BOOK OF ABSTRACTS

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Cephalopod Research Across Scales: From Molecules to Ecosystems

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Table of contents

Oral presentations

Session 1: Systematics and Biogeography

November 12, 0845

Molecular systematics of the family Sepiidae

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Relationships within the family Sepiidae are not well resolved, with most species being allocated to a single genus Sepia. Herein we construct a molecular phylogeny for Sepiidae based on three mitochondrial genes (16S, COI and 12S) and including more than 50 species. We attempted to also sequence COXIII and H3, but the former was saturated across the family, and we had difficulty to consistently sequence the latter. The resulting phylogeny was not fully resolved, especially at deep nodes, but it did recover some important groupings. Supporting findings of previous studies, we found Sepia officinalis more closely related to Sepiella spp. than most other Sepia species, rendering Sepia paraphyletic. We also found Metasepia to be nested within a fairly well supported clade of other Sepia species. Where possible we sequenced the type species of previously proposed subgenera, and we discuss the systematics of the family within this framework, and in the light of our current understanding of sepiid morphology.

November 12, 0900

Cephalopod biodiversity of the Kermadec Islands: implications for conservation

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In order to establish the Kermadec–Rangitāhua Ocean Sanctuary, which will protect a large, unique, near-pristine northeastern section of New Zealand's marine environment, an improved understanding of the marine biodiversity of this area is required. The cephalopod biodiversity of the Kermadecs was previously directly assessed only once (over a century ago), although two updated species checklists (based on uncritical literature reviews) were published in the interim. A recent biodiversity survey of the Kermadec Islands collected over 150 cephalopod specimens, providing the first opportunity to assess locally occurring taxa using integrative taxonomy. Specimens were examined, morphologically identified, and DNA barcoded. DNA sequences were analysed using the Barcode Index Number (BIN) system in the Barcode of Life Data System (BOLD). The present results nearly double the previously known cephalopod biodiversity of the Kermadecs, adding 27 to the previously reported 42 species (bringing the total to 69); three cephalopod orders are also reported from this area for the first time. The BIN analysis highlighted several taxa that are badly in need of revision, including some monotypic genera that now appear to contain multiple species, and at least five species that appear to be new to science. The Kermadec region also hosts 33 cephalopod species that are not known to occur elsewhere in New Zealand waters. The results of this study strongly support the establishment of the Kermadec–Rangitāhua Ocean Sanctuary, which would offer protection to the pelagic, deep-sea taxa reported herein.

November 12, 0915

Spatio-temporal variation and abundance of the red octopus (*Octopus maya* in the northeast zone of the Campeche Bank

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The red octopus (*Octopus maya*) is an endemic species of the Campeche Bank. Its habitat depths range from 0 to 60 m. Nevertheless, the knowledge about many aspects of population dynamics of the species at depths greater than 20 m is scarce. This study aims to identify spatio-temporal variations and population structure of the red octopus between 30 and 60 m deep on the northeast zone of the Campeche Bank, to present the first results on spatial-distribution of *O. maya* outside the conventional study area (western shelf, around 20 m deep). Four research cruises were undertaken in May-June, July and December 2016 and January 2017, respectively. Sampling was done in 30 stations monthly visited. Size (mantle length, ML) and sex of the 151 specimens collected was achieved for each station. Relative abundance in terms of catch per unit area (CPUA, org./km2) of 30 stations per month was calculated. The distribution type was figured by estimating how many octopuses of each species on average per station were caught and the percentages of probability distribution. From these values, parameters of the negative binomial distribution p, k1 and k2 were estimated. *O. maya* showed spatio-temporal variations by size and sex. Octopuses presented a latitudinal size gradient, with the smallest ones inhabiting shallow zones (30 m) and the largest ones on deeper waters. The largest specimens were found in May and those of sublegal sizes (ML < 11 cm) in July and December. The proportion of males was significantly higher than that of females in May and January, while females outstripped males in December. *O. maya* spatial distribution showed to be clumped in May, July and January, while the species was distributed in all the study area in December. The average of CPUA was lower in May (10 org./km2) and it was higher in December (24 org./km2). The observed spatio-temporal variations could be explained by the active displacements that this species makes during its growth.

November 12, 0930

Loliginid squids in the coasts of Tabasco, Mexico

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A total of 498 squids of the family Loliginidae were reviewed. Squids were caught on board of smaller vessels dedicated to shrimp fishing in four localities of the coast of Tabasco: Sánchez Magallanes, Chiltepec, Puerto Ceiba and Frontera during the months of February, March, April and May of 2017. The taxonomic identification was made according to the keys of Cohen (1979), Roper et al. (1995) and Jereb et al. (2010); while the morphometric analysis followed Roper and Voss, (1983) modified by Granados-Amores et al. (2014). The size, weight and sex were determined for the squids. The degree of sexual maturity was determined based on the guidelines of Jackson et al., (1997) and Sauer et al., (1990) assigning stages of sexual maturity from 1 to 4. Three species were identified: 1) Lolliguncula brevis, 365 individuals of which 211 were females and 104 males. The sizes of the females fluctuated from 40 to 118 mm of ML, with a weight between 5.2 and 73.51 g. On the other hand, the males had sizes of 35.5 to 132 mm of ML, with weights of 4.44 to 59.37 g. Regarding the degree of maturity, 84 females were observed in I, 38 in II, 57 in III and 32 in IV, while males 28 in I, 43 in II, 24 in III and 8 in IV. It was not possible to determine the sex of 51 individuals. 2) *Doryteuthis pealei*, 129 individuals in total, 71 females and 54 males. The length and weight ranges were 73-164 mm of ML and 18.23 to 132.68 g, 97.6 -154.5 mm of ML and weight of 37.3 to 80.47 g respectively. We observed 12 females in I, 13 in II, 14 in III and 32 in IV. In the males it was found that 7 were in II, 16 in III and 32 in IV. To 4 individuals it was not possible to determine sex. 3) *D. plei*, with only 4 individuals of which one was female, one was male and 2 undifferentiated. Squids *D. pealeii* and *D. plei* come from shrimp boats that fish off the coast of Tabasco. This work contributes to the knowledge of the distribution of this squid family in the southern part of the Gulf of Mexico.

