</sup>University of Florida

Introduced cane toads, *Rhinella marina*, are present in tropical locations world-wide, and can have significant negative impacts on native fauna. We have developed a trap for adult toads that uses an auditory and food lure, and we have used this trap in a variety of situations, in established populations, and as a sentry device. We have examined the importance of the toad call used on the lures in relation to capture success in different geographic locations, have deployed traps for moderate periods and measured toad population size change, and have deployed traps in locations where toads may be detected in incursions. We have found that traps attract mature females and males regardless of the calls used as attractants, but calls can be improved in attractiveness, and 'tuning' the calls for local female preferences is best for removing adult females. We have found that deploying traps for 10 weeks in the dry season, allowed us to remove approximately 40% of a marked population. Employing traps as sentry devices was successful in areas that are patrolled regularly (ports), but less successful in wilder areas because the burden of checking traps was prohibitive. In general, traps will not eradicate toads, but can be used as successful population suppression or sentry devices with calls engineered for the local population and regular checking.

### Revealing patterns in cane toad chorusing using long-term acoustic monitoring

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<sup>1</sup>Townsville

Frog chorusing reflects breeding activity, and thus can be used to monitor and detect reproduction of a range of species. Advances in automated recording, increased electronic storage, and improved acoustic analysis tools means automated, long-term acoustic monitoring is becoming feasible. The invasive cane toad, *Rhinella marina*, is widespread across northern Australia, but factors influencing breeding are poorly known. To describe the patterns of calling by cane toads, and the influence of environmental variables on toad chorusing patterns, we used continuous acoustic recording over a 19-month period covering two wet seasons at multiple breeding dams in a tropical savanna region in Australia. We used automated call recognition software and visualisation with false-colour spectrograms to reduce the amount of manual listening required to reveal nightly chorusing patterns. Cane toad chorus activity occurred somewhat more frequently in the wet season but also continued throughout the dry season. Nightly chorusing patterns varied widely among study sites under the influence of similar weather conditions, and were highly episodic, occurring in runs. The most important factor influencing the tendency to call was calling the previous night. Responses to factors other than weather are important in driving chorus activity of cane toads.



## Can endocranium shape reveal the sensory ecology of 'aquatic' snakes?

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The endocranium has been used as a proxy for brain shape in many studies, across all vertebrate taxa. Yet, the endocranium of snakes remains poorly studied despite its high degree of compartmentation, which makes it a perfect model to study the relationship between brain morphology and ecology. Snakes have invaded diverse habitats and show a high degree of morphological, behavioral and sensory adaptations to environmental constraints. The sensory ecology of aquatic snakes is particularly interesting, as the return to an aquatic lifestyle in snakes has independently evolved multiple times. Moreover, aquatic snakes inhabit bodies of water with a wide range of physical and chemical properties, such as fast clear streams, ponds, muddy wetlands, brackish water, and even open ocean. Thus, although aquatic snakes occupy the same water medium, their sensory systems must adapt to a broad range of environmental properties. To study this specialization, we here compare the endocranium shape of 36 'aquatic' species that are both phylogenetically and ecologically diverse. We investigate potential relationships between sensory-relevant properties of the environment and the shape of the endocranium. We use 3D geometric morphometrics on high resolution  $\mu$ CT scans to obtain the endocranium shape of collection specimens, that we compare in a phylogenetically informed context. The goal of this study is to identify links between different areas of the endocranium and properties of the aquatic microhabitats. The broader aim of this study is to infer the habitat and sensory modalities of extant and extinct species based on the morphology of the endocranium.

## Visual Adaptation of Full and Semi Aquatic Sea Snakes

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Sea snakes have completely adapted to the aquatic environment relative to their terrestrial ancestors. Compared with all other aquatic species among Amniota group, only sea snakes have closely related species that are half-aquatic lifestyle. The underwater light environment is different from that of land: short and long wavelength of light is filtered by absorbance with water and light intensity is reduced by scattering with the water molecule. In my research, I attempted to clarify the visual adaptation of sea snakes by comparison between the absorption wavelength of their visual pigments and the light environments in their habitats.

I measured absorption wavelengths of the reconstituted visual pigments consisting of A1-retinal and opsin protein encoded by RH1 gene. The absorption spectrum of the RH1 pigment is around 484 nm at the maximum in the terrestrial and semi-aquatic species, whereas at around 493 nm in the full-aquatic species. In addition, the absorption spectra of LWS visual pigments reconstituted from the LWS sequence from semi—aquatic species was shifted toward blue compared with that of full-aquatic species. The predicted absorption of LWS pigments is different among the LWS sequences from semi-aquatic species, though they do not show difference in their living habitats. In addition, the habitat of full-aquatic species with a small difference in absorption of RH1 pigments are living in light environments with large differences (e.g. coral reef, sand, muddy pond). Therefore, I will try to clarify the effect of ambient light differences on visual adaptation of sea snakes.

## **Ontogeny of the anuran urostyle: the developmental context of evolutionary novelty**

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The evolutionary origin of novel structures in multicellular animals is marked by unique developmental novelties. Having evolved nearly 200 mya, the anuran urostyle is one such morphological novelty. It forms during metamorphosis as the tail regresses and locomotion changes from the axial-driven mode of larvae to the limb-driven mode of adult frogs. Histologically, the urostyle comprises derivatives of a mesoderm-derived coccyx and an endoderm-derived hypochord. However, the development of this novelty is poorly understood. Here, we focus on the ontogenetic changes leading to structural novelty of the anuran urostyle: bone and cartilage formation, cell proliferation and differentiation, cell death, and neuromuscular skeletal changes at the site of urostyle development. Cell proliferation and cell apoptosis assays reveal that lateral margins of the ossifying hypochord, an apomorphy of frogs, undergo rapid cell division. We hypothesize that during hypochord ossification, hypertrophic chondrocytes are involved in matrix resorption and undergo rapid ossification correlated with increase in cell number. Concordant with formation of skeletal elements, slow and fast muscles of the tadpoles undergo secondary myogenesis and de-differentiate into adult muscle types; along with notochordal degeneration, the diameter of the spinal cord changes; and neurons extend along the tail and rearrange around the urostyle. Metamorphic events are correlated with high concentrations of thyroid hormones (TH). When TH was prevented, hypochord formation was disrupted. Finally, we compare the anuran urostyle and contrast caudal fusions of other vertebrates (birds, fish, apes) associated with tail loss.

## **A New Approach to Building the Amphibian Tree of Life**

**Serra Silva A<sup>1,2</sup>**, Wilkinson M<sup>1</sup>

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Previous attempts to build the Amphibian Tree of Life have relied heavily on supermatrices, where large amounts of data are concatenated into a single computationally expensive phylogenetic analysis. With ever increasing numbers of described species, this approach lags behind taxonomic knowledge. Of over 8000 described amphibian species, only half have been analysed in the latest published Amphibian Tree of Life. Taking advantage of the inherent scalability of amphibian orders (Gymnophiona: 212 species; Caudata: ~730 species; Anura: ~7060 species), we aim to test the use of supertree, and hybrid supertree/supermatrix, approaches to building the Tree of Life. Preliminary phylogenetic analyses of caecilians showed that supertree analyses are considerably faster (2.5 vs.10 days), but produce less resolved trees than concatenated analyses. To increase supertree resolution we are attempting to increase gene tree resolution prior to the supertree analyses, and testing a hybrid tree building approach, where supermatrix analyses are run on small subsets of closely related taxa, and the resulting phylogenies summarized with a supertree method. Continuing work on gene tree optimization, and analyses of salamander and frog data, should show the suitability of a modular tree building approach. Additionally, the use of a supertree or hybrid approach would make the integration of molecular and morphological data in phylogenetic analyses easier, and allow for more extensive taxon sampling. In short, we hope to show that supertree and/or hybrid approaches can be as resolved as supermatrix analyses, and able to close the gap between amphibian taxonomy and systematics.

**Did *Bd*-CAPE drive the Kihansi spray toad (*Nectophrynoides asperginis*) to extinction in the wild?**

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The global panzootic lineage of *Batrachochytrium dendrobatidis* (*Bd*) has contributed to the devastating decline of amphibian populations worldwide. Recent experimental evidence has suggested that the major population crash and ensuing extinction in the wild of the Kihansi spray toad, (*Nectophrynoides asperginis*) can be partly ascribed to *Bd* infection and chytridiomycosis. Here, we utilised deep sequencing and qPCR to advance our understanding of the extinction event and further characterise the *Bd* infection that occurred during the population crash.

*Bd*-positive spray toads were collected from the Kihansi gorge and the neighbouring Udagaji gorge, with zero *Bd* detected in specimens collected prior to *N. asperginis* decline. Archived toads were subjected to a novel lineage specific qPCR diagnostic to preliminarily identify the *Bd* lineage present at the time of the population decline. Subsequently, a shotgun sequencing approach optimised for analysis of ancient DNA was utilised to deep sequence DNA from two qPCR positive animals, confirming the presence of *Bd*-CAPE lineage.

Our finding demonstrates that *Bd*-Cape was associated with toads at the time of the *N. asperginis* population crash and may have had a principal role in the species extinction in the wild.

## Hooked on humans? *Daboia palaestinae* in reptile assemblages in natural versus anthropogenic habitats in Israel

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Reptile assemblages of Israel's Mediterranean zone include almost 50 chelonian and squamate species. While some species show an affinity to anthropogenic habitats, all are assumed to originate from natural habitats. Israel's natural habitats have been and are undergoing vast environmental changes, hence shifts in habitat preferences and distribution of reptiles are expected. We aim to shed light on distributional patterns of the venomous Palestine Viper (*Daboia palaestinae*), commonly considered as being drawn to human settlements.

We mapped observational and museum data of vipers from the Israel Nature & Parks Authority (INPA) database, the herpetological collections of the Hebrew University of Jerusalem and Tel Aviv University, and the authors' observational databases. We analyzed reptile assemblages at natural habitats of numerous sites we had surveyed for INPA over the last two decades.

The distribution map of *Daboia palaestinae* shows strong affinity to human settlements and agriculture in most landscapes analyzed. Surprisingly, viper observations comprised only 1.51% of total observations in natural habitats (>12,000 observations). Preliminary analysis of snake catcher (licensed professionals) data shows that vipers consist 31% - 55% of snakes captured in anthropogenic settings (>1,700 captures). We empirically demonstrate a strong bond between *Daboia palaestinae* and human settlements in Israel. This species supposedly evolved in Mediterranean woodlands, we suspect distributional changes have occurred due to anthropogenic changes in natural habitats. We suggest broad, long-term studies to illuminate factors driving these patterns, plus public awareness and education programs to prevent snakebite as well as killing of vipers by humans.

## **Applying assisted reproductive techniques to a multi-year study into biotic factors influencing *Necturus* captive breeding**

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Over five years three captive *Necturus* species (♂.♀; *maculosus* [3.1], *beyeri* [7.8], *alabamensis* [3.5]) were subjected to seasonal changes in water temperature and day-length. Assisted reproductive techniques including ultrasound, fecal hormone analysis and exogenous hormone administration were employed to better understand basic reproductive biology and advance breeding efforts for these fully aquatic and highly seasonal species. Monthly ultrasound exams tracked egg development, with dates of spermatophore deposition and oviposition recorded. Exogenous hormone trials in males and females occurred during courtship/spermatophore deposition and egg-laying seasons, respectively. Males were treated (i.p.) with LHRH (n=13; 0.9-4.0 ug/g body weight), LHRH + metoclopramide (n=3; 0.3-0.4 ug/g + 8.3-11.1 IU/g body weight), or sterile water control (n=5). Three LHRH-treated males deposited spermatophores 12-39 days post-treatment. Two control males deposited spermatophores. Females were treated (n=21) with a single i.p. injection of LHRH (1.0-2.74ug/g body weight) or sterile water control (n=13) between 3/31-6/3. Seven LHRH-treated females oviposited 3-13 days post-LHRH (3/31-4/23) compared to eight controls that oviposited eggs (4/5-5/7). Fecal hormone (Testosterone, Progesterone, Estrogen) metabolite measurement validated for each species via enzyme-immunoassay techniques permitted generation of longitudinal endocrine profiles and insight into the effect of environmental and physiologic manipulations on gonadal hormone production. Hormones fluctuated seasonally in a manner consistent with published annual activity and reproductive patterns of *Necturus*. Hormone peaks (Testosterone and Progesterone) temporally coincided with observed reproductive events. Results bring to light mechanisms underlying *Necturus* reproductive potential and output that will help guide captive propagation efforts to improve fertile offspring production from these unique salamanders.

**Diversification of melanosome shape is linked to physiological shifts in vertebrate taxa**

**Shawkey M**<sup>1</sup>, Peteya J<sup>2</sup>, Gao K<sup>3</sup>, Quanguo L<sup>4</sup>, Clarke J<sup>5</sup>, D'Alba L<sup>1</sup>

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<sup>5</sup>*University of Texas at Austin*

Recent studies of fossil integument have demonstrated that melanosomes are preserved in fossils. The morphology of these melanosomes correlates with melanin-based colour in birds and mammals and has been used to predict feather colour in extinct dinosaurs. This correlation is absent in non-dinosaurian reptile integument, in which melanosomes are instead characterized by a uniformly sub-spherical morphology. Melanosomes from anamniote vertebrates (i.e. amphibians) have yet to be morphologically and chemically characterized despite their significance to our understanding of broad patterns of melanin-based colour evolution. Here we examined melanosome morphology and chemistry in extant anamniotes as well as fossil agnathans and amphibians from China with skin preservation. We show that melanosomes in both fossil and extant anamniotes are uniformly ovoid and demonstrate low morphological disparity. These patterns are similar to those of melanosomes of basal amniotes and thus small, ovoid melanosomes are likely the plesiomorphic condition for vertebrates.

Low diversity in integumentary melanosome agnathans, in amphibians and in heterothermic amniotes shows the uniqueness of convergent increases in melanosome diversity in mammals and pennaraptoran dinosaurs. These new data show these increases are unlikely to be related to the evolution of novel integument types or the loss of other colour production mechanisms. Instead, consistent with studies within extant amniotes, our data support conserved low diversity in earlier vertebrates and increased diversity with shifts in the melanocortin system.



**The tenses of existence of the skink *Eugongylus rufescens* (Shaw, 1802) (Scincidae)**

**Shea G<sup>1</sup>**

<sup>1</sup>University Of Sydney, <sup>2</sup>Australian Museum Research Institute

The skink *Eugongylus rufescens* was described as *Lacerta rufescens* by the British naturalist George Shaw in 1802. As with many of Shaw's species, the species was described as a composite from several previous literature sources. While the name is currently applied to a widespread large skink distributed from the Maluku Archipelago to the Solomon Islands and north to Micronesia, the original description gives a type locality of Arabia and Egypt, and some of the European Islands. The complex pathway by which the application of the name has changed is defined, changing "what was" to "what is". The current application of the name still remains a composite of multiple species, and a neotype will be designated to further restrict the name in the future to a species from the southern Maluku Archipelago.

## The evolution and development of microcephalic sea snakes

Sanders K<sup>1</sup>, Sherratt E<sup>1</sup>

<sup>1</sup>*The University of Adelaide*

Snakes exhibit a diverse array of body sizes and shapes despite their elongate, limbless body plan. Among living snakes, arguably the most extreme shape changes along the pre-cloacal body axis are seen in fully aquatic sea snakes (Hydrophiinae) in the genera *Microcephalophis* and *Hydrophis*. These 'microcephalic' sea snakes have tiny heads and dramatically reduced forebody girths that can be less than a third of the girth of their hindbody. This morphology has evolved multiple times in species that specialise on hunting eels that reside in burrows. This talk will highlight the findings from several of our recent papers where morphological comparative analyses were used to understand the development and evolution of the microcephalic sea snakes' unique body plan. From data on skull shape, body shape, and axial morphology (size and number of vertebrae), we find several congruent patterns suggestive of heterochronic changes underlying the microcephalic sea snake morphotype. Our findings suggest that convergent evolution of microcephalic sea snakes has evolved through external selection pressures acting on developmental pathways, such that via shifts in growth and timing, dramatic morphologies can repeatedly evolve.

## Using MicroCT to investigate morphological evolution of elapid snake skulls

**Sherratt E<sup>1</sup>**

<sup>1</sup>*The University Of Adelaide*

X-ray microtomography (microCT) has undoubtedly revolutionised studies of morphological evolution by providing opportunity to visualise in situ the internal anatomy of museum specimens. Here I will demonstrate the power these data have to uncover patterns of morphological variation related to ecological opportunity as well as growth, using examples from my own research on elapid snakes. I will also discuss the challenges that are particular to scanning snake skulls, which are often highly kinetic and subject to preservation biases, and inside heads that are small relative to their elongate bodies.

**Expedition in search of Asian pit-vipers through China: field survey and systematic study (Viperidae, Crotalinae)**

**Shi J<sup>1</sup>**

<sup>1</sup>*Institute Of Vertebrate Paleontology And Paleoanthropology*

Asian pit vipers are small venomous snakes distributed mainly in Asia, which have very high species diversity and radiated into various habitats, such as subfrigid forests (*Gloydius halys*), plateaus (*G. monticola*), islands (*G. shedaoensis*) and deserts. There had still been some unclarified taxonomic problems and some unknown species to be described despite the publication of more than 10 species. In order to clarify the unsolved problems, we carried out a series of expeditions in search of Asian pit-vipers through China from 2012 to 2018. Morphological and molecular phylogenetic studies had been conducted and preliminarily clarified the taxonomic account of the pit vipers of the southwest China and southeast China: the different populations of “*G. intermedius*” from the northeast, northwest and north of China should be attributed to three different species: *G. h. halys*, *G. cognatus* and *G. stejnegeri*, *G. h. caraganus* should be elevated as species status.

Additionally, two new species of the genus *Gloydius* had been unexpectedly discovered from Qinghai-Tibet Plateau: *Gloydius rubromaculatus* Shi, Li and Liu, 2017 and *Gloydius angusticeps* Shi, Yang, Huang, Orlov and Li. *Gloydius rubromaculatus* sp. n. was observed to be insectivorous: preying on moths (Lepidoptera, Noctuidae, *Sideridis* sp.) in the wild. This unusual diet may be one of the key factors to the survival of this species in such a harsh alpine environment.