November 12, 0945

A revision of the Pacific Cranchiidae: Untangling a giant knot of squid

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The Cranchiidae are an abundant family of squid, with representative in every ocean. Despite this, knowledge remains limited on the behaviour, ecology, phylogeny and systematics of the majority of species within this family. The earliest recognised specimen from the family Cranchiidae was identified in 1817; however, since that time, taxonomists have struggled to resolve the systematic position of many taxa within the family. A combination of insufficiently detailed type descriptions, damaged material, dramatic ontogenetic changes, and morphological similarities have made the Cranchiidae one of the most systematically unstable teuthid families. A recent revision of cranchiid taxa in the Pacific Ocean has revealed the presence of additional taxa within several genera; in total, 31 species were encountered, of which at least six appear to be new to science. For known taxa, this research aimed to provide further description and illustration to aid in the future identification and differentiation at a species level.

November 12, 1015

Temporal variation of statolith elements for Jumbo squid *Dosidicus gigas* off the Peruvian Exclusive Economic Zone

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Trace elements in statolith were widely used to evaluate the environmental history of squid and distinguish the population structure. In this study, thirteen elemental signatures in the nuclear and postnuclear zone of statoliths for *Dosidicus gigas* collected off Peruvian EEZ (Exclusive Economic Zone) were quantified using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). The Sr/Ca ratios in the core had significant differences between summer and winter spawning cohorts, and decreased gradually from summer to winter. Ba/Ca ratios in the core varied significantly among three spawning cohorts, and increased gradually from summer to winter. In the postnuclear zone of statolith, only the Ba/Ca ratios were significantly different among the three spawning cohorts. Compared to the trace elements in the postnuclear zone of statolith, elemental signatures in the nuclear zone were more effective to distinguish the summer and winter spawning cohorts of squid. Variability of statolith elememts in different season was found, and elemental signatures in early ontogenetic statoliths could be used to reconstruct the environmental history of early ontogenetic stage of squids.

November 12, 1030

A New Species of *Sepia* (Cephalopoda: Sepiidae) from Japan with a Note of Unusual Sexual Display

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An undescribed Sepia species found in the coastal waters of Sagami Bay, about 50km south-west of Tokyo, is going to be discussed. This is a small cuttlefish similar to *Sepia (Doratosepion) peterseni* at a glance, but Arm II in male not elongated and shell is less acute. Female has shorter and plumper mantle. The most striking scene in this species is behavior of male approaching to female. While he stretching arm tips towards female and trembling, he compressed his mantle dorso-ventrally, becoming the body looks like a thin plate. This time, body color changes to bright red against dull colored female. Such remarkable change of body shape and color has previously never been reported. Molecular phylogenetic analysis based on COI (658bp) showed close relationship among sympatric Doratosepion species.

November 12, 1045

Offshore Influences on Inshore Squid: Linkages Between Water Mass Dynamics and *Doryteuthis pealeii* Distribution

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Longfin inshore squid (*Doryteuthis pealeii*) move inshore in spring from winter habitat at the edge of the continental shelf, and move offshore in fall. The species supports commercial fisheries throughout most of its range in shelf waters of the northeast US. Inter-annual variability (IAV) in landings presents challenges for fishermen and managers due to the fisheries' "boom-or-bust" nature. Annual inshore biomass indices (1978-2012) from surveys in Nantucket and Vineyard Sounds (Massachusetts, USA) were tested for temporal patterns and relationships with indices related to ocean temperatures and circulation patterns, including the North Atlantic Oscillation (NAO) and the Gulf Stream North Wall Index (GSNWI). NAO and GSNWI were most correlated with inshore biomass indices. Multivariate analyses indicated that the relationships among variables changed over time, and the above variables explained relatively little IAV in inshore biomass. Gulf Stream warm-core rings (WCRs) vary inter-annually in numbers, locations, and types of interactions with outer continental shelf waters. Shoreward incursions of WCR water on-shelf and seaward entrainments of shelf water off-shelf may affect longfin squid thermal habitat, causing changes in summer inshore abundance and over-wintering longfin squid abundance near the shelf slope front. The dynamics of squid distribution, abundance, and the associated fisheries change rapidly. Fishery-dependent indices of *D. pealeii* and shortfin squid (*Illex illecebrosus*) seasonal distribution and abundance inverted between 2016 and 2017. An unusual southerly extension of cold Scotian shelf water occurred along the outer shelf in spring 2017, possibly affecting squid thermal habitat and distribution patterns. It remains a challenge to untangle relationships between environmental effects on distribution and abundance, the associated effects on availability to surveys and fisheries, and implications for perceptions of stock abundance and distribution.

November 12, 1100

Cephalopod paralarvae in the Northern System of the Humboldt Current (NSHC)

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In recent decades studies of cephalopods have increased in the Peruvian sea, especially after jumbo flying squid *Dosidicus gigas* became the second resource with greater landings in the Northern System of the Humboldt Current. Most studies have been related to the status of the population level, as well as aspects of its biology and distribution. However, little is known about the state of paralarvae biodiversity of this group.This work presents the knowledge of the diversity at the level of paralarvae in the Peruvian sea based mainly on works that are found as gray literature, internal reports of the Instituto del Mar del Perú (IMARPE), summaries of works presented in congresses, thesis, as well as the taxonomic revision of undetermined specimens of the scientific collection of the Laboratory of Zooplankton and Secondary Production of IMARPE. 24 taxa have been registered and the most frequent species were those of the family Argonautidae, Octopodidae and Ommstrephidae, which showed patterns of distribution and abundances related to specific oceanographic conditions and distance to the coast. It is worth noting, the new record of 2 species *Megalocranchia sp.* (Family Cranchiidae) and *Idiosepius sp.* (Family Idiosepiidae); related to subtropical, tropical and equatorial waters; and marine pastures and mangroves respectively. These have not been registered in the list of 37 adult cephalopods, since they are holoplantonic species.