## Is Rick Shine really a Cognitive Neuroscientist in Disguise?

### Shine J<sup>1</sup>

<sup>1</sup>*The University Of Sydney*

Professor Shine has published over 1,000 papers, written several books and has left aside a legacy in which he has proposed creative and rigorous solutions to a wide range of problems at the intersection of herpetology and ecology. Perhaps less well-known is Rick's recent involvement in cognitive neuroscience. In work conducted with his son, a neuroscientist at the University of Sydney, Rick has played an integral role in crafting ideas about the organisation of the brain, both in humans and across phylogeny.

In this talk, I will summarise two recent projects:

The first project (published in *Frontiers in Psychology*) suggests a testable hypothesis for the relatively recent augmentation of human intelligence -- we argue that standing up catalysed the delegation of well-learned tasks to automaticity, thus freeing up the massive processing power of the brain to focus on the more challenging aspects of a task.

The second project (published in *Nature Neuroscience*) demonstrates that, of all of the billions of neurons in the human brain, functional brain activity only really utilises a handful of dimensions to accomplish cognitive tasks. In many ways, these patterns are reflective of our evolutionary history, and suggest important constraints that link humans to our evolutionary ancestors through the limits imposed by our neurobiology.

## **A life in science, as seen in the rear-view mirror of an increasingly slow-moving vehicle**

**Shine R<sup>1</sup>**

<sup>1</sup>*Macquarie University*

Our job as researchers is to try to make sense of nature, and we do this by transforming complex realities into simplistic narratives. In this talk, I'll apply the same approach of oversimplified story-telling to review how my career unfolded, and to speculate on why things worked out as they did. Strangely, many of the important determinants of my personal scientific trajectory were accidents of history rather than the result of strategic planning. Chief among those factors has been an enduring fascination with small creatures that crawl, hop and slither. A second peculiarity has been my addiction to understanding WHY such creatures look and act the way they do (as opposed to asking, for example, HOW they do such things). A third reflection is that success in science requires a diverse array of skills, including many abilities that I lack – but (to my surprise) that doesn't seem to have mattered very much. For example, my mediocrity in data-gathering and analysis has been disguised by students and collaborators who have filled those gaps with their own talents. The array of people we call "scientists" are remarkably diverse in terms of their motivations, approaches, abilities and interests. By celebrating that diversity, we can do better science and have a lot more fun.

## **When research zeal collides with family life**

### **Shine T<sup>1</sup>**

<sup>1</sup>*Macquarie University*

To succeed in research, a person has to devote a vast amount of time and energy to that pursuit. And inevitably, that affects his or her ability to play other roles. What are the main conflicts that arise between research and family life, and how can we balance those competing demands? In other words, how can a family encourage-help-bludgeon a researcher into also being a (reasonably) normal human being? I will discuss those issues based on a longterm dataset (more than 40 years) I have gathered as the wife of a herpetologist. Crucially, Rick and I found ways to combine family activities with herpetological experiences. Although our family holidays almost always took us to places full of reptiles, we still spent quality time with each other because family members became active members of the research team. That influenced the choice of study systems; for example, harmless snakes on tropical beaches were a win-win solution. The institutions that employ scientists often espouse family-friendly values, but the reality was that we had to work out our own tactics for finding a healthy blend. The enduring zeal of herpetologists makes life challenging for their loved ones, but also creates opportunities for enjoyable collaborations at the interface between science and family life.

## **Experimental eradication of invasive Brown Treesnakes with an automated bait manufacturing and aerial delivery system**

**Siers S<sup>1</sup>**, Goetz S<sup>1</sup>, Nafus M<sup>2</sup>, Hileman E<sup>2</sup>, Yackel Adams A<sup>2</sup>, Collins A<sup>3</sup>, Gosnell R<sup>3</sup>

<sup>1</sup>USDA Wildlife Services National Wildlife Research Center, <sup>2</sup>USGS Fort Collins Science Center, <sup>3</sup>USDA Wildlife Services

The accidentally-introduced Brown Treesnakes on Guam has caused substantial ecological damage and economic burden. High snake densities pose high risk of accidental introduction to other islands and preclude recovery of Guam's native vertebrates. Ground-based deployment of acetaminophen-treated dead neonatal mouse baits has proven effective at reducing Brown Treesnake abundance at local scales but is not practical for landscape-scale suppression over challenging and remote terrain. In response, the USDA Wildlife Services National Wildlife Research Center and Applied Design Corporation developed an Aerial Delivery System (ADS) for the automated production and aerial application of a new bait cartridge system for lethal control of Brown Treesnakes in large and remote forest habitats. When fired from the helicopter-borne dispensing module, cartridges open in flight to expose baits and tangle in the forest canopy, where they are available to foraging Brown Treesnakes. A single payload contains 3,600 bait cartridges; dispensed at a rate of up to four per second, we can treat 30 ha of forest at the maximum application rate of 120 bait/ha in 15 minutes of firing time. Experimental snake eradication has begun within a 55-hectare snake barrier on Guam, with the intention of evaluating the feasibility of eradication and providing snake-free habitat for ecological restoration evaluation. We provide an overview of the ADS and results of the first year of post-treatment snake abundance monitoring. This transformative technology holds great promise for increased biosecurity and recovery of habitat for Guam's native species.



## The Role of Reproductive Technologies in Amphibian Conservation Breeding Programs

**Silla A<sup>1</sup>**

<sup>1</sup>*University Of Wollongong*

Conservation breeding programs have been established for several threatened amphibian species globally to assist species recovery and decelerate declines. Despite captive breeding playing a fundamental role in the conservation of several threatened species, the overall success of captive breeding programs for amphibians remains low, with many programs failing to reliably and predictably initiate breeding behavior, achieve high rates of fertilization, generate viable offspring, or maintain genetic diversity and adaptive capacity of captive populations over time. Reproductive technologies have the potential to bypass the behavioral and physical impediments to natural mating that animals in captivity often encounter, offering enormous potential to enhance the propagation and genetic management of threatened species. An overview of the role of reproductive technologies in amphibian conservation breeding programs will be provided, highlighting recent research developing hormone therapies to induce spawning in the critically endangered Northern Corroboree frog, *Pseudophryne pengilleyi*. Synthetic gonadotropin-releasing hormone can be effectively used to induce spawning in *P. pengilleyi*, with 100% of male–female pairs ovipositing in response to an optimal dose of 0.5 ug/g GnRH-a. Spawning can also be effectively induced following the topical application of GnRH-a to the ventral pelvic region. Topical application of reproductive hormones eliminates the need for specialized training in amphibian injection. Therefore, refinement of these protocols will allow Reproductive Technologies to be adopted by a greater number of captive facilities globally to enhance the recovery of threatened species.

### **Investigating molecular adaptations in dendrobatid poison frogs.**

**Siu-Ting K<sup>1,2</sup>**, Constantinides B<sup>3</sup>, Torres-Sánchez M<sup>4</sup>, Wilkinson M<sup>5</sup>, O'Connell M<sup>6</sup>, Creevey C<sup>1</sup>

<sup>1</sup>*Queen's University Belfast*, <sup>2</sup>*Museo de Historia Natural, UNMSM*, <sup>3</sup>*Leeds University*, <sup>4</sup>*University of Kentucky*, <sup>5</sup>*Natural History Museum, London*, <sup>6</sup>*University of Nottingham*

Numerous studies have shown that in Dendrobatids, alkaloid toxins are acquired by “sequestration from diet”, i.e. the uptake and storage of toxins or their chemical precursors from their prey. In order to avoid being affected by the ingested toxins, the most toxic dendrobatids have evolved mutations in key voltage-gated membrane proteins. However, for less toxic dendrobatids and other frog species, we still do not know the metabolic and molecular adaptations that enable their toxin resistance. To address this, we generated novel whole transcriptomic data from the skin and liver tissues 3 dendrobatid species and 3 outgroup anuran species caught in a rainforest in Peru. Following quality assessment, assembly and gene orthology identification, we carried out differential expression analyses to identify tissue-specific gene expression unique to the poison frogs. To understand our results of gene expression in an evolutionary context, we also conducted a genome-wide analysis for positive selection. We identified novel genes involved in voltage-gated channel activity with signatures of positive selection in some dendrobatids but which did not present differential expression. Furthermore, we also identified differentially expressed genes involved in neuron membrane proteins which did not present signatures of positive selection. We found no signatures of positive selection or differential up-regulation in detoxification genes in the analysed species. These results suggest a model of gradual toxin resistance that involves both metabolic and molecular adaptations and a mechanism for how this trait evolved in Dendrobatid poison frogs.

## Inadvertent paralog inclusion drives artefactual topologies and timetree estimates in Lissamphibia.

**Siu-Ting K<sup>1</sup>**

<sup>1</sup>*Queen's University Belfast*, <sup>2</sup>*Museo de Historia Natural, UNMSM*

Increasingly large phylogenomic datasets including transcriptomic data from non-model organisms have allowed controversial and unexplored evolutionary relationships in the tree of life to be addressed but also risks inadvertent inclusion of paralogs in the analysis. While this may be expected to result in decreased support it is not clear if it could also drive the retrieval of highly supported artefactual relationships. Many groups, including the hyper-diverse Lissamphibia, are especially susceptible to these issues due to ancient gene duplication events, small numbers of sequenced genomes and because transcriptomes are increasingly applied to resolve historically conflicting taxonomic hypotheses. We tested the potential impact of paralog inclusion on the topologies and timetree estimates of the Lissamphibia using published and de novo sequencing data including 18 amphibian species, from which 2,656 single-copy gene families were identified. A novel paralog filtering approach resulted in four differently curated datasets, which were each used for Bayesian inference, maximum likelihood and quartet-based supertree phylogenetic reconstructions. We found that paralogs drive strongly supported conflicting hypotheses within the Lissamphibia (Batrachia and Procera) and older divergence time estimates even within groups where no variation in topology was observed. All investigated methods, except Bayesian inference with the CAT-GTR model, were found to be sensitive to paralogs, but when filtering was applied convergence to the same answer (Batrachia hypothesis) was observed. This is the first large-scale study to address the impact of orthology selection using transcriptomic data and emphasises the importance of quality over quantity particularly for understanding relationships of poorly sampled taxa.

## Ecological and Environmental Drivers of Global Diversity Patterns in Lizards

**Skeels A**<sup>1</sup>, Esquerré D<sup>1</sup>, Cardillo M<sup>1</sup>

<sup>1</sup>*Australian National University*

Lizard communities vary greatly in their diversity both within and across taxonomic groups, yet the mechanisms that allow closely related species to coexist in sympatry are often elusive. To investigate the ecological and environmental drivers of global lizard diversity, we analyzed patterns of species and functional diversity in ten ecologically distinct and globally distributed clades encompassing nearly all known lizard species. Using recently published spatial, phylogenetic, and phenotypic datasets, we built spatially explicit structural equation models to ask whether species diversity was directly or indirectly related to ecological divergence or similarity of species within communities, as well as features of the environment, including productivity, temperature, aridity, and topography. Our results show that high species diversity is achieved via different pathways in different lizard clades, but there is a prominent trend for high diversity communities to be comprised of functionally similar species in warm regions. Cold winter temperatures appear to place the strongest constraints on richness by preventing functionally similar species from co-occurring, which might be a result of resource competition in harsh environments. Our study highlights the ecological idiosyncrasies and generalities that allow diversity to accumulate and helps to explain the unique global diversity patterns of one of the worlds largest vertebrate radiations.

### **Surviving in the Suburbs: the Gibbs line and amphibian conservation**

**Skelly D<sup>1</sup>**, Freidenburg K<sup>1</sup>, Shepack A<sup>2</sup>

<sup>1</sup>*Yale University*, <sup>2</sup>*Florida International University*

Most amphibian species do not persist in developed landscapes such as suburban neighborhoods. During the 1990's James Gibbs observed the relationship between amphibian distribution and development, describing for a number of species a sharp drop in occupancy along transects of increasing human density. The "Gibbs line," this threshold between species presence and absence, offers an opportunity to study the factors that either promote or undermine amphibian populations. Properly considered, these patterns may help us understand how to design future suburban development to better promote the welfare of amphibians and other wildlife. Our studies suggest that the Gibbs line can move little across long periods of time even when development is an ongoing process. We also found that colonization debt is a demographic process that should be considered alongside extinction debt. A field experiment revealed that the quality of wetlands in developed landscapes may not be limiting distributions implying that future research should focus on limitations to movement and adult habitat proximity and quality. Overall, the demographics of amphibians in suburban environments illustrate the multidimensional, unintended influences of humans on other species. An intentional approach, taking into account what we can learn from existing development is a critical priority for amphibian conservation.

## Diversification and biodiversity dynamics of the Arabian squamate reptiles

**Smid J<sup>1,2</sup>**, Sindaco R<sup>3</sup>, Tamar K<sup>4</sup>, Aghova T<sup>1,2</sup>, Simo-Riudalbas M<sup>4</sup>, Carranza S<sup>4</sup>

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The Arabian Peninsula is a distinct and geographically well-delineated landmass with strong biogeographic links to Africa, Asia, and the Mediterranean. The peninsula hosts an unprecedented diversity of squamate reptiles, yet our knowledge of their distribution patterns and evolutionary history lags behind other regions and groups of vertebrates. While the interior deserts are relatively poor in terms of species richness, the isolated mountain ranges that rim the peninsula host rich diversity of habitats and often unique species. To comprehend how this biodiversity contrast was generated we need to understand the dynamics between speciation and extinction across different regions of Arabia. With a detailed knowledge on the geographic distribution of all Arabian squamates and with a nearly fully-sampled phylogeny of all described and cryptic species, we aim at investigating the mechanisms of their diversification dynamics in space and time. Using phylogenetic measures of biodiversity, we identify regions that conserve ancient diversity, in other words regions with slow speciation rates where the composition of the biota is a result of phylogenetic overdispersion. At the same time, we find regions with increased speciation rates that produce phylogenetically clustered communities produced by local radiations. We also analyze the level of phylogenetic similarity between the isolated mountain ranges along the peninsula to assess whether the rich squamate faunas of the mountains originated independently as in situ radiations or are a result of an increased dispersal into these areas.

## Improving and Using a Chromosome-Scale Assembly of the Enormous (32 Gb) Axolotl Genome

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The axolotl (*Ambystoma mexicanum*) has served as an important model for studying diverse areas of inquiry including: evolution, development and regeneration. However, its large genome (~32 gigabases) presents a formidable barrier to genetic analyses. Building on long-term efforts, we recently adapted an established mapping approach, using low coverage sequencing from 48 siblings. This cost-effective approach allowed us to increase the contiguity of the assembly by three orders of magnitude beyond the previous draft assembly and assemble the axolotl genome into 14 scaffolds. Fluorescence in situ hybridization was used to verify the structure of these 14 scaffolds and assign each to its corresponding physical chromosome. This new assembly covers 27.3 gigabases and encompasses 94% of annotated gene models. Individual assembled chromosomes range in size from 0.66 to 3.17 gigabases and are each the size of entire vertebrate genomes.

We also present recent updates and ongoing studies that have further improved this assembly and have used it to further our understanding of the axolotl's genome evolution, developmental biology, and key genomic changes that have occurred over its recent evolutionary history in nature and over 150 years of laboratory domestication. This chromosome-scale assembly has also permitted the implementation of a cost-effective and bulked-seggregate RNAseq strategy that permits rapid identification of mutant genes within this large genome. These include the genes underlying two mutant phenotypes that have evaded genomic characterization for several decades: cardiac (which impacts heart development) and short toes (which impacts limb development/regeneration).

## Microscopic and Macroscopic Photography: Bringing the Small to the World

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Conventional microscope systems have severe color, resolution and depth-of-field limitations that interfere with the operators' ability to make clear observations. Recent technical innovations in photography are generating contextually strong results that are more consistent with human perception than was previously experienced.

Focus stacking is the image generation technique used to generate 2D imagery and 3D models that are completely in focus, color accurate and high resolution. The mechanical process uses a motorized stage that moves the object relative to the camera. Images are automatically captured to record thin, overlapping focal sections used to create and show total depth of field for the targeted specimen. The computer process distinguishes sharp from blurry as represented by the image. The blurry areas of each image are discarded and the sharp areas are blended together until the entire image is displayed completely in focus; hence the term, focus stacking.

These technologies allow operators to non-destructively recreate visually striking images that combine the structural detail of an SEM, with the color detail of a microscope, without having to prepare or alter the specimen for analysis. Focus stacking is shown to generate more robust observations, which can be shared professionally and interpreted by larger groups of students and lay audiences.



**Identifying sex-linked markers in *Litoria aurea*: a novel approach to understanding sex chromosome evolution in an amphibian**

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Few taxa exhibit the variability of sex-determining modes as amphibians. However, due to the presence of homomorphic sex chromosomes in many species, this phenomenon has been difficult to study. The Australian frog, *Litoria aurea*, has been relatively well studied over the past 20 years due to widespread declines largely attributable to chytrid fungus. However, it has been subject to few molecular studies and its mode of sex determination remained unknown. We applied DArTseq™ to develop sex-linked single nucleotide polymorphisms (SNPs) and restriction fragment presence/absence (PA) markers in 44 phenotypically sexed *L. aurea* individuals from the Molonglo River in NSW, Australia. We conclusively identified a male heterogametic (XX-XY) sex determination mode in this species, identifying 11 perfectly sex-linked SNP and six strongly sex-linked PA markers. We identified a further 47 moderately sex-linked SNP loci, likely serving as evidence indicative of XY recombination. Furthermore, within these 47 loci, a group of nine males were found to have a feminised Y chromosome that significantly differed to all other males. We postulate ancestral sex-reversal as a means for the evolution of this now pseudoautosomal region on the Y chromosome. Our findings present new evidence for the 'fountain of youth' hypothesis for the retention of homomorphic sex chromosomes in amphibians and describe a novel approach for the study of sex chromosome evolution in amphibia.

## Longevity is not influenced by metabolic rates across the vertebrate tree of life

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The 'rate-of-living theory' postulates that life expectancy is a negative function of the rates at which organisms metabolise. Factors accelerating metabolism, such as high body temperature and active foraging, lead to organismic 'wear-out'. This reduces lifespan through an accumulation of biochemical errors and the build-up of toxic metabolic by-products. This theory is a keystone underlying our understanding of life history trade-offs, but it has been recently questioned. Because this theory has never been globally tested, in a phylogenetic framework, across both endotherms and ectotherms, we aimed to test several of its predictions. Using a global-scale dataset spanning life span data of 4,065 land vertebrate species, we performed the most comprehensive test of the fundamental prediction underlying the rate-of-living theory to date. We investigated how metabolic rates, and a range of factors associated with them, relate to longevity. Our findings strongly reject the predictions of the rate of living theory. RMR, FMR, seasonality, and activity times, as well as reptile body temperatures and foraging ecology, are unrelated to longevity. In contrast, lower longevity across tetrapod species is associated with high environmental temperatures. We conclude that the rate of living theory is incorrect and suggest that life expectancy is driven by selection arising from external mortality factors. A simple link between metabolic rates, oxidative damage and lifespan is false. Importantly, our findings lead to a novel hypothesis: that rapid climate change imposes strong selection for rapid life history evolution. Increasing global temperatures may thus result in accelerated senescence rates, especially in ectotherms.

## Population genetics of timber rattlesnake populations in Northeastern United States

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Understanding how genetic variation is distributed within and among populations of a species is widely used to make conservation management recommendations. Peripheral populations often have lower genetic diversity than core populations and may benefit from artificial gene flow for future population persistence. Using 13 microsatellite loci we quantified genetic diversity in 16 peripheral Timber Rattlesnake (*Crotalus horridus*) populations in the northeastern United States. Most of these populations are all within the geographic periphery of the range, with some in the core area of the range. Populations were highly differentiated from each other. There was no correlation between genetic distance and geographic distance. Seven clusters of individuals were identified with each cluster corresponding to a geographic region. This finding suggests that genetic drift has led to population differentiation, and likely overwhelmed natural selection. Within the largest peripheral population, there appears to be a metapopulation structure, with gene flow among nearby den regions. For future population persistence, assisted gene flow or 'genetic rescue' might provide a viable management action for the most-at-risk populations. If assisted gene flow were to be implemented, results presented here should serve as a guide for determining which populations are genetically diverse and large enough to serve as the best donor populations and which are most imperiled.

**Of poisons and parasites—the defensive role of tetrodotoxin against infections in newts**

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Newts of the genus *Taricha* have long been studied in regards to their toxin, Tetrodotoxin (TTX). Primarily, research has focused on the role of TTX as defense against their predators, garter snakes (*Thamnophis* sp.). Newt TTX levels have been studied intensively, and a great level of variation has been found. Much of this variation is not well explained simply by the presence of predators. Recent studies suggest that many of these compounds defend animals from parasites and other pathogens in addition to their predators. This study was done to investigate the relationship between TTX concentration and parasite infection. We collected a total of 345 adults newts (*Taricha torosa* and *Taricha granulosa*), measured their TTX levels, and assessed their micro- and macroparasite infections. As in other studies, there was wide variation in the amount of TTX in individual newts (range: 0 - 60 ug/cm<sup>2</sup> of skin). Newts were also found to have 18 unique taxa of helminths, fungi, viruses, and protozoans. Furthermore, TTX was found to be a negative predictor of overall parasite richness as well as infection by chytrid fungus and ranavirus, and factors such as species, sex, and size also influenced infection levels. These results suggest that TTX not only defends newts from predators, but also defends newts from parasitic infections.

**Analysis of ultraconserved elements supports African origins of narrow-mouthed frogs**

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Narrow-mouthed frogs (Anura: Microhylidae) are globally distributed and molecular data suggest the rapid evolution of multiple subfamilies shortly after their origin. However, several subfamilial relationships remain unexplored using phylogenomic data. Here we analysed 1,746 nuclear ultraconserved elements, a total matrix of 400,664 nucleotides, from representatives of 11 of 13 microhylid subfamilies. Species tree and maximum likelihood analyses unambiguously supported Hoplophryinae as the earliest diverging microhylid subfamily and Chaperininae as a junior synonym of Microhylinae. Given the emerging consensus that subfamilies from mainland Africa diverged early, microhylids have likely occupied the continent for over 100 million years.

## Cryptic and Failed Speciation in Sulawesi Flying Lizards: Part 2, Evidence for Mito-nuclear Dobzhansky-Muller Incompatibilities

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Three species of flying lizards (*Draco*) occur on Sulawesi. In part 1 of our presentation, we showed that these three species are composed of 12 deeply-divergent mitochondrial clades, with 9 of these mitochondrially divergent clades representing distinct cryptic or non-cryptic species. In two instances, we discovered deeply divergent mitochondrial boundaries at which there is no detectable barrier to nuclear gene flow in an ~1200-locus data set. We propose that these divergences reflect failed speciation events. According to our model, the mitochondrial boundaries represent the interface at which once-independent lineages came into secondary contact and merged, leaving the mitochondrial genome and one or more nuclear-encoded genes that interface with mitochondrial genes 'hung up' at this interface. Such Dobzhansky-Muller mito-nuclear incompatibilities are expected when once-isolated lineages come into secondary contact, as mitochondrial genes evolve relatively rapidly and compensatory changes are expected in nuclear-encoded co-adapted gene complexes to maintain organellar function. For 28 *Draco beccarii* samples spanning the 10.5% mitochondrial boundary on Sulawesi's SE Peninsula, we screened 603 nuclear-encoded genes known to interact with the mitochondrion, including ~90 genes in the OXPHOS pathway. Association tests found several genes with amino acid (AA) replacement substitutions strongly associated with the mitochondrial boundary, consistent with our hypothesized model. When considering the top-50 associated SNPs in the 603-gene data set, 16 were AA replacement substitutions. The same association test on the ~1200 locus data set detected four AA replacement substitutions, three of which are actually genes that interact with the mitochondrion (including two in the OXPHOS pathway).

### **Low embryonic heat tolerance increases vulnerability to climate change in low-latitude lizards**

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Heat tolerance at the immobile embryonic stage is expected to be very important in determining species vulnerability to climate change, but we still know little about how geographic variation of embryonic heat tolerance alters projections of species distributions. Here we experimentally determined the mean and developmental plasticity of embryonic upper thermal tolerance (EUTT) for three latitudinally-distributed populations of a lacertid lizard and incorporated the latitudinal pattern of EUTT into mechanistic models to predict population vulnerability to climate change. Our study demonstrated that EUTT decreased as latitude declined in the lizard. Moreover, the reaction norms of EUTT in response to developmental temperatures showed constant, bell-shaped and decreasing patterns at high, medium and low latitudes respectively, suggesting further decreases in EUTT at medium and low latitudes as developmental temperatures increase. Species distribution models predicted that the centroid of suitable habitats for this lacertid species would move northward in response to ongoing climate change. After incorporating latitudinal variation and reaction norms of EUTT, even lizards within areas predicted to remain highly suitable are expected to experience increases in heat stress over time, particularly at medium and low latitudes. Our study reveals geographic variation and developmental plasticity in EUTT and highlights its importance for predicting species vulnerability and distribution shifts in response to climate change.

## The relevance of bioacoustic monitoring for reassessing conservation status of bush frogs in Western Ghats

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Among amphibians, anurans are the most vocal, especially during the breeding season. The caller, usually males, produce conspicuous calls, to advertise their location to conspecific females demonstrating their readiness to mate, and to other rival males showing their willingness to defend their territory. Bush frogs of genus *Raorchestes* is the largest group of diminutive tree dwelling amphibians with as many as 54 extant species currently known from the Western Ghats. A vast majority of these Indian bush frogs are known only from their type locality (> 50 %) and are either listed as 'Data Deficient' or remain 'Not Evaluated' as per the IUCN criteria (> 50 %). Between 2012 - 2017, during the months of June to September, I conducted call based surveys across the Western Ghats to understand the distribution pattern of these amphibians. Data was collected for 42 out of the 54 identified species of bush frogs from the Western Ghats. The results of survey revealed several new localities for many of these bush frogs, especially those which were till date known from single localities. The data is also significant for those species which have been listed in 'threatened' categories based on their area of occurrence and distribution. This highlights the urgent need for the reassessment of conservation status of these bush frogs from the Western Ghats and implies how the use of acoustic monitoring can be very useful to monitor/identify new localities of these diminutive amphibians.



## Salamander population persistence from a spatially explicit cross-ecosystem perspective

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The concept of meta-ecosystem has been paid attention in recent years, which integrates a set of ecosystems comprising of connections between adjacent ecosystems of different habitats through resources and those between distant ecosystems of similar habitats via dispersal. Meta-ecosystem concept was developed mainly by ecosystem ecology, but it should be applied for a variety of metapopulation ecology, particularly for organisms exhibiting ontogenetic habitat shifts.

We targeted the salamander *Hynobius tokyoensis* that habits small mountain streams in the Kanto area of Japan. At the local scale, we evaluated habitat quality for both of larval and adult salamanders considering resource-based connection. At the regional scale, we evaluated habitat connectivity via dispersal using circuit theory that assumes random movement in heterogeneous landscapes and genetic analysis.

As a result, deciduous forests are considered to be suitable for both larval and adult salamanders; deciduous forests appear to enhance water temperature and prey abundance in the larval period. At the regional scale, forest continuity appears to increase dispersal between streams and raise population size. Thus, changing forest environments would have negative influence on the population of the salamander at both of local and regional scales, and they would be recipients of meta-ecosystem services.

## Site occupancy and eDNA concentrations of eastern hellbender (*Cryptobranchus a. alleganiensis*) in a large river

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Detection of threatened aquatic organisms using eDNA in a large river poses some challenges because of their microhabitat use, low eDNA concentrations due to their low abundance, and stream current preventing eDNA diffusion laterally across the stream. The eastern hellbender in Susquehanna drainage in Pennsylvania, USA, serves as a good model to examine sampling designs to overcome these challenges. Although their distribution was thought to be limited to the tributaries, our preliminary survey detected hellbender eDNA in the main stem. The aim of the present study was to examine the importance of sampling season, stream depth, vertical location along the stream stretch, and lateral location across the stream in detecting hellbender eDNA using occupancy modeling. In 2017, we collected water samples from four vertical locations, each of which contained five sampling sites spaced across the river. Sample collection was repeated monthly from July through October. We also collected water samples from three tributaries. We repeatedly detected hellbender eDNA from multiple sites, corroborating the evidence of hellbender existence in the main stem. The best supported occupancy model contained “lateral location” and “depth” as site occupancy parameters and “breeding season” as detection probability parameter. eDNA concentrations were also significantly higher during the breeding season and in the left side of the river. The concentrations were significantly higher in the tributaries than in the main stem. These results emphasize the importance of microhabitat use and breeding season in developing an eDNA sampling design in a large river where eDNA concentrations were relatively low.

**Ecological divergence of the eastern newt, *Notophthalmus viridescens***

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Polyphenism has been hypothesized to facilitate species diversification. In salamanders, this hypothesis has been tested from the view that metamorphic and paedomorphic adults may diverge from each other, which has received little support to date. We adopted a novel approach for the investigation of ecological divergence via polyphenism by testing for divergence between metamorphic adults that are adapted to different environments via differential polyphenic expression. By using the eastern newt subspecies (four subspecies currently designated based on body size, dorsal patterning, and life history) as models, we examined 1) ecological factors responsible for variation in polyphenic expression among the newt subspecies, 2) reproductive isolation between the parapatric subspecies that differ in polyphenic expression, and 3) the role of Pleistocene refugia in the ecological divergence among the newt subspecies. Overall, our findings suggest that 1) pond hydrology is one of the primary ecological factors driving the inter-subspecific divergence in polyphenic expression, 2) the body-size difference between the subspecies importantly affect sexual selection processes, resulting in nonrandom but not completely assortative mating patterns between the subspecies, and 3) isolation during Pleistocene through refugial retreat did not play a predominant role in the ecological divergence among the newt subspecies. Instead, the ecological divergence has likely occurred during postglacial expansion to the current distribution ranges. While there are strong supports for the polyphenism-facilitated ecological divergence, sexual selection processes may be halting completion of the ecological speciation among the eastern newt subspecies.

## **Cattle and Spears: damages of an uninvited guest to the Mediterranean herpetofauna**

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While studying the effect of grazing on the Mediterranean ecosystem using reptiles as an indicator, we accidentally encountered the potency of a relatively new predator in the system – the Cattle Egret (*Bubulcus ibis*). Therefore, we explored the impact of this eruptive waterfowl, which often escort cattle, on the lizard assemblages in Northern Israeli woodlands.

We integrated data on predator functioning and its effect on prey species abundances by: (1) mapping the distribution of egret nesting colonies; (2) exploring egret food menu during nesting seasons using vomit analyses in 10 large nesting colonies near woodlands; (3) sampling lizard assemblages using direct observations in sites along a distance gradient from these colonies; and (4) monitoring egret activity in study sites using direct observation and camera traps.

We found that: (1) nesting colonies are widespread; (2) lizards composed ~ 20% of egret diet; (3) sites in proximity to egret colonies had species-poor lizard communities; and (4) egrets were more abundant in grazed sites in proximity to colonies (<7 Km).

Our study demonstrated the wide spread distributions of Cattle Egrets in the Israeli Mediterranean ecosystem, and their direct impact on lizard communities, distinctly in cattle grazed woodlands. In context of the ongoing debate on the role of grazing, our results highlight a trade-off between the maintenance of habitat heterogeneity by this traditional land use practice, and the increased risk of predation to wildlife caused by the subsequent egret infestation.

### Fine-scale evolution of aposematism in poison frogs

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Evolutionary origins of chemical defense in poison frogs are associated with the appearance of brightly colored warning signals and shifts in diet, the source of their defenses. The defended phenotype varies substantially in *Epipedobates* poison frogs, offering the opportunity to parse apart how aposematism, the pairing of an anti-predator defense with a warning signal, evolves. With a dataset encompassing 300 individuals representing all seven *Epipedobates* species, we estimated patterns of change in diet, chemical defense, and warning coloration over evolutionary time. Preliminary data show that a moderate level of chemical defense evolved in a cryptic ancestor of the clade. Then, slight to moderate conspicuousness evolved several times, in *E. darwinwallacei*, *E. anthonyi*, *E. machalilla*, and *E. tricolor*. One of these species, *E. anthonyi*, possesses relatively increased chemical defenses, and several of its populations also display high conspicuousness (i.e., bright red or white coloration). Unexpectedly, patterns in diet did not correlate with shifts in chemical defense or warning signals, at least at the scale of our analyses. We suggest that chemical defense in *Epipedobates* evolved prior to and likely drove multiple origins of warning signals, which were then augmented in one species with increased defenses. The role of diet in the origin of aposematism remains unclear, suggesting that changes in chemical defense are more likely attributed to modifications in the physiological ability to sequester chemicals rather than shifts in dietary access to chemicals. These results reveal potential evolutionary scenarios and processes that favor the origin of complex traits like aposematism.

## Unraveling the role of body size in thermal maxima

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In recent studies on the critical thermal maximum (CTmax) of lizards in our laboratory, we observed a body size effect under hypoxic conditions, where larger lizards have lower CTmax. We followed this up by testing four species of lizards under normoxic and hypoxic conditions, and found that snout-vent length is negatively related to the CTmax under hypoxic conditions in large-bodied lizard species. This could be a true body size effect, or it could be an artifact of how CTmax is measured. When studying thermal tolerance, we measure a lizard's body temperature via the cloaca. However, brain temperature may be more germane to CTmax than cloacal temperature, since high temperatures are thought to impact the central nervous system. The higher thermal inertia of larger lizards could mean that brain-cloaca temperature differentials are impacted by body size. To test this hypothesis, we measured brain and cloacal heating rates simultaneously. We found that brain temperatures were consistently lower than cloacal temperatures, and that smaller lizards had larger brain-cloaca temperature differentials than larger lizards. Further, running the same protocol on dead lizards revealed that they had lower brain-cloaca temperature differentials than live lizards, indicating that lizards actively cool their brains via physiological regulation. Our data suggest that body size can greatly impact the data we obtain in CTmax studies, and we provide cautionary information to researchers measuring this variable.

## The role of paleogeographic history in the evolution of Afro-Arabian squamate reptiles: a large-scale perspective

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Since the beginning of the split of the Arabian and African plates at the end of the Eocene, the geological history of the area comprises a complex succession of temporary separation and reconnection of landmasses. This geological history has been shown to have a crucial impact on the regional fauna, shaping its biogeographic patterns. Specifically, the evolutionary histories of several reptile genera from north Africa and Arabia have been independently explored and discussed within this geological context. However, an integrative study incorporating all those reptile groups is still lacking. Here, we assemble a comprehensive dataset including most of the squamate genera present both in Africa and Arabia with the objective of investigating large-scale biogeographic patterns in a comparative framework. We reconstruct phylogenetic relationships among more than 500 species with 5 mitochondrial and 6 nuclear loci, and estimate divergence times with eleven calibration points, including fossils and biogeographic events. With this broad dataset, we are able to reconstruct and compare the evolutionary history of 22 genera of squamate reptiles across north Africa and Arabia. By including data on species traits, we associate phylogenetic and morphological diversification with paleogeographic and paleoclimatic events affecting these regions. As a result, we can describe how the Afro-Arabian geologic and climatic history has shaped the diversity and evolution of desert-dwelling squamates.