November 12, 1115

How old are giant squids? First approach to ageing *Architeuthis* beaks

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The *Architeuthis* giant squid is one of the largest and most enigmatic marine species. Their age estimation remains controversial with the many estimates of maximum age ranging from 1 to many years. Successful results on ageing cephalopod beaks for some benthic (octopod) and pelagic (oegopsid) species support using these structures for age estimation. Remains of *Architeuthis* specimens are occasionally reported floating in waters off the Canary Islands (Central-East Atlantic). We analysed the beaks of 8 individuals with reconstructed ML between 823 and 1418 mm and caught in 1995-2006. The beaks were measured, weighted and sectioned in the Rostrum area (Rostrum Sagittal Sections-RSS) for microstructure analysis. Inner surfaces of Lateral Walls (LWS) and Crest (CS) were also examined in upper jaws. Preliminary results showed a constant sequence of micro-increments along the RSS. Lower jaws resulted more feasible due to the higher erosion and tighter agglomeration of micro-increments of upper jaws. Using oblique light, a sequence of thick increments was found in the CS. Most of them continued along the LWS, where usually several micro-increments were visible in between. The micro-increment sequence was clear in some areas of LWS although it was usually difficult to follow throughout the entire LWS. As previous validation studies showed no significant differences between RSS and LWS, we used the latter when micro-increments were visible and RSS was incomplete or damaged. Statoliths were mainly unavailable or showed the likely effect of increment agglomeration to make reliable comparisons for now. Assuming daily deposition in *Architeuthis* beaks, our age estimations between 480 and 670 days suggest a rapid growth, averaging 2.1 ± 0.4 mm ML d -1. Maximum ages were estimated applying these results to the largest measured specimens (e.g. 2400 mm ML) obtaining ~3 years of age. Analysis of more different-sized individuals would allow a more accurate age and life-span estimations of this legendary squid.

November 12, 1130

A Squid for All Salinities? Distribution of the Euryhaline Squid *Lolliguncula brevis* in South Carolina

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The Atlantic brief squid, *Lolliguncula brevi*s, is the only cephalopod known to tolerate salinities of less than 20 ppt for extended periods of time, having been recorded in salinities as low as 18 ppt. This loliginid is found along coastlines from Nova Scotia to southern Brazil, where it is a considerable portion of the estuarine faunal biomass. Despite its unique physiology, wide distribution, and sheer abundance, its ecology and ontogeny are not well-studied. We examined the distribution of *L. brevis* in South Carolina estuaries to gain insights into which selective pressures drove the species to evolve euryhalinity. As part of a crustacean monitoring program, squid were collected from inshore and coastal waters in South Carolina across salinity gradients over the course of two years. Individuals were measured, sexed, and assigned a maturity score. The data contain evidence that *L. brevis* inhabits waters with salinities as low as 13 ppt. Our results for shifts in maturity scores across salinity gradients support the hypothesis that coastal waters are used for mating and that estuaries are used for nurseries. Results also indicate that dorsal mantle length and weight are weak predictors of maturity and that they should be used only with caution as proxies for ontogenetic stage in future studies on this ecologically important species.

November 12, 1145

Systematic position of Pygmy *Octopus* "Octopus" *fitchi* Berry 1953 and the larger striped octopus (Cephalopoda: Octopodidae)

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In the last decade systematics of family Octopodidae has changed drastically. An example of this is the polyphyletic genus Octopus which for long time had been considered the most speciose within family but this diversity is due to its condition of catch-all genus. Nowadays most species of Octopus have unresolved generic status and only nine species belong to Octopus sensu stricto. "*Octopus" fitchi is* a shallow water pigmy octopus from Panamic province with scarce information about it. The larger pacific stripped octopus (LPSO) is frequently misidentified with *"Octopus" chierchiae*. Although there are several studies about this species, no formal description has been published still. Both species has an uncertain systematic position. In this study we support the distinction of these two taxa from Octopus and the monophyly of Octopus sensu stricto based on mitochondrial and nuclear markers. The topologies shows a well-supported monophyletic clade constituted by the species of Octopus sensu stricto, *O. bimaculatus, O. bimaculoides, O. insularis, O. hummelincki, O. maya, O. mimus, O. cf. oculifer, O. tetricus,* and *O. vulgaris* as the basal species of the genus. *"O." fitchi* is closely related to Paroctopus whereas LPSO is the sister taxon of "O." tehuelchus clade. However *"O." fitchi* and LPSO are genetically and morphologically different to those related taxa and we recommend describing new genera for each.

November 12, 1330

Global patterns of coastal cephalopod richness: hotspots and latitudinal gradients

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The present study aimed to identify, for the first time, the global hotspots of coastal cephalopod richness, namely cuttlefishes (families Sepiidae, Sepiolidae, Sepiadariidae and Idiosepiidae), squids (family Loliginidae) and octopuses (family Octopodidae), and to assess their latitudinal gradient of species richness (LGRS). A presence/absence database was created according to Marine Ecoregions of the World, which revealed that the most diverse ocean was the Pacific (with 212 species), followed by the Indian (151 species) and Atlantic (103 species) Oceans. Within the 232 ecoregions considered, the highest cephalopod richness value was reached in the Central Kuroshio Current ecoregion, with 64 species, followed by the East China Sea (59 species) and the Eastern Philippines (48 species). Nonetheless, the location of the hotspots varied greatly among the different taxonomic groups. Regarding the association between latitude and species richness, the peak of cephalopod richness varied among oceans, and only the squids showed a unimodal distribution with latitude. In contrast, cuttlefish, octopods and all cephalopods together revealed bimodal distributions, always with the major peak of species richness in the northern hemisphere. These findings highlight the notion that the shape and symmetry of LGRS are not universal – i.e. there are no single causal predictors to explain the latitudinal zenith locations within the same taxa.

November 12, 1345

Larval and juvenile cephalopods from the North Atlantic

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Larval descriptions of cephalopods are poor and by using DNA barcoding, a global bio-identification system for animals, along with morphological investigation we can confirm identifications. We conducted three cruises from Galway, Ireland to St John's Newfoundland, in the years 2014, 2015 and 2016, with our sampling efforts focussed at stations located across a warm-core mesoscale eddy on the western part of the transect. Plankton trawls were hauled from just below the deep scattering layer (DSL) to the surface, while a pelagic trawl and a multinet were deployed directly in the DSL based on acoustic data. In total, 305 cephalopods were collected, most of which came from the pelagic trawl; only 21 were obtained from the multinet and plankton nets. Universal primers were used to amplify the Folmer region of cytochrome oxidase subunit I (COI) as a DNA barcode and compared to sequences already available on Genbank.The majority of specimens were squid, but notable exceptions included the sepiolid *Heteroteuthis dagamensis*, the pelagic octopod *Haliphron atlanticus* and a paralarva of the benthic octopod *Eledone cirrhosa*. One squid of particular note was Ancistroteuthis lichtensteinii, which may have been transported north in the warm-core eddy. So far, we have found good agreement between the morphological identifications (by MV) and sequence data, with most specimens conforming to known species. However, several specimens warrant further investigation. For example, the COI sequence of a species that morphologically resembled Todarodes sagittatus was clearly distinct from other COI sequences of that species on Genbank and specimens that were only identified *as Brachioteuthis sp*. and *Taoniinae indet* require further identification. These identifications can help improve our knowledge of cephalopod distribution and the association of larval and juvenile cephalopods with particular water bodies.

November 12, 1400

Vertical and small-scale distribution of cephalopods in the Charlie-Gibbs Fracture Zone of the Mid-Atlantic Ridge

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In 2009, NOAA surveyed the nekton fauna of the fracture zone halfway between Iceland and the Azores as a small-scale follow-up to a previous large-scale Norwegian expedition. Midwater sampling by NOAA with a Norwegian Krill Trawl resulted in 64 discrete-depth samples from 12 stations at depths from near-surface to 3000 m. An additional seven bottom samples were collected with a large trawl at depths of 2000-3500 m. The expedition collected 471 cephalopods in ca. 24 species. In the midwater series, few cephalopods were taken in the deepest nets. However, some presumably deep-living cephalopods were collected much shallower than expected. Because this happened at stations above shallow peaks of the ridge, this phenomenon is unlikely to be a result of sampling contamination in the discrete-depth samples. The only species sufficiently common and abundant for detailed analysis of depth distribution was *Gonatus steenstrupi*. The most abundant species in the bottom trawl was *Mastigoteuthis agassizii* (also collected in the bathypelagic to upper mesopelagic), followed by four species of cirrate octopod (two of which were also taken in midwater samples).

November 12, 1415

Distribution and Abundance of Cephalopod Paralarvae on the Northeast US Shelf

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Cephalopod paralarvae were identified from plankton collections from the Ecosystem Monitoring program of the NOAA NEFSC to examine the species composition, seasonality, and habitat use. Paralarvae were identified from almost 1400 stations that were conducted on shelf-wide surveys from Cape Hatteras, North Carolina to Cape Sable, Nova Scotia during multiple seasons from 2010 to 2012. Twenty-one taxa of cephalopods from 13 families were identified. Cephalopod paralarvae were most abundant in the Middle-Atlantic Bight, Southern New England, and Georges Bank regions of the Northeast US Shelf and least abundant in the Gulf of Maine. The neritic species, longfin inshore squid (*Doryteuthis pealeii*), was the most abundant and comprised over 75 % of the total abundance of paralarvae collected. Longfin inshore squid were collected during all seasons, but most abundant from July to October with the preferred habitat on the inner-shelf (< 60 m) with surface water temperatures from 16 – 25 o C and salinities less than 33. The other taxa were also most abundant from July to October with surface water temperatures > 22 o C and salinities > 34 and dominated by oceanic taxa that were most abundant on the outer-shelf (> 80 m). We estimated Longfin inshore squid ages based on wild paralarval European squid (*Loligo vulgaris*) growth rates to calculate hatch dates. More than 85 % of the Longfin inshore squid collected were estimated to have hatched during July to October, and along with abundance and size distribution patterns indicate spawning on the Northeast US Shelf occurred mainly in the summer.

Session 2: Fisheries, stock assessment and management

November 12, 1430

Could possible environmentally driven changes in the South African Chokka squid spawning behaviour be affecting the sustainability of the fishery?

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The squid fishery, which primarily targets *Loligo reynaudii* locally known as Chokka squid, is the fourth largest commercial fishery in South Africa. Catches in this fishery are associated with spawning aggregations and consequently, patterns in the spatial and temporal distributions of squid catches are presumed to be directly correlated with variations in the spawning behaviour of Chokka squid. In 2013 an 80% decline in Chokka squid catches was recorded, leading to uncertainty in squid population stability as well as the socio-economic security of some 27 000 workers dependent on the South African squid fishery. Understanding the nature of these fluctuations is imperative to better conserve marine near-shore food web stability and sustain a valuable marine resource. We quantified the monthly/annual catch records of the South African squid fishery along the Eastern Cape coastline during 2006-2018 and discuss our findings, with specific reference to 1) the temporal and spatial distribution of Chokka squid catches using reported catch location data, aerial surveys and Vessel Monitoring System data, 2) satellite environmental data, such as chlorophyll-a concentration and sea surface temperature.