## What mechanism sets thermal tolerance in reptiles and amphibians?

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Much recent theoretical and empirical work has sought to describe the physiological mechanisms underlying thermal tolerance in animals. Leading hypotheses can be summarized as either subcellular components (i.e. proteins or membranes) or organ systems (i.e. oxygen and capacity limited thermal tolerance) failing at high temperatures. Published data from decades of research on the physiology of amphibians and non-avian reptiles suggest that both mechanisms are important with hierarchical effects. Thus, we propose an integrated framework, which we call Hierarchical Mechanisms of Thermal Limitation (HMTL), to explain how subcellular and organ system failures interact to limit performance and set tolerance limits at high temperatures, which we further integrate with the commonly-used thermal performance curve paradigm. We use model simulations to illustrate novel predictions of the HMTL framework, and to weigh the potential ecological relevance of this mechanism. We highlight that the HMTL mechanism will restrict the capacity of animals to seek relief from elevated temperatures by moving to high elevations because reduced oxygen partial pressures result in a lowered thermal optimum and peak performance. We hope that this framework spurs innovative experiments and models to better forecast the effects of global change on reptiles and amphibians.



**Phylogenetic relationship of *Cyrtodactylus pulchellus* complex in Thailand: Implication for taxonomic status**

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Thai-Malay Peninsula region has the complexity geology, providing various habitats for geckos. Several taxa have been discovered and recognized as species complex including *Cyrtodactylus pulchellus*. To reveal the phylogenetic relationship and evaluate the taxonomic status of the *C. pulchellus* complex in Thailand, molecular analyses of three focal species (*C. lekaguli*, *C. macrotuberculatus* and *C. phuketensis*) were conducted using mitochondrial ND2 gene. The Bayesian Inference showed strong support, revealing two major clades, (I) the *C. lekaguli* group, and (II) the *C. macrotuberculatus* and *C. phuketensis* group. The genetic distance between these two major clades was high (>15.0%) whereas that within clade II (*C. macrotuberculatus* and *C. phuketensis*) was low (<3.0%). Additionally, morphometrics and morphological characters of these two species are indistinct. Therefore, the taxonomic status of *C. macrotuberculatus* and *C. phuketensis* should be re-evaluated using a combination of molecular and morphological evidence and additional samplings should be conducted across their geographic distribution.

### Adaptation and evolution of Tiger Snakes

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Island populations have long been of interest to evolutionary biologists because of the rapid adaptation possible in response to the novel environments of islands and island-like ecosystems. Novel phenomenon such as changes in body size, head size, and evolution of venom, are often observed on islands in comparison to conspecifics on the mainland, which is especially true of the tiger snake, *Notechis scutatus*, found on islands across southern Australia. Tiger snakes are known to have evolved differences in body size (both gigantism and dwarfism), head size (on recently colonised islands), and possibly venom (in response to prey differences). We investigate evolutionary and adaptive molecular mechanisms involved in these phenomena, using a newly published tiger snake genome and recently generated methylomes.

## Niche differentiation of two coexisting tadpoles on Emei Mountain

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Exploring the mechanisms of species coexistence is a central issue in ecology. Despite theory has been provided, little attention has been paid to the coexistence of amphibians, especially tadpoles. In the present study, Emei Mountain was selected as the study area, and *Leptobrachium boringii* and *Quasipaa boulengeri* tadpoles were selected as the models to test how tadpoles coexist in mountain streams. Specifically, we quantified 1) functional niche difference, 2) micro-habitat utilization difference, and 3) trophic niche difference between the two tadpoles. Our results indicated that the observed functional richness of *L. boringii* (12.59%) was lower than that of *Q. boulengeri* tadpoles (46.67%). Functional identity was significantly different between them based on PERMANOVA analyses ( $P < 0.001$ ). And functional overlap between them was extremely low (1.47%). Micro-habitat utilization were also different between the two tadpoles. *Q. boulengeri* mainly inhabited in the environment where the stream was deep and the water-velocity was slow. While the *L. boringii* lived in the stream with a certain velocity and shallow environment. Although trophic niche size was not significantly differed, the trophic overlap (SEAC) was 16.55% between the two tadpoles, indicating that they prefer specific food resources. Overall, *L. boringii* and *Q. boulengeri* tadpoles differed in terms of functional, spatial, and trophic niche, which allowed the coexistence of them. We believe that this study provides an important information for the protection of endangered amphibians and biodiversity.

### **Nestled in the city: nesting behaviour enhances embryo survival in urban areas**

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<sup>1</sup>*Auburn University*, <sup>2</sup>*Australian National University*

Lizards occupy diverse habitats around the world from dense forests to arid deserts, which pose different challenges for nesting females because embryo development is directly affected by nest microhabitats. For lizards that occupy urban environments, the nesting situation for females is exacerbated as ground temperature (due to the urban heat island effect) can extend beyond tolerable limits for developing embryos. Despite this, many species have established populations in urban areas outside their native ranges and habitats. Without physiological adaptations of embryos to markedly hotter conditions, maternally-selected nest sites may enhance embryonic survival. In Puerto Rican crested anoles from an urban area in Miami, mothers' nest in microhabitats that are, on average, cooler than microhabitats that are not used for nesting. To test the hypothesis that mothers choose sites with thermal environments that facilitate embryo survival, we incubated eggs from a captive colony of crested anoles under thermal regimes that mimic 1) maternally-selected nests in urban areas, 2) sites not used for nesting in urban areas, and 3) nests sites in a nearby forest. We found that thermal regimes of maternally-selected urban nests yield higher egg survival than those of sites that were not used. Survival did not differ between urban and forest nest treatments. However, eggs incubated under urban treatments developed faster and hatched earlier than those incubated under forest thermal regimes. Our study revealed that maternal nest sites in urban areas shield embryos from potentially lethal conditions, which contributes to the successful colonisation of anoles in Miami.

## Identification of divergent serpentoviruses in free-ranging invasive pythons in southern Florida, United States

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Established invasive pythons in southern Florida, such as the Burmese python (*Python bivittatus*), have significantly impacted native ecosystems. Aside from direct predation and competition, invasive species can also introduce nonnative pathogens that can negatively affect native species. The genus *Serpentovirus* (order Nidovirales) is composed of positive-sense RNA viruses primarily found in reptiles. Some serpentoviruses, such as shingleback nidovirus, are associated with mortalities in wild populations, while others, including ball python nidovirus and green tree python nidovirus, can be a major cause of disease and mortality in captive animals. To determine if serpentoviruses were present in invasive pythons in south Florida, oral swabs were collected from both long-term captive and free-ranging in situ pythons. Swabs were screened for the presence of serpentoviruses by reverse transcription PCR. Sanger sequencing of PCR amplified products identified multiple divergent sequences, circulating in the invasive pythons across their range. Clinical signs and postmortem lesions consistent with *Serpentovirus* infection were observed in a subset of sampled pythons. Though the risk these viruses pose to native species is currently unknown, the potential for spillover to indigenous herpetofauna is of concern and warrants further investigation.

## Using environmental DNA sampling to delimit a European newt invasion in Australia

Tingley R<sup>1</sup>

<sup>1</sup>Monash University

Early detection and rapid response (EDRR) efforts require an ability to detect populations in the early stages of invasion. Environmental DNA (eDNA) sampling—detection of extracellular DNA from environmental samples—has the potential to revolutionise the initial stages of EDRR. In this talk, I will outline how my research group has used this emerging survey technique to monitor the spread of a recently established invader, the European smooth newt *Lissotriton vulgaris vulgaris*, in Melbourne, Australia. Initial field-based comparisons between a traditional sampling technique (bottle trapping) and eDNA sampling demonstrated that per-sample eDNA detection probabilities were much higher (0.29–1.0) compared to per-trap estimates (0.01–0.26). Optimisation analyses revealed that eDNA sampling was also more cost-efficient than trapping, once species-specific eDNA primers had been developed. I will conclude by illustrating how we are currently using eDNA sampling to delimit the extent of the Melbourne newt invasion, in order to determine the most appropriate management response. Collectively, our findings demonstrate that eDNA sampling can be a sensitive and cost-efficient technique in the EDRR toolkit.

## Restinga - The North Coast of Bahia Sand Dune Herpetofauna, Brazil

Tinoco M<sup>1</sup>

<sup>1</sup>*Environmental Planning Graduate Program - Ucsal*

North Coast of Bahia Restinga ecosystems, given the strong anthropogenic pressure and megadiversity, is perhaps one of the most threatened portions of this ecosystem in Brazil. It forms in a tiny portion some beautiful landscapes such as: dunes, forests, enclaves of cerrado, semiarid, perennial and temporary lagoons. This entire landscape, together, houses over 600 vertebrate and thousands of plant species under an ongoing anthropogenic threat, which altogether makes of it a conservation hotspot. Amphibians and reptiles are present with over 170 vanishing species. The restinga, once a living landscape for ancestral tribes, later a stage for Europeans struggling to enrich in these plains, then to traditional communities, resulting in large latifundia, and nowadays carries on being colonized by great corporations of hotel resorts, condominiums and its own, why not saying, unnatural urban growth. Together, they bring about suppression of natural habitats, introduce tens of invasive species, cause fires, and deplete natural resources. We are not only concerned here to illustrate or promote a little of the knowledge about the ecology and conservation of the herpetofauna within the region, but to provoke, through these fantastic animals, ways to promote the conservation of the restinga. Nowhere else on this planet we can witness the merging of four great biomes, where we witness the union of countless elements of the Atlantic Forest, Caatinga, Cerrado and Marine "biome" in an incomparable way. We hope this product, framed here, will return as the protection of this global importance biodiversity hotspot which calls out for help.

**Conservation of Miyako grass lizard, *Takydromus toyamai*: life history traits, population genetics, and conservation measures**

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*Takydromus toyamai* is a green-colored grass lizard endemic to the Miyako Islands in southwestern Japan. This species is believed to have been common in many places in the past. Currently, however, facing serious threats to population decline, it is listed as critically endangered in the Japanese Red List. According to our surveys on local distribution and current status using field census and questionnaire, this lizard was still occasionally found in various ubiquitous habitats including disturbed grass fields nearby human vicinity. This suggested low habitat requirement of this species. A mark-and-recapture study also revealed that *T. toyamai* has high growth rate (0.3 mm/day), long period of recruitment (near half a year), and early sexual maturity (less than one year). Given its association with transient grass fields, these results implied that populations of *T. toyamai* potentially had high tolerance against environmental disturbances, although its high-density areas were actually very scarce. Field observations in high-density areas indicated that they appeared in some restricted sites along, otherwise seemingly uniform and continuous environment. Results of population genetic analyses suggested that gene flows were restricted between some neighboring local populations. These results imply that one possible cause of its scarcity seems to associate with its high fidelity. In this presentation, we also introduce other conservation activities; e.g., evaluating vegetation structure in high- and low-density areas, mowing experiments, and social education with the cooperation of the local government and high school. Integrating all, we seek effective and feasible schemes for future conservation measures of this species.



**Relict distribution of *Microhyla* in the Ryukyu Archipelago: High diversity in East-Asia maintained by insularization**

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The Ryukyu Archipelago, located at the southwestern part of Japan, is known as a group of continental islands and harbors many endemic taxa, supposedly reflecting its fairly long isolation from the Eurasian continent, Taiwan, and the Japanese main islands. *Microhyla okinavensis* has been known as an endemic member of the terrestrial fauna of this archipelago. Molecular phylogenetic analyses using samples from nearly all island populations of the species and representative samples of other east Asian congeneric species revealed that *M. okinavensis* consists of four distinct subclades, of which the Amami, Okinawa, and Miyako subclades, though exhibiting distinct genetic differentiations from each other, formed a monophyletic group (clade A). The remaining Yaeyama subclade was exclusively sister to *M. mixtura* from inland China, forming another monophyletic group (clade B), rendering *M. okinavensis* in the current definition paraphyly. These results, as well as estimated dates of divergence from related taxa, indicate that *M. okinavensis* actually includes more than one distinct species. The species currently recognized as *Microhyla okinavensis* and *M. mixtura* are considered as relictual species with disjunct distributions most probably caused by replacement with *M. fissipes* in intervening areas.

**Let the little guys speak! Anuran community ecology and calling acoustics in windfarms**

**Trowbridge C<sup>1</sup>**, Withers K<sup>2</sup>, Litzgus J<sup>1</sup>

<sup>1</sup>*Laurentian University*, <sup>2</sup>*Brookfield Renewable*

To combat climate change, alternative “green” energy sources are being introduced, like wind energy. Although green energy sources reduce reliance upon fossil fuels, they may introduce new sources of habitat alteration and noise pollution. Negative impacts of habitat destruction on wildlife are well-documented, while impacts of noise pollution is a growing concern as a new non-physical threat to the environment. Many anuran species exhibit altered acoustic characteristics and behaviours in response to noisy environments, such as road traffic, yet few studies have considered wind energy as a source of disruptive noise. In May 2018 we began studying anuran community ecology and acoustics within Prince Wind, a Northern Ontario windfarm located close to relatively pristine wetlands. We are measuring anuran diversity in habitats adjacent to wetlands using transect surveys and anuran chorus characteristics (intensity, diversity, and dominant frequency) using nightly audio recordings with SM4 Song Meters in 4 turbine and 4 control sites located >1.5 km from turbines. If wind energy facilities present a new source of noise pollution to anurans, then we expect wetlands within the windfarm to have lower species diversity and lower chorus intensity. Preliminary analyses indicate similar diversity and number of species calling in both turbine and control wetlands, with 4 different species recorded in both treatments. Amphibian populations are declining globally, so understanding and discovering new possible threats and mitigation options are essential to their conservation. We will discuss our full results and how they could influence amphibian conservation under the demand for green energy alternatives.

**Finding needles in haystacks: using niche models to locate previously unknown populations of sea snakes**

**Udyawer V<sup>1</sup>**, Somaweera R<sup>2</sup>, Nitschke C<sup>3</sup>, D'Anastasi B<sup>4</sup>, Sanders K<sup>3</sup>, Webber B<sup>2</sup>, Hourston M<sup>5</sup>, Heupel M<sup>6</sup>  
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Strategies aimed to conserve and manage rare species are often hindered by the lack of data needed for their effective design. Incomplete and inaccurate data on habitat associations and current species distributions pose a barrier to effective conservation and management for several species of endemic sea snakes in Western Australia which are potentially declining. Here we used an environmental niche modelling approach to understand habitat associations and identify suitable habitats for five of these taxa (Short-nosed sea snake, Leaf-tailed sea snake, Dusky sea snake, Brown-lined sea snake and Shark Bay sea snake). Models were used to predict species-specific habitat suitability across coastal waters along the North West Shelf of Western Australia, in order to prioritise future survey regions to locate unknown populations of these rare species. Models were also used to quantify the effectiveness of current spatial management strategies (i.e. Marine Protected Areas) in conserving important habitats for these species. Defining accurate geographic distributions for rare species is a vital first step in defining accurate extent of species occurrence and range overlap with regional threatening processes.

## Origins of lizard diversity in the continental island of Sri Lanka: insights from scincid lizards

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Understanding the origins of island faunas is a primary goal in evolutionary biology and biogeography. Sri Lanka is a continental island at the tip of southern India with an exceptional diversity and endemism of lizards (78%). Yet, relatively little is known about the origins of this remarkable diversity in the island. Of the 106 species of lizards, 32 are skinks belonging to 8 genera. To understand the biogeographic origins and patterns of diversification in Sri Lankan skinks, we investigated the phylogenetic relationships of *Eutropis* and *Lankascincus*, two of the most speciose genera of skinks in Sri Lanka. Our dated molecular phylogenetic analyses with Ancestral area reconstructions reveal that members of the genus *Eutropis* have colonized the island at least four times from India independently between 6.5-0.5 million years ago (MYA). However, the common ancestor of *Lankascincus* and its south Indian sister lineage, *Ristella* colonized the island more than 6.7 MYA from India triggering an in-situ radiation ~2.8 MYA which sequentially gave rise to six main lineages of *Lankascincus*. Majority of the *Lankascincus* species diversified within the southern Wet bioclimatic zone (annual rainfall >2000 ml) of Sri Lanka. Several species of *Lankascincus* shows recent (0.8-0.5 MYA) genetic divergence among their isolated populations distributed in different mountains which was most likely driven through isolation in the mountain tops due to climate change driven fragmentation of the contiguous habitat. Thus, our findings indicate that a combination of colonization and in-situ radiation have been responsible in determining the skink diversity in this continental island.

## Positive Perceptions of the Enigmatic Eastern Hellbender Salamander: Content Analysis of Historical to Current Media

Unger S<sup>1</sup>

<sup>1</sup>*Wingate University,* <sup>2</sup>*Eastern Band of the Cherokee Indians*

Print and online media may reflect changing perceptions about reptiles and amphibians when viewed in a historical context and as conservation programs bring about increased awareness of declining species management. To determine whether public perceptions of a species with a proven history of public misunderstanding and persecution, the hellbender salamander, change according to societal interests over time, we conducted a content analysis of 288 newspaper articles over the past 153 years of coverage through Conservation Era's, including: Exploitation (1850-1899), Protection (1900-1929), Game Management (1930-1965), Environmental Management (1966-1979), and Conservation Biology (1980-2016). In addition, we examined trends in more recent online media coverage. As measured by article frame (valence values), we detected an increase in positive perceptions about hellbenders in newspapers after 1980, which coincides with the Conservation Era. Among the most frequently-used words used to describe hellbenders pre-1980's, include "ugly" and "a monster" and "repulsive", whereas more recent words describing hellbenders include "unique" and "rare." Many articles before the Conservation Era included surprisingly informative natural history. Conservation efforts clearly had impacts on online media coverage, which increased following the federal listing of Ozark hellbenders and their successful captive rearing by the St. Louis Zoo in 2011. These results are encouraging for conserving this enigmatic salamander.

## Generation of amphibians from cryopreserved sperm; proof of capacity for development beyond metamorphosis

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The continued global decline of amphibians has sparked a movement among reproductive and conservation biologists to use artificial reproductive technologies (ARTs) to assist with recovery and preservation efforts. Within Australia, seven species have succumbed to extinction with several species at risk of extinction. Thus, sperm cryopreservation and IVF techniques have been investigated for several pelodyadids, with a focus on the Eastern sedge frog, *Litoria fallax*, the bleating tree frog, *Litoria dentata* and the endangered green and golden bell frog, *Litoria aurea*.