November 12, 1445

Genome-wide SNPs reveal complex fine-scale population structure in the California market squid fishery (*Doryteuthis opalescens*)

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Marine fisheries are critical for supporting local economies and sustaining local and global food security. In California, market squid (*Doryteuthis opalescens*) is one of the largest and most valuable fisheries. Thus, understanding stock structure is crucial for informing effective conservation and management. This study aimed to determine whether multiple breeding stocks of *D. opalescens* occur within the California fishery using a combined genomic and morphometric approach. A total of 662 single nucleotide polymorphisms (SNPs) were sequenced from 156 individuals sampled across five months (May-September 2014) representing northern (Monterey) and southern (Southern California) regions of the fishery (n = 8). Results revealed low levels of genetic differentiation between all geographic and temporal sampling groups ($FST=0.008$, $p>0.05$), but no significant differentiation between all northern and southern samples ($FCT=-0.006$, $p > 0.05$). Closer inspection of sampling groups using discriminant analysis of principal components and pairwise comparisons of genetic differentiation reveal unexpected complex patterns of fine-scale temporal population structure. Specifically, we observe that some spawning groups recruiting to spawning grounds at different times are genetically distinct. These results lend preliminary support to the existence of smaller genetically distinct cohorts that continually spawn in California, as opposed to the prevailing notion that spawning occurs in two asynchronous peaks in the northern and southern regions of the fishery. Fine-scale, complex structure observed in *D. opalescens* highlights the need for further investigations into the life history of this important fisheries species.

November 12, 1500

Ageing techniques and genetic studies: towards an improved management of squid populations

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Knowledge of recruitment to a fishery is particularly important in short-lived species, such as most cephalopods, in which there is a complete turnover of biomass every 1 or 2 years. Our study deals with the age, growth, and mortality estimation of loliginids paralarvae (*Alloteuthis media, Alloteuthis subulata* and *Loligo vulgaris*). Seventeen oceanographic surveys - between June and November 2017- were carried out. Four 24h surveys were undertaken along Cies and Ons islands, five 12h night surveys around the Ría de Vigo and seven 24h surveys along the northern coast of Galicia (NW Spain, NE Atlantic). A total of 208 loliginids were found from the north and south of Galician coast; all of them were genetically identified. All the individuals that were not damaged were measured to analyse morphometric differences between individuals. For these analyses, it was used a LEICA M205 stereomicroscope and LEICA Application Suite (LAS) image analysis system. Mantle length (ML), Total length (TL), Head length (HL), Fin length (FL) and right and left Tentacles length (RTeL, LTeL) were dorsally measured. Mantle lengths of the loliginid paralarvae ranged from 1,486 and 6,845 mm. Afterwards, statoliths were removed and growth increments counted. The genetic identification of each animal will serve to properly estimate growth rate by loliginid species since the previous studies with paralarvae dealt at family level. The results will be discussed in order to evaluate the influence of the different life cycles of each loliginid species in the management of these socioeconomically important squids in Galician waters.

November 12, 1530

Boom or bust: oceanographic factors that influence *Illex illecebrosus* (Northern shortfin squid) abundance on the U.S. fishing grounds

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Illex illecebrosus (Northern shortfin squid) is a nerito-oceanic species that inhabits a broad, 15-degree latitudinal range which encompasses subtropical, temperate and boreal waters of the Northwest Atlantic Ocean (NWA). Consequently, individuals experience a wide range of oceanographic conditions during their short, sub-annual lifespans. Directed fisheries occur along the edges of the continental shelves and upper slopes of the Northeast U.S. and Nova Scotia and inshore off Newfoundland, during summer through fall, when on-shelf abundance is highest. Abundance on the U.S. shelf is highly variable and characterized by rare, generally single, boom years which cannot be taken advantage of under the current multi-year fixed quota system. In addition, a fixed quota may also result in recruitment overfishing during low abundance years. Oceanographic conditions have a major influence on *I. illecebrosus* population dynamics, including abundance, and the early life history stages inhabit the highly dynamic environment of the Gulf Stream and its adjacent water masses. Recent rapid, ocean warming may have affected the GS dynamics and consequently, recruitment. For example, the GS moved southward from its mean latitudinal position during 1993-2013 which may have affected the transport patterns of *Illex* paralarvae and juveniles to the fishing grounds. Impacts of these changes on abundance have not been assessed. In addition, an adaptive management approach is needed for the resource; a pre-fishery abundance forecast model that accounts for environmental factors that regulate abundance on the U.S. shelf. As a precursor to developing such a forecast model, our study identifies a set of oceanographic indices that influence relative abundance on the U.S. shelf and upper slope. We also determine the strengths of these relationships on seasonal to inter-annual time scales in order to identify the best pre-fishery predictors of *I. illecebrosus* abundance on the U.S fishing grounds.

November 12, 1545

Catches of *Sepia officinalis* in the small-scale cuttlefish trap fishery off the Algarve coast (southern Portugal)

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The trap fishery for the cuttlefish (*Sepia officinalis*) is performed seasonally off the Algarve coast. This highly sustainable and low impacting small-scale fishery constitutes a locally important socio-economic activity during spring and summer. Undamaged catches and highly fresh individuals further contribute to the considerable price reached by *S. officinalis*. In the Algarve coast, during the study period (2009 – 2014), cuttlefish mean catches reached 446 tons year-1 and represented a mean value of 1.9 M€ year-1. Despite its relevance, the lacking information on this fishery prompted a study aiming to characterize its catches. Fishing surveys were carried out from 2009 to 2014 onboard an artisanal fishing vessel operating cuttlefish traps, a gear of rectangular shape comprising a steel frame covered with 35 mm square plastic mesh. A total of 86 fishing surveys were performed in the main fishing season: May to September. Fishing grounds were located on sandy bottoms at depths ranging from 8 to 10 m. Fishing operations involved 6311 cuttlefish traps during the whole study period, with a gear catchability of 37% and a mean of 0.9 individuals caught per trap. Overall, 5883 *S. officinalis* were caught, being only recorded 282 dead individuals inside the traps (total mortality rate of 4.8%). The mean fishing yield (standardized CPUE's in number and weight) was 46.6 individuals and 11.3 kg.50 traps-1. Overall, males (n = 3188) outnumbered females (n = 2695), producing a male-biased sex ratio (1M: 0.85F). On average, females were slightly larger and heavier (124.4 ± 22.9 mm ML; 253.1 ± 148.8 g TW) than males (121.9 ± 24.0 mm ML; 230.7 ± 143.1 g TW). Accordingly, the length-frequency distributions displayed significant differences between sexes, with 89.6% of females and 85.8% of males accomplishing the minimum landing size (100 mm ML). The bycatch comprised 38 taxa and represented 21.1% in number and 15.9% in weight of the total catches using cuttlefish traps.