Previous studies of several *Litoria* sp. have utilised 15% v/v dimethyl sulfoxide (DMSO) in combination with 10% w/v sucrose as a cryoprotectant. However, trials with *Litoria fallax* and *Litoria dentata* have indicated an improved response to cryopreservation when the sucrose levels are lowered to a hypotonic level (1% w/v), with post-thaw recovery of forward progressive motility at ~45% and vitality at ~82% in *Litoria dentata*.

Using this approach, the first successful IVF from cryopreserved sperm in an Australian amphibian species was achieved using *Litoria fallax*. Giving validation to the biobanking approach, a mature male was produced. More recently, using sperm stored for four years, several *Litoria aurea* adults have been produced from cryopreserved sperm frozen using 15% v/v DMSO in combination with 10% w/v sucrose as cryoprotectants. The average forward progressive motility (~24%) and vitality (~53%) recovered post-thaw for *L. aurea* in this treatment was lower than that achieved in *L. dentata*. These studies provide valuable knowledge and progression of the ARTs for Australian pelodyadid species.

### Sex-biased dispersal in the Asp viper

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Sex-biased dispersal is the consequence of differences in costs and benefits of dispersal between sexes. This phenomenon is essential to maintain genetic diversity within and among populations. Several studies demonstrated female-biased dispersal in mammals and male-biased dispersal in birds. In reptiles, male-biased dispersal seems to be prevalent, but only a few studies were conducted in this group until now. Moreover, most of these studies considered only populations in a single location, although one study demonstrated distinct patterns of sex-biased dispersal between populations of different locations. We investigated sex-biased dispersal in the Asp viper (*Vipera aspis*) in four locations in Switzerland using microsatellite markers. We hypothesised that males disperse farther, whereby females are more spatially autocorrelated and show a stronger isolation by distance than males. In three of the four investigated locations, a trend for male-biased dispersal was detected, while in one location female-biased dispersal was found. A possible explanation for this difference is the higher level of fragmentation of the latter location. Our study demonstrated the importance to test for sex-biased dispersal in different habitats and that general conclusions about patterns of sex-biased dispersal should be drawn with caution if studies are conducted in a single location.

## Metabarcoding as a tool to determine feeding behavior of the European pond turtle

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Knowledge of feeding strategy and food preferences is one of the milestones of the natural history of a species and is essential to optimise conservation programs. The European pond turtle (*Emys orbicularis*, L. 1758) is the only freshwater turtle living in Switzerland and ranked as critically endangered on the Swiss Red List. Its diet is still unclear, considered by some studies as carnivorous, often scavenger and sometimes vegetarian. We conducted analyses of *Emys*' diet by developing a new method of global DNA amplification and metabarcoding analysis, using universal PCR primers to determine the species occurring in the faeces.

The analysis of the diet of this species was conducted during its whole activity period (April to September) in the natural reserve of Moulin de Vert (Geneva, Switzerland) to determine if there is a shift in food intake during whole activity period and if diet varies between adults/juvenile and males/females. Furthermore, four different populations were sampled during the month of July to detect possible difference in food consumption. Moreover, this study not only determined the nutritional needs for the European pond turtle, but also demonstrated that this species is not a threat to its environment (predation on other threatened species such as amphibians). Globally, we were able to demonstrate, using the European pond turtle, that the genetic analyses of faeces could be an efficient tool to determine trophic networks with a very high level of precision.



## Effects of maternal diet on reproduction and development in turtles

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Maternal effects are the nongenetic influences mothers have on offspring phenotype. They include nest site choice, egg brooding, genomic imprinting, and the nutrients and hormones mothers allocate to their eggs. The nutrients mothers allocate to their young depend on the nutritional resources available to her during reproductive allocation. We used a well-studied set of populations of declining Murray River Short-necked Turtles (*Emydura macquarii*) to investigate how maternal diet differences influence reproductive success, embryonic development, and hatchling phenotype. *Emydura macquarii* is a generalist that consumes green algae, aquatic plants, periphyton, aquatic invertebrates, fish, and carrion. We studied *E. macquarii* in four populations in north-central Victoria that differ in adult diet. We show that females from more herbivorous populations primarily eat filamentous green algae and exhibit higher body condition indices than do females from more carnivorous, and potentially food-limited, populations. These constraints are associated with reduced clutch size. Clutches from the same food-limited populations also experience lower hatching success rates, and hatchling growth rates differ among populations across an interaction with incubation temperature. Our data indicate the potential for resource limitation to impact fitness via both direct reductions on clutch size and indirect impacts on developing embryos and offspring. We are continuing to test this hypothesis by comparing embryonic metabolism and egg and hatchling nutritional composition among populations. In addition to advancing knowledge of maternal effects, our results provide initial understanding of how environmental constraints on reproduction translate to fitness impacts on offspring of a declining species.

## **New Zealand border and post-border detection and response strategies**

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<sup>1</sup>*Bioresearches (A Babbage Company)*, <sup>2</sup>*Biosecurity New Zealand*

Global appreciation of the significance of herpetological invasive species is growing. New Zealand is an isolated archipelago that relies heavily on international trade. As cross-border freight volume grows so too have the interception rates of reptiles and amphibians. New Zealand has an international reputation as a leader in biosecurity and exotic organism management and NZ Legislation provides an effective framework for border management, controls on importation, and rapid response to incursions of exotic disease and organisms. Biosecurity New Zealand (BNZ) is tasked with leading New Zealand's biosecurity system; a multi-layered defence including offshore activities, importation regulations, strict border measures, and active surveillance programmes. These are backed up by a post-border incursion-response system to rapidly manage incursions of invasive species or provide for effective long-term management. Success is demonstrated by fact that, while more than 3000 individuals comprising > 150 species of amphibian and reptiles have been intercepted entering NZ over the past 19 years, only one amphibian has successfully established, and this species is currently subject to an intensive eradication programme. BNZ, relies on the expertise of technical experts in the fields of taxonomy and invasive herpetofauna management, to provide timely species determinations, risk analyses and advice on detection and eradication methods and operational strategies. The BNZ intercepted herpetofauna database and reference specimen collection—the largest of its type in the world—is paramount for elucidating pathways for exotic herpetofauna arrival and assessing the risk of exotic parasites and pathogens to native herpetofauna. Measures are constantly evolving towards improving the biosecurity system.

**NZ conservation management of Data Deficient and Data Poor lizard taxa: progress, challenges, future directions.**

**Knox C**<sup>1</sup>, van Winkel D<sup>2</sup>, Lettink M<sup>3</sup>

<sup>1</sup>*Wildland Consultants Ltd*, <sup>2</sup>*Bioresearches (Babbage Consultants)*, <sup>3</sup>*Fauna Finders*

New Zealand is home to a unique and diverse herpetofauna that includes more than 104 lizard taxa, of which nearly half (~48%) are considered 'Data Deficient' (DD) or carry 'Data Poor' (DP) qualifiers under the NZ reptile conservation threat status assessment. Research funding is generally prioritised for nationally threatened taxa, for the purpose of understanding drivers of decline and/ or the development of conservation management strategies. However, the importance of understanding the ecology and population status of very poorly known taxa and assigning appropriate conservation threat statuses is fundamental for determining the level of management they require. Here we highlight case studies where recent progress has increased our knowledge of the biology, ecology and threat status of DD and DP lizards. We also discuss the challenges faced when working with these taxa, including taxonomic uncertainties, working in challenging environments, difficulties estimating population trends, ascertaining causes of decline and understanding how to arrest declines. Identifying knowledge gaps is also crucial for directing future research to ensure outcomes contribute to the elevation of DD taxa and remove uninformative DP qualifiers.

## Alpine newt delimitation, containment and eradication strategies

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New Zealand has historically suffered relatively few incursions or establishments of exotic herpetofauna. However, detection of the alpine newt (*Ichthyosaura alpestris*) in 2013 initiated a rapid incursion response by Biosecurity New Zealand and Department of Conservation. Delimitation efforts confirmed the presence of large established population that was confined an area of c. 30 hectares. Risk analyses regarded the newts to be a serious biological threat to indigenous *Leiopelma* frogs as vectors of chytrid fungus and other pathogens. A commitment to contain and eradicate the newt was made in late 2013 and an operational strategy is currently overseen by a technical advisory group (TAG) and supported by a proactive communication plan for all landowners affected by the eradication programme. This strategy has gone through several iterations over the last six years and has been largely adaptive in its approach to delimitation, containment and the application of eradication tools. It has relied heavily on research into the development of tools and techniques such as containment fencing, escape-proof pit-fall traps, sentinel ponds, lures, detector dogs, habitat removal methods, and skeletochronology. Newt captures have declined dramatically over the course of the eradication effort to the degree that knock-down has been achieved. The greatest test lies ahead as the eradication progresses into the mop-up phase targeting the last individuals, and finally the confirmation of absence. Considerable ongoing commitment from stakeholders will be necessary to achieve eradication; which will be a global first for invasive Urodela.

## Linking In-Situ and Ex-Situ Populations of Threatened Amphibians using the National Amphibian Genome Bank

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Although captive assurance colonies have been established for many North American threatened amphibian species, most of these programs are unsustainable and currently unable to maintain >90% of the populations' gene diversity required to avoid inbreeding depression. As part of the overall toolbox for addressing amphibian collections viability, we linked in-situ and ex-situ amphibian populations for long-term sustainability and genetic management through the use of assisted reproductive technologies (ART) and genome resource banks (GRB). For this study, we collected and cryopreserved sperm in the field from two federally-listed species, *Lithobates chirocahuensis* (n=62) and *Peltophryne lemur* (n=10), and conducted in-vitro fertilization (IVF) on eggs from captive females at zoological institutions. Average pre-freeze sperm motility ranged from 63-65% in both species. Sperm were cryopreserved in the field and banked at the National Amphibian Genome Bank at Mississippi State University. IVFs using frozen sperm were conducted at Fort Worth Zoo and Omaha's Henry Doorly Zoo using captive females which were first examined with ultrasound to determine the stage of oocyte maturity, and then treated with exogenous hormones to induce spawning. Post-thaw sperm motility ranged from 28-43% in the both species. Fertilization of 200-300 eggs using frozen-thawed sperm was achieved in both *P. lemur* and *L. chirocahuensis* yielding viable tadpoles. This study demonstrates a first step in both the collection of genetic materials in-situ from wild amphibian populations, and its integration into captive breeding programs for federally-listed endangered species, providing a means to increase captive collections genetic diversity without removing animals from the wild.

**Filling in the phylogenetic gaps: targeted taxon sampling clarifies relationships of Indian agamids and snakes**

**Deepak V<sup>1</sup>**

<sup>1</sup>*Natural History Museum*

Robust phylogenies are important for testing evolutionary and biogeographic hypotheses. Incomplete taxon sampling in phylogenies can be a problem, especially when evolutionarily distinct lineages are under-sampled. The Indian subcontinent and particularly the biodiversity hotspots of the Western Ghats and Northeast India harbour evolutionarily distinct lineages of herpetofauna. Collaborative research has aimed to fill in some of the gaps in the phylogenies of agamids and snakes of these regions. Most of these studies included multilocus sanger sequence dataset which complimented existing phylogenies. Surprising results include a new subfamily of snakes (Xylophiinae) from Western Ghats, a new genus (*Smithophis*) and five new species of snakes from India. Long unclear phylogenetic relationships in agamids (subfamily: Draconinae) have been resolved with the increased taxon sampling, and four new genus (*Microauris*, *Monilesaurus*, *Sarada*, *Cristidorsa*) and ten new species of agamids have been identified and described. These studies have lasting impact because they highlight the biodiversity of this region and clarify biotic origins and systematic relationships of snakes and agamids.

## Ecomorphological diversity in natricine snakes

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Natricine snakes (Colubridae: Natricinae) originated in Asia and dispersed into North America, Africa and Australasia. They are diverse, with surface, burrowing, and (semi)aquatic ecotypes that have evolved multiple times independently. Given this scenario we asked: Is external morphological variation explained most by habit, diet, phylogeny or geographic distribution? Morphological data were generated for 22 linear measurements (15 head and 7 body measurements) from 1,148 specimens covering 73% (177 species) of all known natricines and 28 specimens of 13 non-natricine snake species. Dietary and natural history data were collated from the literature. Measurements were size corrected and subjected to multivariate analysis. The smallest body sizes occur in burrowing species and the largest in aquatic ecotypes. Burrowing taxa have the most distinctive morphospace occupation among the three main ecotypes. Burrowing ecotypes from Asia and from North America have almost no overlap in morphospace. There is partial overlap in morphospace among aquatic species from Asia, North America and Africa. Among aquatic taxa there are two independent lineages of Asian aquatic burrowers that have unique morphospace occupation. Surface dwellers broadly group into semiaquatic and terrestrial ecotypes but with substantial morphospace overlap, and with little geographic substructuring. Other than for some molluscivorous lineages, ecotype explains morphospace occupation to a greater degree than does dietary specialism.

## Are local molecular clocks misleading? – The case of Mediterranean amphibians

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Since the paradigm of the molecular clock was introduced more than 50 years ago, numerous improvements have been made to its application, which have led to seemingly accurate estimates of split times. However, divergence time estimation is influenced by a variety of parameters, e.g. the genome used, sequence length, the number of genes, taxon sampling, data partitioning, the clock framework, the choice of time priors or even the software used. The choice of calibration points is considered the most crucial step in molecular clock analysis. In practice, most time trees focus on a reduced number of taxa, sometimes restricted to a focal geographic region. From a taxonomic point of view, they can be considered as 'local clocks'. Here, we compare the previously published results of 94 molecular clock studies on Mediterranean amphibians (covering 112 local and 12 global calibrations) with a recently published taxonomically 'global' time tree that combined sequence data on 5 mitochondrial and 4 nuclear genes from 201 intraspecific lineages of all species. A comparison of age estimation of homologous nodes revealed that 77% of all local clocks overestimated divergence times. We discuss how mitochondrial versus nuclear genomes, geological versus fossil calibration, as well as choice of calibration event bias time tree estimation. Inconsistencies in the use of geological priors for time tree calibrations appear to be a major cause of divergent split time estimation.



### Germany, the hotspot of *Bsal* emergence

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Since its discovery 10 years ago, numerous new records of *Batrachochytrium salamandrivorans* (*Bsal*) suggest that this pathogen is extending its range in Europe rapidly. Germany is the 'hotspot' of *Bsal* emergence. Of the next to 50 European sites known by early 2019, almost 40 are located in Germany affecting five urodelan species: *Salamandra salamandra* >20 populations, *Ichthyosaura alpestris* >10 populations, *Lissotriton vulgaris* and *Lissotriton helveticus* 5 and 4 populations, respectively, and *Triturus cristatus* 2 populations (double-counts are possible). Especially, *S. salamandra* suffers from *Bsal* infection, as verified by dramatic population declines and mass mortality events. In one such case co-infection with *Batrachochytrium dendrobatidis* was detected. German records are known from two regions: The Eifel Mountains in the West close to Dutch and Belgian outbreaks; in this region, *Bsal* presence could be dated back to 2004; the Ruhr, where *Bsal* was first detected in 2017 and is currently spreading at alarming rates. At several sites in the Eifel Mountains, historically known to harbor *S. salamandra* and newts, nowadays only newts can be found. Some of these are *Bsal*-positive, suggesting silent *Bsal*-induced salamander declines in the past. At other *Bsal* sites we still find fire salamanders at low densities. Standardized larval monitoring in the southern Eifel Mountains revealed recent breakdowns of fire salamander populations, thus identifying part of the *Bsal* expansion front. Our data give new insight into *Bsal* invasion dynamics. This demonstrate that monitoring is an essential key to understand the threat posed by this pathogen to Europe's salamanders.

## **Integrative taxonomy revealed 23 new taxa of amphibians and reptiles in Eastern Panama**

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Eastern Panama contains the least explored regions in the country with a few roads permitting partial access to lowlands only. From 2011-2014, we conducted field trips to compensate for the lack of information on the diversity of amphibians and reptiles from eastern Panama. Sampling was carried on the principal mountain ranges, including Darién, Jingurudó, Maje, Pirre, Sapó, and San Blas mountains. We barcoded approximately 70% of the amphibians and 50% of the reptiles yet reported from research area. Morphological consistencies and variations were analyzed together with specimens obtained from museum collections. A total of 202 bioacoustic files, comprising 18:23:24 hours of recording, were analyzed. Based on classical taxonomy only (external morphological characters), we identified 65 species of amphibians and 72 species of reptiles. However, by applying an integrative multidisciplinary approach we increased the number of distinct, separate taxa to 79 amphibian and 88 reptile species. Additionally, we report on 23 species that we could not definitely assign to any described species, further increasing the diversity of amphibians by 19.4% and reptiles by 4.8% in eastern Panama. Thus, we initiated several subprojects to resolve taxonomic uncertainties within the genera *Bolitoglossa*, *Diasporus*, *Dactyloa*, *Ecnomiohyla*, *Lepidoblepharis*, and the *Pristimantis caryophyllaceus* and *Anolis tropidogaster* complexes. This is the first regional evaluation of the biodiversity in Panama applying integrative taxonomy.

### **Morphological convergence in bouldering frogs**

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Shared selective pressures can drive evolutionary optimization towards the same solution multiple times, leading to convergence. Therefore, it is common to observe convergent morphotypes in species from different lineages that share a similar ecotype. Australia is home to 18 species of microhylid frogs of the genus *Cophixalus*, restricted to the tropical north-east. While most of these frogs are minute species that live in the leaf-litter of rainforest habitat on cool and wet mountaintops, five recently described species live in a starkly different habitat - giant boulder-fields. The boulder species are all much bigger than the rainforest species, averaging three times their body length and 15 times their mass. Interestingly, phylogenetic data shows that boulder species are far from each other's closest relatives, showing a clear example of parallel evolution in a highly distinct habitat. Here we used a 3D geometric morphometric approach on skull and limb bones of boulder-fields and rainforest species. We tested whether they differ in shape as well as size, their unique morphological adaptations, and the degree of morphological convergence in each bone and overall body shape. We found that morphological convergence was striking between these three independent boulder-field clades. We discuss their morphological convergence in the context of selective pressures imposed by the functional and physiological requirements of boulder fields. We hypothesize that their much bigger size might help reduce water loss through the skin, and work as an anti-predatory mechanism. Similarly, their unique morphology would enable them to successfully navigate through the complicated boulder piles.