November 12, 1600

Seasonal comparison of female reproductive traits in swordtip squid (*Photololigo edulis*) among different geographical populations

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Swordtip squid (*Uroteuthis edulis*) contribute a lot to coastal fisheries in western Pacific. To better manage swordtip squid fishery, it is vital to understand its resource fluctuation under environmental changes through different geographical populations. The age, growth and reproductive traits in female swordtip squid (*Photololigo edulis*) were compared between populations in western Sea of Japan and Taiwanese water. Population in western Sea of Japan was sampled monthly from May 2017 to Feb 2018, and Taiwanese population was sampled n two main spawning seasons (spring and autumn) in 2017. In Japanese population, females were immature in autumn, less than 10% were mature starting in Dec and the ratio of mature individuals was increased from winter to summer. Hatching occurred throughout the year with several peaks in Japanese population similar to Taiwanese population from previous studies. In Japanese population, the minimum mature age was similar in spring and summer cohort, but somatic growth was faster in spring than summer cohort. For Taiwanese population, spring cohort grew faster than autumn cohort in November, while slower than autumn cohort in September. In general, individuals of Japanese population mature in a larger size and older age than Taiwanese population. In Japanese population, potential fecundity of summer cohort appears to be lower than spring cohort, and similar to both spring and autumn cohorts in Taiwanese population. Also, size distribution of ovarian egg showed the similar pattern in Japanese summer cohort with Taiwanese population. Seasonal and latitudinal diversity on biological traits of swordtip squid would be associated with environmental difference.

November 12, 1615

World Octopus Fisheries

Warwick Sauer, Anyanee Yamrungrueng, Augusto C. Crespi-Abril, Biju Kumar, Charlie Gough, Chih-Shin Chen, hristian M. Ibáñez, Chung-Cheng Lu, Deepak Samuel, Delta Putra, Elizabeth Conners, Evgenyi N. Drobyazin, Felipe A. Briceño, Fernando A. Fernández-Álvarez, Geetha Sasikumar, Graham Gillespie, Hideo Sakaguchi, Hidetaka Furuya, Ian G. Gleadall, Jaruwat Nabhitabhata, Jinda Petchkamnerd, Jorge E. Ramos, Juan Arguelles, K.K. Sajikumar, K.S. Mohamed, Kyosei Noro, Leo J. Che, Leo W. González, Manuel Haimovici, Marek Lipinski, Nicola Downey-Breedt, Oleg N. Katugin, P. Krishnan, Roger Villanueva, Rosario Cisneros, Rui Rosa, Sergio A. Carrasco, Stephen Leporati, Steve Rocliffe, Timothy Emery, Toshifumi Wada, Unai Markaida, Vladimir V. Kulik, Zheng Xiaodong, Zöe Doubleday

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World cephalopod landings are increasing, with the highest reported landings in the last decade, reaching a peak of 4.8 million t in 2014. Although Octopuses generally only makes up 10% of total cephalopod landings, a number of octopus fisheries around the globe are of great economic importance at a local level, and FAO production data indicates a highest ever recorded landing of 343 277 t in 2016. On average, 56% of total reported octopus landings are from the Northwest Pacific, followed by 24% in the Central Eastern Atlantic. The remaining regions contribute between 0.07% and 6.5% to global reported landings. Numerous octopus species are exploited around the globe and apart from octopuses of the genus *Enteroctopus* and *Eledone*, almost all belong to the Family Octopodidae. This multi author overview describes the major octopus fisheries around the world, discussing the main ecological and biological features of exploited stocks, fishery characteristics, stock assessment and management measures. Area divisions are based on major FAO fishing areas, with detailed discussion on some 35 species, both targeted and incidentally caught. A review of climate change impacts, fishing methods, ecosystem impacts of octopus fisheries and fisheries management are also included.

Session 3: Life history and role in the ecosystem
November 13, 0800

Development of the dopaminergic system during embryogenesis in *Sepia officinalis* (Cephalopoda) and influence of light

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Cephalopods have a highly centralized central nervous system, composed of a brain and two optic lobes, probably related to their important cognitive abilities. In this central nervous system, large amounts of neurotransmitters are produced including dopamine. Dopamine is involved in visual learning and cognition in many metazoans and might have the same role in Cephalopods. Nevertheless, there are only few data available on the characterization and development of the dopaminergic system in relation to the acquisition of learning abilities in Cephalopods. It has been shown that light induces an increase of dopaminergic neurons in Vertebrates. Therefore, we hypothesized, in *S. officinalis* embryo, a role of the light in brain maturation, before hatching through the egg capsule and after hatching. Indeed, visual learning has been reported in cuttlefish in late embryonic stages when light reaches the embryo.
In order to gain a better understanding of the setting-up of the dopaminergic system, we have characterized the dopaminergic receptors and localized them at different stages of *Sepia* embryos. We also designed a protocol where eggs are submitted to different photoperiods. Then the expression of dopamine receptors and other photosensitive molecules such as cryptochromes and rhodopsin will be quantified through qRT-PCR in central nervous system in the different conditions. This work will give us first information about the role of light in the setting-up of the dopaminergic system and the acquisition of visual learning.