### Biomechanics and morphological patterns in head-first burrowing frogs

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Southwest Australia is a biodiversity and endemism hotspot for herpetofauna. Three unusual Australo-Papuan myobatrachid species occur in sandy habitats across semi-arid and arid regions in the Southwest corner: the turtle frog (*Myobatrachus gouldii*), and the northern and southern sandhill frogs (*Arenophryne rotunda* and *A. xiphorhyncha*). These three species are extremely fossorial, and differ from most other burrowing frogs by burrowing head-first in a forward direction, a behaviour which appears to have resulted in extreme morphological adaptations. Interestingly, the degree of morphological adaptations and fossoriality varies among them, but they are all starkly different from their non-burrowing sister species *Metacrinia nichollsi*. Using diffusible iodine-based contrast-enhanced Computed Tomography (diceCT) we were able to extract data of both bones and soft tissue of these four species to assess morphological adaptation to burrowing across a fossoriality gradient. We used Three-Dimensional (3D) geometric morphometric analyses to identify morphological integration patterns among fore-limb bones. We also assessed 3D muscle architecture associated with forward burrowing behavior. Finally, using Finite Element Analyses we assessed the biomechanical properties of the humerus and radioulna. We discuss their morphological evolution, biomechanics, and soft tissue differences in the context of different soil properties and paleoclimatic events that might have contributed to the morphological adaptations for a fossorial lifestyle in this clade of bizarre frogs. We also show that a combination of different x-ray microCT scanning techniques can provide a greater understanding of morphological evolution, and we hypothesize that our results could help to infer behavioural and ecological habits of extinct taxa.

## Wind speed alters the thermoregulatory behaviour of skinks even when substrate temperature remains constant

**Virens J**<sup>1</sup>

<sup>1</sup>*University of Otago*

Wind speed variation has the potential to dramatically alter thermal environments and rates of evaporative water loss, yet little is known about the effects of wind on the thermoregulation of ectotherms. Mean wind speed, maximum gust strength and wind direction are all predicted to alter dramatically over the next century as the climate changes. Therefore, understanding how wind affects the thermal ecology of ectotherms will be important in predicting how species will be affected by climate change. In this study we examined how different windspeeds affected the basking behaviour of a small heliothermic New Zealand skink (McCann's skink, *Oligosoma maccanni*). We designed an experiment in which skinks could choose to either bask in a wind tunnel or move to a retreat site that was sheltered from the wind (the equivalent of sheltering under rocks in the wild). Skinks had access to the same temperatures within the wind tunnel for all three treatments. Using bio-loggers, we measured skin surface temperature of the skinks every 2 s over a 90-min period using three windspeeds (0, 2 and 6 m s<sup>-1</sup>). We then calculated the deviation of the skink's body temperature from its set-point range for preferred body temperature. Our results show that wind can dramatically affect how skinks thermoregulate under controlled conditions, despite equal access to temperatures within their set-point range. Thus, changes in wind conditions alone due to climate change may alter the thermoregulatory behaviour of some lizards, which will have implications for their wider ecology.

**A non-invasive in situ underwater DNA sampling method applied to the olm (*Proteus anguinus*)**

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We developed a non-invasive, in situ underwater method of DNA sampling and preservation that can be applied during diving in less than a minute of animal handling. We applied the method on a Herzegovinian population of the olm (*Proteus anguinus*, Proteidae, Caudata), an endangered aquatic cave-dwelling vertebrate, which makes it an excellent model to test the method under the harshest conditions. We sampled 22 adult individuals during five dives. We extracted sufficient quantity and quality of DNA from all individuals more than a year after sampling. We amplified 10 species-specific microsatellite loci, previously tested in four Croatian populations covering a large part of the species' distribution and revealing high genetic variability. PCR success varied between 6 – 10 loci with a median of 7. Fragment length analysis was successful on 9 loci. We found that a single allele was present in every locus in our population. We propose that our method can be widely used to sample endangered populations, or in projects where the disturbance of individuals must be kept minimal for conservation and scientific purposes.

## Rediscovering the Axolotl as a Model for Paedomorphosis

Voss R<sup>1</sup>

<sup>1</sup>*University of Kentucky*

The Mexican axolotl (*Ambystoma mexicanum*) is an important model organism in biomedical research. Much current attention is focused on the axolotl's amazing ability to regenerate tissues and whole organs after injury. However, not forgotten is the axolotl's equally amazing ability to thwart aspects of tissue maturation and retain juvenile morphology into the adult phase of life. Unlike close tiger salamander relatives that undergo a thyroid hormone regulated metamorphosis, the axolotl does not typically undergo a metamorphosis. Instead, the axolotl exhibits a paedomorphic mode of development that enables a completely aquatic life cycle. The evolution of paedomorphosis allowed axolotls to exploit relatively permanent habitats in Mexico, and preadapted axolotls for domestication and laboratory study. In my talk, I will review our work concerning the genetic basis of paedomorphosis and then argue the need to move beyond endocrinology-guided approaches to understand the axolotl's hypothyroid state. With the recent completion of the axolotl genome assembly and established methods to manipulate gene functions, the axolotl is poised to provide new insights about paedomorphosis and the role of thyroid hormone in development and evolution.

### How does it end? Shifts in chytridiomycosis disease dynamics over time

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Infectious diseases rarely end in extinction. Yet the mechanisms that explain how epidemics subside are difficult to pinpoint. We are characterizing host-pathogen interactions long after the emergence of a lethal fungal pathogen (*Batrachochytrium dendrobatidis*) in a tropical amphibian assemblage. In Panama, a subset of amphibian host species are recovering, but the pathogen is still present and is as pathogenic today as it was almost a decade ago. In addition, some amphibian species appear to be less susceptible than they were before disease emergence. Some evidence suggests that some hosts have defenses that are more effective now than they were prior to initial outbreaks. These results suggest that host recoveries cannot be attributed to pathogen attenuation and may be due to shifts in host responses and susceptibility. These findings provide insights into the mechanisms underlying disease transitions, which are increasingly important to understand in an era of emerging infectious diseases and unprecedented global pandemics.



## Prior pathogen exposure and a preliminary vaccine increase amphibian resistance to chytridiomycosis

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The amphibian disease chytridiomycosis, caused by *Batrachochytrium dendrobatidis* (*Bd*), presents a complicated challenge for captive breeding and headstarting programs that aim to introduce amphibians to sites where *Bd* is present. One possible solution for increasing the success of these efforts is to immunize animals before release. Immunizations against chytridiomycosis have been tested with limited enduring success in a few species, but the utility of vaccines to broadly mitigate chytridiomycosis in the wild is not known. For our experiments we used two species of ranids, the northern leopard frog (*Rana pipiens*) and the relict leopard frog (*Rana onca*), both of which have experienced drastic declines in the southwestern United States. For both species, the potential of successful reintroductions are likely limited by the presence of *Bd*. Therefore, we aimed to increase resistance of these species to chytridiomycosis using two approaches: (1) exposing frogs to a live *Bd* isolate that has previously demonstrated hypovirulence towards one of our study species; and (2) exposing frogs to a virulent *Bd* isolate followed by clearance with itraconazole. We found that both approaches led to drastically reduced *Bd* infections in previously exposed frogs as compared to naïve controls. Importantly, both approaches led to significantly greater survivorship. At this time our approach has not yet been field tested, but a proof-of-concept field experiment is currently being conducted to evaluate the potential importance of this conservation tool.

## **Strategy and tool development for invasive plague skinks (*Lampropholis delicata*) on Aotea, Great Barrier Island**

**Wairepo J<sup>1</sup>**, MacKay J<sup>1</sup>

<sup>1</sup>*Wildland Consultants Ltd*

Since their discovery at Tryphena Wharf in 2013, plague skinks on Aotea Great Barrier Island have been managed by Auckland Council in an attempt to understand their population range, contain them, and, most recently determine the feasibility of eradication.

Together with the Department of Conservation, Auckland Council have committed to undertake a range of experimental trials to develop and evaluate novel management tools and strategies. Between September 2017 and May 2019, trials were run over two seasons, and included the construction of a skink containment fence, the use of avian bio-control, lure-based trapping trials, heat treatments, spatial-survey modelling of eradication requirements, and the development of plague skink detector dogs. We describe the outcome of these trials to-date, key learnings, and next steps.

### **Walking the line: balancing the needs of wildlife and development**

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New Zealand's lizard fauna comprises more than 104 extant described or proposed species currently recognised, of which approximately 84% are considered to be At Risk or Threatened. New Zealand is in a period of significant growth, with population growth rates increasing on average by 0.91% each year for the last decade. Whilst essential in supporting the growing needs of New Zealanders, the resulting increase in infrastructure development (e.g. for housing, roading, mining and agriculture) has led to significant habitat loss and subsequent declines of lizard populations in both urban and rural environments. Despite their protection under New Zealand legislation (particularly the Resource Management Act 1991 and the Wildlife Act 1953), managing lizard populations in the face of this 'development-boom' is challenging and relies upon a suite of mostly-untested mitigation tools. Challenges include difficulties in obtaining high-quality data, a shortage of suitably-qualified lizard specialists, management of introduced mammalian predators, and unsympathetic clients. Additionally, there is often a lack of cohesion between territorial authorities (councils) and other government departments, and a failure to monitor compliance of permit conditions or enforce penalties for non-compliance. Opportunities to achieve better conservation outcomes for lizards include: (i) identifying a national management model that is adaptable to regions and species-specific requirements; (ii) improving communication pathways between government, councils and developers; and (iii) the use of development funding to fill knowledge gaps.

## Ancestral chytrid pathogen remains hypervirulent following its long co-evolution with amphibian hosts

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Many amphibian species around the world, except in Asia, suffer morbidity and mortality when infected by the emerging infectious pathogen, *Batrachochytrium dendrobatidis* (*Bd*). A lineage of the amphibian chytrid fungus isolated from South Korean amphibians (*BdAsia-1*) is evolutionarily basal to recombinant global pandemic lineages (*BdGPL*) associated with worldwide amphibian population declines. In Asia, the *Bd* pathogen and its amphibian hosts have co-evolved over 100 years or more. Thus, resilience of Asian amphibian populations to infection might result from attenuated virulence of endemic *Bd* lineages, evolved immunity to the pathogen, or both. We compared susceptibilities of an Australasian amphibian, *Litoria caerulea*, known to lack resistance to *BdGPL*, with those of three Korean species, *Bufo gargarizans*, *Bombina orientalis*, and *Hyla japonica*, after inoculation with *BdAsia-1*, *BdGPL*, or a blank solution. Subjects became infected in all experimental treatments, but Korean species rapidly cleared themselves of infection, regardless of *Bd* lineage. They survived with no apparent secondary effects. By contrast, *L. caerulea*, after infection by either *BdAsia-1* or *BdGPL*, suffered deteriorating body condition and carried progressively higher *Bd* loads over time. Subsequently, most subjects died. Comparing their effects on *L. caerulea*, *BdAsia-1* induced more rapid disease progression than *BdGPL*. The results suggest that genomic recombination with other lineages was not necessary for the ancestral *Bd* lineage to evolve hypervirulence over its long period of co-evolution with amphibian hosts. The pathogen's virulence may have driven strong selection for adaptive immune responses in endemic Asian amphibian host species.

**Disease and disturbance: investigating the factors that drive amphibian reservoir host occurrence and distribution**

**Wallace D<sup>1</sup>**

<sup>1</sup>*Deakin University*

Pathogens such as amphibian chytrid fungus pose a substantial threat to biodiversity. Reservoir host species, which carry a pathogen but do not become ill, can increase the risk to susceptible sympatric species by maintaining and facilitating the spread of pathogens within a system. Determining habitat use by reservoir host species - in particular where this overlaps with susceptible species - will improve prediction and mitigation of disease risks. We surveyed 98 sites across the range of the critically endangered Baw Baw frog, *Philoria frosti*. We then used occupancy modelling to identify factors influencing the occurrence of two common frog species; *Crinia signifera*, a known chytrid reservoir host, and *Litoria ewingii*, of unknown susceptibility.

*Litoria ewingii* occurrence increased with disturbance intensity, while *C. signifera* occupancy was not significantly associated with the disturbance index. Occurrence of both *C. signifera* and *L. ewingii* was negatively influenced by principal component one, which included negative interactions with elevation and habitat connectivity variables and a positive interaction with canopy cover. Based on the distribution of *C. signifera* and *L. ewingii*, forested areas with high canopy cover likely represent refugia for *P. frosti* from reservoir hosts, and are potential reintroduction sites. This study therefore highlights the value of understanding reservoir host occurrence and distribution.

### Longwall mining and amphibian populations – a case study

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Underground longwall mining may degrade stream habitat by the formation of subsidence, which can cause surface water to be diverted underground through fractures in the bedrock. Longwall mining therefore represents a potential threat to stream-breeding amphibians such as Littlejohn's tree frog, *Litoria littlejohni* – an amphibian species with an extremely patchy distribution. To examine the impacts of longwall mining, we have conducted auditory and mark-recapture surveys for *L. littlejohni* frogs and dip-netted for tadpoles. Streams were classified into four experimental treatments: under-mined sites within an active mine lease area, non-mined sites within the mine lease area, sites exposed to downstream mining impacts within the mine lease, and control sites where no mining has occurred. In the first of its three-year duration, our study revealed that abundance of both *L. littlejohni* tadpoles and frogs was higher within the control stream sites, whereas *L. littlejohni* tadpoles occupied a higher proportion of ponds within the mine lease area. This could be attributed to reduced breeding habitat availability within the mine sites due to subsidence-driven dewatering, as the average number of ponds was approximately half that of those within the control streams. Frog species richness was also higher within control streams, compared to under-mined streams. Overall, these preliminary results indicate that longwall mining may reduce breeding habitat availability not only for *L. littlejohni*, but for other stream-breeding amphibians. Ultimately, understanding the impacts of longwall mining will help inform sustainable mining practices and allow for more effective mitigation methods to be implemented into the future.

## Maternal effect as an adaptation to drought in a desert-dwelling oviparous lizard

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Reptiles are the most diverse vertebrates in arid habitat. Several studies on arid species have addressed the adaptive strategies of adults to water insufficiency. For oviparous species, the embryo is the most fragile stage. How the eggs survived the drought and if maternal effects play an adaptive role is an intriguing question in evolutionary ecology; however, has seldom been investigated.

Here, we conducted reciprocal transplant experiment with a factorial design (high and low maternal water conditions × high and low moisture for egg incubation) in two (mesic and arid) habitats of an oviparous lizard (*Eremias argus*) to test the competing hypotheses of maternal effects: environmental matching, drought compensation, drought pathology, and no-compensation hypotheses.

We found that the maternal effects varied between habitats. The compensation hypothesis is supported in the arid habitat; the female increased clutch size and made their egg develop faster without compromising hatching success and offspring survival under low water condition. While the no-compensation hypothesis is supported in the mesic habitat, the female decreased egg mass without improving their egg hatching success and offspring survival under low water condition. Besides, the female from mesic habitat suffered from higher parasite load and death rate due to lower immune function under low water condition.

Our study highlights the evolutionary significance of maternal effects on drought adaptation in oviparous reptiles. Furthermore, maternal effects of lizards from mesic habitat would isolate embryo from the impact of drought incident temporally, with a price of mothers' immune function.

**Implications of continuous amphibian diversity monitoring in Daweishan of tropical SE Yunnan, China**

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Tropical montane ecological systems with complex topography are recognized as a major reason for biodiversity hotspots in the Indo-China Peninsular. Long term spatial and temporal data in amphibian species composition within a region is essential to implement effective conservation strategies and design regional protected areas. The present study used 5 years of monitoring data to compare the impacts of human disturbances on amphibian diversity. The results showed that although there was a large difference in the species assemblage of amphibians between lower and higher elevations of Daweishan Mountains, there were no significant differences in the number of species and individuals which were recorded at mature or larval stages. The atmosphere and water temperature were the major factors that regulate the diversity distribution of amphibian taxa in the Daweishan region of SE Yunnan, China. Disturbances from tropical agriculture expansion in the lower elevations and tourism activities in the higher elevations showed similar effects on amphibian diversity distribution. Specific to amphibian diversity conservation in Daweishan, the present study demonstrates that the importance of protecting the existing tropical forest from fragmentation in the lowlands and preventing further human disturbances in the continuous evergreen broad-leaf forests in the highland are equally important.



**Degrees of change: within and between population variation in phenological reaction norms in a lizard**

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As the earth warms, populations of individuals will be faced with novel climatic environments to which they may not be adapted. In the short-term, populations can be buffered against the negative effects of altered environmental conditions via phenotypic plasticity and, in the longer term, via adaptive evolution. However, the nature and direction of these population responses will be dependent on the degree to which responses vary among individuals (within-population variation in plasticity), which is likely to vary between populations. We have estimate of patterns of among-individual variation in plastic responses across multiple populations in only a few systems. This lack of data limits our ability to accurately predict the consequences of climatic change for population and species persistence. We use a 16-year data set from climatically distinct populations of the viviparous skink, *Niveoscincus ocellatus*, to address this. Specifically, we tracked over 2600 litters, including repeated observations of over 600 females, to examine inter- and intra- population variability in the response of parturition date to environmental temperature under a random regression framework. Populations shared a common population-mean reaction norm but differed substantially in the degree to which reaction norms varied among individuals. These results show that even where populations share a common mean-level response we cannot assume that that they will be affected similarly by altered environmental conditions. If we are to accurately assess how changing climates will impact species and populations, we require estimates of how plastic responses vary both among and within populations.

## Apex reptilian predators in Northern Australia: can landscape CTA training curb cane toad impacts

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In tropical Australia, we have underestimated the critical ecological roles of large reptilian predators. In floodplain environments, a single Yellow-spotted monitor, *Varanus panoptes*, (SVL 0.6m) can consume up to three kilograms of prey and grow nearly a kilogram each month. Given that these animals can be found in high densities, it's not surprising that their rapid population declines (>90%) have been implicated in meso-predator release and trophic cascades; massively amplifying the destructive ecological impact of cane toads (*Rhinella marina*) across northern Australia. It's clear that large reptiles must be protected, to preserve the ecological integrity of the systems being invaded.

We can reduce cane toad impacts by training native predators to avoid toads before they invade an area, using 'Conditioned Taste Aversion' ('CTA'). Akin to eliciting a food poisoning response, if animals have a non-lethal dose of toad toxin, they can quickly learn to avoid the large and fatally toxic toads at the frontline. In some predators, CTA increases population survival by up to 50%.

We have translated single-species field trials into a landscape-level conservation strategy, targeting multiple reptilian predators at once. We apply various CTA cues (metamorph 'teacher' toads and toad-meat sausages with nausea inducing compound) in areas of high biodiversity ahead of the cane toad invasion.

We created 'The Cane toad Coalition'; a powerful consortium of research, government, conservation, and indigenous organisations collaborating to deliver the largest cane toad mitigation strategy to date. I discuss the early results of the field research and it's wider implications.

## Effects of population density and timing of hatching on phenotypic selection in an invasive lizard

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<sup>1</sup>Auburn University, <sup>2</sup>University of Minnesota

Intra-specific competition is strongly influenced by population density and can have profound effects on individuals and populations. For example, variation in population density can influence phenotypic variation via numerous mechanisms (e.g. natural selection, phenotypic plasticity). Prior research in our lab and at our field site indicates that adult lizard density strongly influences hatchling survival and modifies behaviors. In addition, the timing of hatching strongly influences offspring survival, possibly due to seasonal shifts in competitive interactions. To address these issues, we conducted a large-scale field experiment where we spatially manipulated adult population densities and leveraged naturally occurring temporal changes in hatchling density to evaluate the effects of conspecific densities on early life phenotypes and survival in brown anoles (*Anolis sagrei*). We released marked hatchling lizards onto small islands where we experimentally manipulated the population densities such that there were either high or low densities of adult anoles. We released these hatchlings early in the season when hatchling densities are naturally low, and later in the season when hatchling densities are naturally high. We sampled the populations at the end of the breeding season (prior to winter) and again the following spring, and repeated the study in the subsequent year. Preliminary results suggest that early-hatched individuals had greater survival than those that hatched late in the season, but adult density had no effect on hatchling survival. These data also allow us to investigate the importance of the timing of hatching, and patterns of growth and selection under differing population densities.

**Progress report on herpetological sampling and oVert (Open Exploration of Vertebrate Diversity in 3D)**

**Watkins-Colwell G<sup>1</sup>**, Austin C<sup>2</sup>, Cannatella D<sup>3</sup>, Hanken J<sup>4</sup>, Spencer C<sup>5</sup>, Welton L<sup>6</sup>, Stanley E<sup>7</sup>, Blackburn D<sup>7</sup>  
<sup>1</sup>*Peabody Museum of Natural History, Yale University*, <sup>2</sup>*Museum of Natural Science, Louisiana State University*, <sup>3</sup>*University of Texas at Austin*, <sup>4</sup>*Museum of Comparative Zoology, Harvard University*, <sup>5</sup>*Museum of Vertebrate Zoology, University of California - Berkeley*, <sup>6</sup>*Biodiversity Institute, University of Kansas*, <sup>7</sup>*Florida Museum of Natural History, University of Florida*

In 2017 NSF funded “oVert (openVertebrate): Open Exploration of Vertebrate Diversity in 3D,” which is the first Thematic Collections Network devoted entirely to vertebrate morphological specimens. The primary goal of oVert is to generate and serve high-resolution digital three-dimensional data for internal anatomy across vertebrate diversity. oVert will CT-scan >20,000 fluid-preserved specimens representing >80% of the living genera of vertebrates, providing broad coverage for exploration and research on all major groups of vertebrates. Contrast-enhanced scans will be generated to reveal soft tissues and organs for most of the living vertebrate families, including amphibians and reptiles. This collection of digital imagery and three-dimensional volumes will be open for exploration, download, and use for research and education. These new media will provide unprecedented global access to valuable morphological data of specimens in collections held in the United States. A progress report relevant to herpetological specimens will be provided.

## The impact of non-native trout on endangered and endemic amphibians of the Peruvian Andes

**Watson A<sup>1,2,3</sup>**, Castillo Roque L<sup>1,2</sup>, Damián Baldeón O<sup>2</sup>, Elías R<sup>1</sup>, Wissel B<sup>4</sup>

<sup>1</sup>Denver Zoological Foundation, <sup>2</sup>Grupo RANA Association, <sup>3</sup>University of Canterbury, <sup>4</sup>University of Regina

The Junín giant frog (*Telmatobius macrostomus*) and Junín riparian frog (*Telmatobius brachydactylus*) are endangered and endemic amphibians to the central Andes of Peru. We examined food web dynamics within the Junín National Reserve, the area with the greatest historical records of these frogs, to characterize energy flow and trophic interactions. Specifically, we sampled sites for stable carbon isotopes ( $\delta^{13}\text{C}$ ) to assess energy sources and stable nitrogen isotopes ( $\delta^{15}\text{N}$ ) to measure trophic position of these frogs, and other native and non-native fishes (native pupfish and catfish of the genera *Orestias* and *Trichomycterus*, respectively, and invasive trout *Onchorhynchus mykiss*). It is suspected that trout are outcompeting these frogs for what is considered their main food source (*Orestias* spp.). We used two isotope ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ), two source Bayesian mixing models to determine trophic positions and estimate the contribution of different prey items for amphibian and fish diets. This investigation reveals the impact of trout on native amphibians and fishes at the ecosystem level. Current conservation efforts focus on restoring species habitats; however, if a habitat is restored while predatory and invasive species persist the desired conservation outcomes may not be fulfilled. Understanding the impact of invasive species on native populations is key for conservation efforts to be successful. Improvement of current conservation and management efforts could be adapted toward allocating additional resources to the eradication of invasive species within protected areas. Furthermore, future efforts of captive breeding require this information for successful reintroduction of these species throughout their native range.

## Antibiotic susceptibility profiles of *Salmonella* species isolated from captive amphibians and reptiles in Uganda (via video)

**Watuwa J<sup>1</sup>**

<sup>1</sup>*Elgon Wildlife Conservation Organization & Makerere University Kampala*

Domestication of amphibians and reptiles for tourism purposes and as pets is increasing worldwide. This comes with increased human-amphibian-reptile interactions during capture, transport and feeding in captivity. Studies have reported amphibians among the important sources of zoonotic bacteria including *Salmonella*. Amidst this threat, there are increasing reports of drug resistant *Salmonella* species that could jeopardize treatment in both humans and animals.

Antimicrobial resistance is an emerging public problem that threatens treatment.

This study will establish the prevalence of *Salmonella* in captive amphibians and reptiles to establish the antibiotic susceptibility profiles of the common antibiotics used in treatment of salmonellosis to give a more tangible estimate of the magnitude of the problem to amphibians, reptiles and humans as a zoonotic disease. Also it's hoped this study will provide evidence based guidelines on handling amphibians and reptiles in captivity and spell out public health risk.

Methodology

A cross-sectional study will be carried out in Uganda. Selected amphibians and reptiles will be restrained and sterile cotton swab will be gently inserted and rotated inside the cloaca or swabbed. The cotton swab will be immersed into sample collection tube containing transport media, stored in a cool box and transported to the laboratory for microbial examination.

Significance

This project will directly achieve amphibian and reptile conservation by reducing the risk of cross species disease spread between reptiles, amphibians and people. By implementing an amphibian and reptile health monitoring program we will be able to inform timely interventions that could prevent potentially fatal disease outbreaks.

## **Between a rock and a hard place: conservation of the endangered broad-headed snake**

**Webb J<sup>1</sup>**

<sup>1</sup>*School of Life Sciences, University of Technology Sydney*

The broad-headed snake *Hoplocephalus bungaroides* was once abundant on sandstone rock outcrops in the Sydney region. This species requires sun-exposed rocks for diurnal thermoregulation. Habitat loss, coupled with the removal of 'bush-rocks' for landscaping urban gardens, has contributed to the species decline. In 1999, the government listed bush-rock removal as a key threatening process, yet landscape suppliers can legally sell bush-rock, and this industry threatens extant broad-headed snake populations. In addition, reptile collectors harvest wild broad-headed snakes for the underground pet trade. To mitigate these threats, in 2008 the NSW National Parks and Wildlife Service installed locked gates on a fire trail to protect a significant population of broad-headed snakes. Here I address two questions: (1) has the installation of locked gates deterred reptile collectors and reduced habitat disturbance? And (2) Do snake poachers threaten the viability of extant snake populations? To answer these questions, I analysed data from my 27 year mark-recapture study of broad-headed snakes. Using program MARK, I estimated survival rates of snakes at replicate sites on an unprotected and protected plateau. I then ran population-viability models to assess the likelihood of local extinction. These analyses showed that the collection of snakes can rapidly drive the snake population on the unprotected plateau to extinction. To conserve broad-headed snakes, wildlife managers will need to protect additional rock outcrops. Ultimately, if we want to conserve rock-dwelling reptiles, we need to educate the public, and encourage them to lobby councils and state government to outlaw the sale of bush rocks.

## Is vegetation mapping a suitable proxy for predicting occupancy of threatened frogs?

**Webster G<sup>1</sup>**, Clulow S<sup>1</sup>, Bower D<sup>2</sup>

<sup>1</sup>Macquarie University, <sup>2</sup>University of New England

Threatened species with restricted distributions often conflict with human activities, such as development. Often, impact assessments will determine the extent of habitat for threatened species using vegetation communities, assuming that the species occupies those habitats. For threatened amphibians however, vegetation communities might be a poor proxy for predicting occupancy due to a greater reliance on microhabitat requirements such as hydrological regimes and structure, rather than particular vegetation assortments. We tested these assumptions for a newly described threatened frog with a restricted distribution, Mahony's Toadlet (*Uperoleia mahonyi*) from New South Wales, Australia. Presently known from only nine localities along the NSW coast, it is thought to be a habitat specialist; most sites where it has been found contain specific vegetation types, soil and hydrology. But will vegetation alone accurately predict occupancy for the purpose of environmental impact assessments? We produced hypotheses of distribution based upon vegetation communities, bioclimatic data and soil types and determined detection probability at known sites prior to broad-scale surveys. We then allocated randomised survey sites to each of the predicted area of occupancy for each hypotheses, allowing comprehensive occupancy modelling. We will present our findings and discuss the suitability or otherwise of using each approach for assessing human land-use conflicts for threatened amphibian species.



## Genetic pattern of the eastern golden frog (*Pelophylax plancyi*) in the megacity, Shanghai

**Wei X<sup>1</sup>**, Ma S<sup>1</sup>, Yue Q<sup>1</sup>, Mu Z<sup>1</sup>, Huang M<sup>1</sup>, Hua P<sup>2</sup>, Wang Z<sup>1</sup>

<sup>1</sup>*School of Life Sciences, East China Normal University,* <sup>2</sup>*School of Ecological and Environmental Sciences, East China Normal University*

Urbanization is one of the most important factors that results in the global decline of amphibian biodiversity. Understanding how amphibian species are influenced by different stages of urbanization is therefore significantly important to realize the functional mechanism of urbanization to amphibians. A typical urbanized ecosystem like Shanghai City experienced more than a thousand years of slow progressing history followed by rapid and intensive modern urbanization development in recent decades. We chose the eastern golden frog (*Pelophylax plancyi*) for study, which is the most abundant and widely distributed native species in Shanghai. Using Cyt-b, microsatellites and SNPs, we obtained three datasets in total 407 individuals from 15 sampling sites across the urbanization gradient. All populations suffered bottleneck several hundred years ago (511±89 years). Fifteen populations were assigned into 8 genetic clusters, showing a consistent distribution pattern with the urbanization gradient; moderate gene flow existed from populations in peripheral suburban areas to those in urban areas, but was absent between populations in urban areas. The genetic diversity had no significant difference among clusters, and all populations had an average effective population size of 518. This study indicated that urbanization can continuously influence amphibian populations since the early stage of urban development. Population decline started before modern urbanization stage, while the modern urbanization process contributes to current genetic structure. Nevertheless, amphibian populations can persist in isolated habitats in urban areas. Thus the maintenance and improvement of fragmented habitat patches to support local populations is essential to the amphibian conservation in heavily urbanized areas.

**Understanding limits to recruitment can help inform chytrid fungus mitigation strategies.**

**West M<sup>1,2</sup>**, Todd C<sup>3</sup>, Gillespie G<sup>4</sup>, McCarthy M<sup>1</sup>

<sup>1</sup>University Of Melbourne, <sup>2</sup>National Environmental Science Programme, Threatened Species Recovery Hub, <sup>3</sup>Arthur Rylah Institute, <sup>4</sup>Northern Territory Department of Environment and Resources

Understanding species' differing population-level responses to threats can be key to identifying conservation options for declining populations. However, this is difficult when multiple threats are implicated. Chytrid fungus (*Batrachochytrium dendrobatidis*: *Bd*) is implicated in at least 500 amphibian population declines globally, although few tangible options exist to mitigate pathogen impacts. Other threatening processes including non-native predators, can contribute to amphibian declines and may exacerbate *Bd* impacts. We disentangled the impacts of *Bd* and non-native predatory fish upon two stream breeding frog species with differing conservation statuses to reveal vital rates that are crucial for species persistence. *Litoria spenceri* are threatened and historically occurred across a 300-1100m asl elevational gradient in south-eastern Australia. *Litoria lesueurii* are not threatened and sympatric with *L. spenceri* at some sites. Using mark-recapture derived demographic rates known to correlate with climate and elevation, discrete-time deterministic matrix population models were constructed for each species at high, moderate and low elevation sites, and under multiple management scenarios. Our study reveals that age to maturation, clutch size and egg-year 1 survival influenced interspecific and intraspecific population-level responses of the two frogs to *Bd* impacts. Importantly, our results highlight that a population's capacity to counteract *Bd*-mediated adult mortality is clearly constrained by other threats and environmental interactions that limit species recruitment. Strategies to mitigate *Bd* impacts need to consider these interactions and constraints on recruitment.

## Does temperature mediate social conflict in a family-living skink?

Moss J<sup>1</sup>, **While G**<sup>1</sup>

<sup>1</sup>*University Of Tasmania*

Climate change will dramatically alter environmental conditions experienced by natural systems over the coming decades. It is imperative that we understand how these changes will impact evolutionary outcomes. This is particularly true in the context of social evolution, as environmental stressors can dramatically alter how individuals interact, inducing abrupt transitions between cohesive and dissolute states. Despite this, we know almost nothing about how climate change will mediate fundamental social processes. *Egernia*, a group of Australian skinks that live in family groups of varying complexity, represents a key step in the evolution of structured social living. Pivotal to this transition is monogamy. Long-term monogamy favors cooperation by increasing relatedness between family members. In contrast, female multiple mating, polyandry, exacerbates intrafamilial aggression. Rising temperatures could increase mating frequency in ectotherms because extended activity periods promote higher intersexual encounter rates. However, the broader implications for this are unknown. How will temperature affect social interactions at the individual level? Further, what consequences will result for social cohesion at the population level? We will address these questions by exposing experimental populations of family-living *Liopholis whitii* to conditions that mimic a range of climate change scenarios. We will then assess outcomes for genetic monogamy and family conflict. These findings will be combined with a 15-year dataset on free-living *L. whitii* to evaluate the broader links between climate, monogamy and family dynamics. Drawing from these multiple lines of inquiry, we will establish how directional climate change shapes the evolutionary trajectory of social interactions.

## A Neogene carettocheliid turtle from Sarawak, Malaysia

**White A<sup>1</sup>**

<sup>1</sup>BEES, University of New South Wales

Carettocheliid turtles are today only represented by a single species, *Carettochelys insculpta*, found in Australia and Papua New Guinea. Carettocheliids historically had much wider distribution being found in Europe, Asia and North America in the Cretaceous and much of the Cenozoic. They disappeared from the northern continents but persisted in south-east Asia in the Miocene with fossils known from Myanmar, Thailand and Papua New Guinea. A late Pleistocene entry into Australia is regarded as inferred. The discovery of a partial skull of a hitherto undescribed carettocheliid turtle in Sarawak attests to the regional diversity of these turtles in the Neogene. The Sarawak turtle shows a number of unique morphological features that are suggestive of a distinctive and unusual life style for carettocheliids. These will be discussed in this paper.

### **Lifetime fitness, sex-specific life history, and the maintenance of a polyphenism**

**Whiteman H<sup>1</sup>**, Lackey A<sup>2</sup>, Moore M<sup>3</sup>

<sup>1</sup>*Murray State University*, <sup>2</sup>*SUNY-Binghamton*, <sup>3</sup>*Case Western Reserve University*

Polyphenisms such as facultative paedomorphosis can reveal the evolutionary and ecological processes that initiate and maintain diversity within populations. We examined lifetime fitness of two morphs in a polyphenic population of Arizona Tiger Salamanders using a 27-year data set with 1,317 adults and 6,862 captures across 8 salamander generations. Larval salamanders develop into aquatic paedomorphs that retain larval traits and stay in their natal pond or terrestrial metamorphs. To evaluate the adaptive significance of this polyphenism, we compared lifetime reproductive success of each morph as well as how life history strategies and spatiotemporal variation explained fitness. We found sex-specific differences in lifetime fitness between morphs. For males, paedomorphs had more reproductive opportunities than metamorphs when we accounted for the potential advantage of larger males gaining access to multiple mates. For females, in contrast, metamorphs had higher estimated egg production than paedomorphs. Life history strategies differed between morphs largely because the morphs maximize different ends of the trade-off between age at first reproduction and longevity. Spatiotemporal variation affected larval more than adult life history traits with little to no effect on lifetime fitness. Thus, environmental variation likely explains differences in morph production across time and space but contributes little to differences in lifetime fitness between morphs and sexes. Our long-term study and measures of lifetime fitness provide unique insight into the complex selective regimes potentially acting on each morph and sex. Our findings motivate future work to examine how sex-specific selection may contribute to the maintenance of polyphenism.