November 13, 0815

Early life-history stages provide insights on cephalopod fauna inhabiting oceanic islands in the Juan Fernández and Desventuradas Ecoregion

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Southeast Pacific (SEP) oceanic islands are characterized by their extreme isolation and high degree of endemism, with a mixture of tropical, subtropical and temperate species. To date, most research has been focussed on species composition and distributions, with few information available on early life-history traits. Pelagic and planktonic systems in SEP oceanic environments not only play a crucial role in marine trophodynamics, but also provide important information on species' dispersal capacities; nonetheless, detailed information exist only for some meso and macroplanctonic communities in general, and for some groups in particular (decapods and fishes). In this study, we provide new records of early life-stages of cephalopods based on planktonic collections carried out during October and November 2016 around three oceanic islands: San Félix, San Ambrosio (Desventuradas: 26.3°S, 79.8°W) and Alejandro Selkirk (Juan Fernández Archipelago; 33.7° S, 80.7° W), which are part of the Nazca-Desventuradas Marine Park. Twenty-four paralarvae and juveniles were obtained and identified based on morphological characters (i.e. mantle length [ML], chromatophore patterns, number of suckers on arms and tentacles). Six families were recorded, including Octopodidae, Tremoctopodidae, Brachioteuthidae, Onychoteuthidae, Loliginidae and Lycoteuthidae. The 87% of individuals (Onychoteuthidae, Brachioteuthidae and Lycoteuthidae) corresponded to juvenile stages of around 4 – 12 mm ML, and the 13% to newly hatched paralarvae and early-juvenile stages (Octopodidae, Loliginidae and Tremoctopodidae) between 1.0 – 4.0 mm ML. A DNA barcoding approach using the mitochondrial Cytochrome Oxidase I (COI) gene validated the identity of *Onykia aff. robsoni*, *Brachioteuthis picta* and *Octopus mimus*, with three specific identities remaining to be evaluated. These records provide new insights on cephalopod's geographic distributions around poorly studied SEP oceanic islands, suggesting that these environments may be

appropriate spawning grounds for benthic and pelagic species. (Funding: FONDECYT-CONICYT research grant #11170617 to S.A. Carrasco).

November 13, 0830

Live observations of deep-sea cephalopods in the Western Antarctic Peninsula

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In early 2017, manned submersible explorations were conducted in the Western Antarctic Peninsula in aid of filming a deep-sea documentary for NHK Enterprises. These dives occurred over the course of three weeks, at depths of up to 1000m. Among other creatures, at least nine cephalopod taxa were filmed, including four octopus species (*Pareledone spp., Megaleledone setebos*, and *Cirroctopus glacialis*) and five squid species, including the first live observations of *Kondakovia longimana*. While these animals' behaviour was almost certainly impacted by the submersible's presence, the high-definition footage still provides valuable observations of these animals in situ in their remote and little-explored environment. This footage, shown for the first time, will include several observations not included in the final documentary (scheduled to air to the public in early 2019).

November 13, 0845

Use of compound specific isotope analysis of individual amino acids to infer trophic ecology of cephalopods

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Except for muscular species of economic interest, the trophic ecology of most cephalopods is poorly known. Such a lack of information is particularly acute for oceanic squids although very abundant. This is mainly because they are difficult to catch and also because they cut their prey into tiny fragments before ingestion, making difficult the identification of their diet when, by chance, stomach contents are available. In this context, stable isotopes (SI) are widely used to infer their trophic ecology, δ13C and δ15N being good respective indexes of habitat and consumer diet. SI are analysed on bulk tissues, generally on mantle or buccal muscle but beaks from stomach contents of their predators are also of considerable interest. However, δ15N values from the beaks are systematically lower by 4-6‰ in comparison with muscles because of their chitinous composition. Indeed, chitin is depleted in 15N relative to the proteins. In this study, we measured both δ13C and δ15N on 16 individual amino acids (aa) obtained by the hydrolysis of the proteins of the beaks collected from predator's stomachs, as this method allow to cope with the chitin effect and to get the trophic enrichment in 15N independently of the fluctuation of δ15N isotopic baseline. We selected 4 oceanic squid species, *Kondakovia longimana* and *Taningia danae* and the iconic giant and colossal squids *Architeuthis dux* and *Mesonychoteuthis hamiltoni* for the study. In beaks, 8 trophic aa were detected (vs. 10 in muscles) and 6 source aa (vs. 5 in muscles). We found little variation for most of the aa between lower and upper beaks but differences between beak and muscles reached up 6‰. Despite this, trophic position calculated as the difference between trophic and source aa were similar for both tissues. This result shows that the present method can be applied to beaks to infer cephalopods trophic ecology, with important perspectives including the use of museum collections to make retrospective investigations.

November 13, 0900

Application of statolith barium isotopic marking technique and chemistry composition on the dispersal and migration of *Sepioteuthis lessoniana* in Taiwan

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Sepioteuthis lessoniana is an important fishery species that widely distributes in the offshore-inshore neritic waters of Indo-Pacific Ocean. In order to establish its larvae dispersal and movement pattern in natural environment, first, we examine the efficiency of barium stable isotope (137Ba) mass-marking techniques and to evaluate the optimal enriched isotope concentration and immersion duration for marking statoliths. A significant discrimination is achieved by treatments of 7-day immersion with a minimum of 0.2 ppm spike concentration. Moreover, 138Ba/Ca, 137Ba/Ca, Pb/Ca and Sr/Ca play the highest classification role (100% correction) of hatchlings in the group of 7-day immersion by different spiked concentrations. We consequently collected egg capsules from northern Taiwan, used 137Ba to mark hatchlings and released to the same location. Barium isotope ratios (138Ba:137Ba) and microchemistry patterns in statoliths were analysed to investigate the movement and to identify its hatching origins. The preliminary results drawn from this study can improve our knowledge on distribution and migration of *Sepioteuthis lessoniana* population in northern Taiwan.

November 13, 0915

Cuttlefish egg: from formation to the embryo hatching.