## Survival and recovery of amphibian populations post *Bd*-emergence can inform strategies for conservation translocations

Whitfield S<sup>1</sup>

<sup>1</sup>*Zoo Miami*

The emergence of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*), in the Neotropics has decimated amphibian biodiversity - causing widespread extirpations and major range contractions of susceptible species. Translocations and reintroductions of amphibians post-*Bd* emergence have generally failed, highlighting a clear need for novel strategies for translocations of amphibians post-*Bd* emergence. In Costa Rica, three decades after emergence of *Bd* caused widespread amphibian disappearances, some highly susceptible species appear to show signs of natural recovery. While rare, these examples of natural recovery illustrate narrow sets of ecological conditions under which co-existence between highly susceptible amphibians and *Bd* are apparently possible. Here, we combine community level pathogen surveillance across seven sites occupied by relict populations of highly susceptible amphibian species with climate data to illustrate a range of survival scenarios for highly *Bd*-susceptible species. Some species may survive in climatic refugia in low-elevation areas with warm climates, though *Bd* prevalence may be either low or high for these species. Other species persist within climatic zones amenable to growth of *Bd* and relatively high community-level infection prevalence, suggesting either resistance or tolerance to infection by *Bd*. We discuss advantages and limitations of using natural recovery and persistence of highly *Bd*-susceptible species as a natural analog for anthropogenic translocations.

## **Influence of incubation environment on lizard learning**

**Whiting M<sup>1</sup>**, Beltrán I<sup>1</sup>

<sup>1</sup>*Macquarie University*

It is well established that the environment an organism experiences during early development has a profound effect on its phenotype. Rick Shine made this connection relatively early in his career and he and his students generated a large body of work on phenotypic plasticity in response to variance in incubation temperature and moisture. More recently, Rick recognised that cognition (brain structure and learning) should also be impacted by incubation temperature and the arrival of Josh Amiel, a new PhD student with an interest in neuroscience, provided the perfect opportunity to test this hypothesis. Josh and Rick showed that lizards incubated at 'hot' temperatures learnt faster than those incubated at 'cold' temperatures. They also showed that fundamental differences in brain structure, including neuron number and density, underpinned these differences. My lab collaborated with Rick and Josh to further study the impacts of incubation temperature on learning and showed that 'hot' lizards were better at learning colour associations in a multi-stage instrumental task. We are currently studying new model systems including *Saiphos equalis*, known for having both oviparous and viviparous populations. In a spatial learning task, viviparous lizards incubated at higher temperatures made more mistakes compared to oviparous lizards from the same incubation treatment. Our results support the hypothesis that viviparous lizards are more vulnerable to global warming. I will talk about the important early work from Rick's lab and more recent work that we are doing to better understand the role of incubation temperature on cognition and the implications for global warming.

## Transcriptomics of viviparity and long egg retention in a bimodally reproductive skink (*Saiphos equalis*)

Foster C<sup>1</sup>, Thompson M<sup>1</sup>, Van Dyke J<sup>2</sup>, Brandley M<sup>1</sup>, Whittington C<sup>1</sup>

<sup>1</sup>The University Of Sydney, <sup>2</sup>La Trobe University

The transition from an ancestral oviparous (egg-laying) mode of reproduction to a derived viviparous (live-bearing) state has occurred over 150 times independently in vertebrates. Transitions between parity modes are physiologically complex, and are likely to be driven by a large suite of developmental and genetic changes. Only a handful of vertebrates are bimodally reproductive. In these species, all squamates, some populations are oviparous, and others viviparous. These species represent recent origins of viviparity, and therefore are ideal for investigating the mechanisms underlying the evolution of viviparity.

We used transcriptomic methods to characterise the gene expression changes in individuals from long egg-retaining oviparous and viviparous populations of the bimodally reproductive three-toed skink, *Saiphos equalis*, across different reproductive stages. Many genes were differentially expressed between non-reproductive and gravid individuals, and both parity modes have clearly different gene expression profiles. During gravidity, viviparous *S. equalis* convergently upregulate many genes associated with the evolution of viviparity in other taxa.

Surprisingly, oviparous *S. equalis* exhibit many of the same temporal changes in gene expression during gravidity, which is different from several other oviparous skinks that do not exhibit strong gravidity-related changes in gene expression. Our results suggest that morphological and physiological changes that are important for the evolution of viviparity might also be important for long egg retention, which is itself an exaptation for viviparity. Collectively, we conclude that while reproductive mode can evolve through changes in gene expression, many physiological changes throughout gravidity are similar in both oviparous long egg retention and viviparity.



## Caecilian genomes

**Wilkinson M<sup>1</sup>**, O'Connell M<sup>2</sup>, McCarthy S<sup>3</sup>, Durbin R<sup>3</sup>

<sup>1</sup>*The Natural History Museum*, <sup>2</sup>*University of Nottingham*, <sup>3</sup>*University of Cambridge*

Caecilians are highly distinctive elongate, snake or worm-like amphibians lacking any trace of limbs. Due to their tropical distribution and cryptic ecology, the mostly soil-dwelling caecilians have long been considered the least well-studied of the extant Amphibia. Although many of the approximately 215 recognised species are poorly known, recently there has been substantial progress in their systematics, including a fairly well-established higher level phylogeny, and some quite unanticipated natural history discoveries. We report here on the genomes of three species representing three families and diverse ecologies and reproductive modes. The Neotropical *Rhinatrema bivittatum* is a member of the Rhinatrematidae (the sister group of all other extant caecilians) and has many ancestral traits including a biphasic life history (with an aquatic larval stage). *Microcaecilia unicolor* (Siphonopidae) is an oviparous direct developer (lacking a larval stage) and a more dedicated burrower, with reduced eyes covered by bone. The African *Geotrupes seraphini* (Demophiidae) is a secondarily zygotrophic species that is viviparous. We summarise our efforts to producing high-quality assemblies for these species and to relate their genomic diversity to the biology of the group and to the ecological, morphological and reproductive diversity within it.

## **Salt tolerance in frogs: a widespread trait conferring adaptive capacity for global change?**

**Williams C<sup>1</sup>**, Hopkins G<sup>2</sup>, Brodie Jr E<sup>3</sup>

<sup>1</sup>*University of South Australia*, <sup>2</sup>*Western Oregon University*, <sup>3</sup>*Utah State University*

Salinity tolerance is reported from more than a hundred frog species, occupying both coastal and inland areas. Given likely increases in salinization as a result of human activity and climate change (both through sea level rise and land clearing activity) there is a need to understand how frogs may be expected to adapt. Furthermore, given the global impact of chytridiomycosis and recent reports of the possible curative effects of salt on infection rates and lethality, understanding frog tolerance to salt and the possible adaptive benefits of life in saline habitats is vital. To that end, we address the question of whether Australian frogs are more salinity tolerant than fauna in other regions, by reason of this country's geological history. We then explore the drivers of salinity tolerance evolution and the ecological benefits and trade-offs of this trait. For instance, beyond the immediate benefit of being able to utilise saline habitat when other waters may be scarce, does salinity tolerance provide benefits in the form of reduced resource competition and fewer predators? How does this trade off with the costs of osmotic stress in these habitats? Furthermore, recent evidence suggests that higher salinities may reduce chytridiomycosis transmission and lethality of infection, but not all frog life stages may be able to survive in water with a salinity high enough to achieve this effect. We thus explore the potential costs, benefits, and drivers of salinity adaptation both in Australia, and more broadly globally, in our efforts to understand this under-appreciated amphibian trait.

### **Better together: collaborative planning in threatened species recovery**

**Wren S**<sup>1,2</sup>, Baker A<sup>3</sup>, Lees C<sup>4</sup>

<sup>1</sup>University Of Otago, <sup>2</sup>IUCN SSC Amphibian Specialist Group, <sup>3</sup>Amphibian Ark, <sup>4</sup>IUCN SSC Conservation Planning Specialist Group

Planning is a necessary part of all threatened species conservation work. Both the plan itself and the way in which the conservation planning stage is carried out can influence the success or failure of a recovery programme. The current aim of the IUCN SSC Conservation Planning Specialist Group is to help ensure that “every species that needs a plan, is covered by an effective plan”. Consequently, CPSG is working closely with IUCN Taxon Specialist Groups and other conservation organisations to increase the coverage of plans. It advocates a step-wise planning process which is science-driven, stakeholder inclusive and supported by neutral facilitation. To date there have been few collaboratively-developed species conservation plans for herptiles, despite the high proportion of threatened species within these taxa and the fact that lack of coordination and collaboration are often cited as obstacles to conservation efforts. This presentation will address the benefits of collaborative conservation planning which explicitly integrates all aspects of a conservation programme and ensures that everyone is working towards a common goal. Steps in the IUCN conservation planning cycle will be outlined, and key points that will improve the effectiveness of the planning process and resultant plan will be discussed. Finally, existing guidelines that can help those embarking on a species conservation planning project will be highlighted. Only with good-quality conservation planning efforts, which engage all relevant stakeholders, will we be able to get ahead of the global extinction crisis, and into a phase of species recovery.

### Looking back and leaping forward: New Zealand native frog translocations and future priorities

Easton <sup>11</sup>, Wren S<sup>1,2</sup>, Germano J<sup>3</sup>, Beauchamp T<sup>4</sup>, Bell B<sup>5</sup>, Bell E<sup>6</sup>, Cisternas J<sup>1</sup>, Dewhurst P<sup>7</sup>, Gibson R<sup>8</sup>,  
Haigh A<sup>9</sup>, Tocher M<sup>10</sup>, Bishop P<sup>1,2</sup>

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New Zealand is home to three endemic Leiopelmatid frog species, all threatened with extinction. To varying degrees, translocations have played a role in the conservation management of all three species. The reasons for release have been: to assess translocation feasibility, to create additional populations for species found in very few locations, to attempt to create a chytrid-free site, and mitigating the impacts of development. Nine translocations have occurred, five of which involved multiple releases or later supplementation. Three translocations have succeeded, one is likely to succeed, one unknown, and four have failed. Much knowledge has been gained through these translocations including frog dispersal/movement, body condition and growth, interactions with other species, and microhabitat requirements. Advances in genetic knowledge and methods for non-invasive sex identification have allowed for more targeted guidelines for future releases. While early releases were often carried out by one or two organisations, more recent releases have extended collaboration, including direct involvement by tangata whenua (Maori tribes at the source/release site), universities, the Department of Conservation and captive or non-profit organisations. These collaborations have led to more respectful cultural relationships as well as improved monitoring, development of translocation protocols/design and disease screening, among other benefits. Climate change will impact current frog populations and tools for appropriate predator control to benefit frogs are still being developed. Many issues are therefore involved in the design and prioritisation of translocations as future tools in the conservation management of New Zealand frogs and in achieving the long-term commitment to make this work.

### Improving early detection and rapid response for cryptic species: a review of current analytical tools

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Successful eradication of invasive species is facilitated by early detection and prompt initiation of control. However, detecting when a colonization event occurs is difficult when species are cryptic or occur at low densities. Also difficult is knowing when to declare success and terminate control activities of a previously extant invasive population. Quantitative tools for population analysis can provide information that is key to smart decision making in both scenarios. In our research efforts to (1) confirm, with a high degree of certainty, the absence of an incipient population of invasive Brown Treesnakes (*Boiga irregularis*) after a confirmed sighting, and (2) declare an eradication successful for Veiled Chameleons (*Chamaeleo calyptratus*), we developed quantitative models capable of providing information on population presence, structure, and dynamics. In the first model we illustrate how to infer the absence of a population when species-specific detection and density estimates are known, or reasonable surrogates exist. In the second model we use removal data to make inference about population size, changes in population size, and movement rates. These models have applicability for many early detection and rapid response (EDRR) challenges presented by invasive herpetofauna.

**Rapid differentiation of sexual signals in Cane toads *Rhinella marina***

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Advertisement calls tend to differ among populations, based on morphological and environmental factors, or simply geographic distance, in many taxa. Invasive cane toads (*Rhinella marina*) were introduced to Australia in 1935 and their distribution has expanded at increasing rates over time. Rapid evolution occurred in morphological and behavioural characters that accelerate dispersal, but the effects of rapid expansion on sexual signals have not been examined. We collected advertisement calls from four populations of different ages since invasion, and analysed the geographic differentiation of seven call parameters. Our comparisons indicate that the toad calls differ among Australian populations. The signal variation was not simply clinal with respect to population age, climate, or morphological differentiation. We suggest that selection on signalling among populations has been idiosyncratic and may reflect local female preferences or adaptation to environmental factors.

**Acoustic communication applied for the control of invasive cane toads *Rhinella marina***

**Yasumiba K<sup>1</sup>**

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Anuran amphibians are highly dependent on acoustic communication to increase their mating success. Both males and females use the variation in male advertisement calls to obtain information about conspecifics, the environment, and potential competitors or mates. A highly invasive amphibian, the cane toad (*Rhinella marina*) was introduced to Australia and continues to have severe negative impacts on native biodiversity. Among several possible methods for the control of cane toads, trapping of adults using an acoustic lure is a practical way to reduce their local population sizes. However, despite its potential for use in controlling this species, the acoustic communication of cane toads has not been well studied, and the use of vocalisations in their mating system is largely unknown. Both sexes of cane toads in Australia respond to conspecific vocalisations with phonotaxis, movement towards the sound source, so we examined the responses of both males and females to natural variation in call characteristics and determined call preferences for both sexes. We also show that there are geographic differences of the calls between populations. Based on these results, we report a more effective trapping method using acoustic lures as a new toad trap.

## The embryos of turtles can influence their own sexual destinies

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Sessile organisms with thermally-sensitive developmental trajectories are at high risk from climate change. For example, oviparous reptiles with temperature-dependent sex determination (TSD) may experience strong (potentially disastrous) shifts in offspring sex ratio if reproducing females are unable to predict incubation conditions at the time of oviposition. How then have TSD reptile taxa persisted over previous periods of extreme climatic conditions? An ability of embryos to move within the egg to select optimal thermal regimes could buffer ambient extremes, but the feasibility of behavioural thermoregulation by embryos has come under strong challenge. To test this idea we measured thermal gradients within eggs in semi-natural nests of a freshwater turtle species with TSD, manipulated embryonic thermoregulatory ability, and modelled the impacts of embryonic thermoregulation on offspring sex ratios. Behavioural thermoregulation by embryos accelerated development and influenced offspring sex ratio, expanding the range of ambient conditions under which nests produce equal numbers of male and female offspring. Model projections suggest that sex-ratio shifts induced by global warming will be buffered by the ability of embryos to influence their sexual destiny via behavioural thermoregulation.



### Hide or escape? Variations in escape behaviour of three North East Asian anurans

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Every species has its own optimal escape strategy to balance predation risks and the energetic cost of fleeing. Here, we tested the importance of microhabitat use in the escape behaviour of three common North East Asian anurans: Mongolian toads (*Pseudepidalea raddei*), Amur brown frogs (*Rana amurensis*), and Japanese treefrogs (*Dryophytes japonicus*). We collected data in 2017 and 2018 in Mongolia, Russia, China and North Korea. We examined flight initiation distance (FID; distance from a potential predator to the point when the individual starts to flee) and distance fled (DF; distance between flight initiation and flight termination points); and collected sex and health index as control factors. *Pseudepidalea raddei* had the highest FID and DF, *R. amurensis* followed and *D. japonicus* had the smallest FID and DF, a pattern matching to that of body size and weight in these species. DF was higher than FID in *P. raddei* and *R. amurensis*, but FID was higher than DF in *D. japonicus*, a potential link to active escape behaviour versus crypticity. For all three species, FID and DF were higher when they fled swimming rather than jumping. For toads, FID and DF were higher when they were on ground rather than water, while for both frogs FID and DF were higher when they were in water compared to ground. These differences are likely explained by difference in threats to the species. Our results indicate that the three sympatric species have different escape strategies, which might also reflect physiological differences.

**Re-assessment of taxonomy and geographic distribution of *Fejervarya cancrivora* complex from Thailand and nearby countries**

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The Crab-eating frog *Fejervarya cancrivora* is widely distributed in Asia and has been recognized as species complex. Previously, two species of the *F. cancrivora* complex, *F. cancrivora* and *F. raja* were reported from Thailand but sometimes *F. moodiei* was also mentioned for their occurrence in Thailand. These species are usually misidentified due to taxonomic complexity within the *F. cancrivora* complex. To clarify their taxonomic status and geographic distribution, the morphological examination included type materials and collected specimens from Thailand and other Asian countries. The phylogenetic relationship was examined based on the mitochondrial 16S DNA gene. The results showed that these two species are placed in different clades. *Fejervarya cancrivora* has high morphological similarity with *F. moodiei* but genetic divergence was high (6.5 –10.5%). The geographic distribution of *F. cancrivora* ranges from the south of Isthmus of Kra of Thailand to Malaysia and Indonesia whereas *F. moodiei* is distributed, ranging from India, Bangladesh, China and throughout Thailand to Philippines. However, the additional specimen collection and molecular analysis should be conducted for determining the taxonomic status of *F. raja*.

## Genetic population assignment as a tool for identifying geographic origin of trafficked freshwater turtles

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Wildlife trafficking can have a devastating impact on the persistence of many rare and charismatic species and is one of the most lucrative transnational crimes. Turtles are some of the most widely trafficked animals, contributing to the illegal trades in pets, food and traditional Asian medicines. Accurately determining the geographic origin of trafficked turtles is essential for repatriation, as well as aiding law enforcement to target poaching 'hotspots' and smuggling routes. The pig-nosed turtle (*Carettochelys insculpta*) is an endangered and CITES listed freshwater turtle endemic to Australia and New Guinea that is trafficked throughout Southeast Asia, China, Europe and North America. Our study examined the population structure of the pig-nosed turtle across its distribution using ~15,000 SNP genetic markers. The pig-nosed turtle has strong genetic structuring, enabling population assignment between Australian and New Guinea rivers, among Australian rivers, and among New Guinea rivers between Papua New Guinea and Papua Province. We have used this population assignment test to determine the geographic origin of several trafficked pig-nosed turtles that have been confiscated in Asia, Australia, Europe and North America over the past 24 years.