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The successive steps of egg-laying in the cuttlefish Sepia officinalis are well described. Ovulation is the first step, with the release of oocytes from the ovarian stroma into the genital coelom. Mature oocytes are stored into the genital coelom until mating. The stereotyped head-to-head behavior triggers oocyte progress to the oral cavity, next to the copulatory pouch where spermatophores are stored. The first capsule layer is secreted by the oviduct gland before the oocyte is released into the mantle cavity. The second layer is mixed to ink and secreted by the nidamental gland inside the mantle cavity. Inside the oral cavity, the embedded oocyte stays around three minutes close to the copulatory pouch to be fertilized. Finally, the egg is included into the egg-mass. All these steps are orchestrated by three classes of regulatory peptides: (1) neuropeptides, (2) ovarian regulatory peptides and (3) sex pheromones. After egg-laying, females and males die, embryo protection is ensured for 8-10 weeks by a multilayer capsule secreted by the accessory sex glands. Thanks to the identification of the gland proteomes, the role of each gland in capsule elaboration is now elucidated. The oviduct gland secretes the inner layer of the egg case and the sex pheromone polypeptides. The main nidamental gland secretes the main polysaccharides and glycoproteins, such as SepECPs (Sepia Egg Case Proteins), involved in capsule formation and in embryo protection. The accessory nidamental gland expresses specific proteins inherent in the structural organization of the gland, and hosts symbiotic bacteria. Similarly, to salivary glands, this gland secretes immune factors associated with gamete and/or embryo protection.

November 13, 0930

Identification and characterization of essential habitats for three cephalopod species in the Galician Atlantic Islands National Park

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We evaluated specific habitat features (bottom substrate type, depth, temperature and season) at random locations in the Cíes archipelago (Galician Atlantic Islands National Park, NW Spain) and to determine their impact on *Octopus vulgaris, Sepia officinalis* and *Loligo vulgaris* habitat use. We performed 113 underwater visual transects (UVT) by scuba diving between April 2012 and August 2015. Habitat features were evaluated as predictors of the presence/absence of spawning dens and egg clusters using Generalized Additive Models. The *O. vulgaris* spawning essential habitats (SEH) was found between 5 and 30 m depth in rocky bottoms from Punta Escodelo to Punta Ferreiro (Monteagudo Island), which surface is 6% of the total marine area of the Cíes islands. We propose a complete protection of this area for exploitation. UVT also showed that there is an *O. vulgaris* hatchery essential habitat (specimens' ≤1000 g) in the sandy bottoms of the Rodas inlet. This small area (2.8% of the total) could be also protected. S. officinalis results revealed two SEH: Bajo de Viños and Piedra del Borrón, hard bottom shoals between 8-13 m covered by sea fans and sea worms and located in the central Cíes islands. We also suggest protecting that small area (0.28% of the total). Very few *L. vulgaris* eggs masses were found with UVT. The SEHs found reveal indicators of two species habitat selection and should help to identify targets for habitat improvement projects and ecosystem management approaches.

November 13, 0945

How in situ observations advance our understanding of life cycles of deep living cephalopods

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The +2:17majority of 700 species of coleoid cephalopods inhabit open ocean waters and the deep sea. However, even in the 21st century still only a fraction of the oceanic cephalopod species has been observed in their natural habitat. Access to biological specimens of oceanic cephalopods, other than the commercial species, relies mostly on the examination of carcasses, either collected from nets, stomach contents or found at the sea surface or washed upon the shores. For many species, adult individuals are never collected. Altogether there exists a large gap in knowledge on the life cycles and behavior of the majority of oceanic cephalopods. In recent years, there is a growing realization that the deep sea provides significant ecosystem services (carbon storage, biodiversity, water column and seafloor resources), and that it is subject to stressors and the impact of anthropogenic activities. As a result, there is an international interest and effort for exploration of the deep sea. Due to the high abundance and large biomasses, oceanic cephalopods play an important and diverse role in oceanic foodwebs and contribute in various ways to these ecosystem services. The combined efforts of seafloor and midwater exploration programs, together with the advancement of underwater technologies, result in in situ observations of open ocean communities, providing novel insights into the biology and ecology of deep-sea fauna including cephalopods. This seminar will give an overview of the most recent advances of scientific research on deep-sea cephalopods life cycles with a focus on data collection using in situ observations from submersibles and camera systems.

November 13, 1015

What have we learned since 2011 about cephalopods of the northern Gulf of Mexico?

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Two recent studies that focused on the deep water column in the Gulf of Mexico (GOM) were the 2011-2016 Offshore Nekton Sampling and Analysis Program (ONSAP) and 2015-2017 Deep Pelagic Nekton Dynamics of the Gulf of Mexico (DEEPEND) program. These programs produced a combined dataset of over 12,500 midwater-cephalopod records for the northern GOM region and this talk summarizes multiple cephalopod-related projects that have been completed. These projects include: (1) a connectivity study of three species using Sanger and Nextgen sequencing between the GOM and the northern Atlantic Ocean; (2) a stable isotope analysis of selected species to evaluate the role of cephalopods in the midwater food web; (3) a temporal examination of polycyclic aromatic hydrocarbon concentrations relative to the Deepwater Horizon spill and their possible effect on cephalopods from 2011-2017; and (4) an update on cephalopod species richness of the GOM highlighting newly discovered cephalopods. The results of these collaborative studies demonstrate the importance of cephalopods in the midwater ecosystem of the GOM.

November 13, 1030

The Boreoatlantic armhook squid (*Gonatus fabricii*) is the essential food resource of male sperm whales in the northern Atlantic Ocean

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In early 2016 the largest sperm whale mortality event to date occurred in the North Sea. A total of 30 sperm whales stranded in six countries within two months. All animals were immature males in good nutritional condition. They were on the way back from their feeding grounds off northern Norway towards the central Atlantic. Why the whales entered the North Sea and headed south to the shallow waters of the Wadden Sea (<35 m) is still not clear. Here, we focus on the prey remains that were sampled from the gastrointestinal tracts of 18 sperm whales that stranded in Germany, the Netherlands and France. Prey categories comprised mainly cephalopod beaks, fish bones and otoliths. The Boreoatlantic armhook squid (*Gonatus fabricii*) was by far the most abundant cephalopod prey in numerical numbers making up 96.4 % of all lower cephalopod beaks counted. The European flying squid (Todarodes sagittatus) was represented by 3.4 %; all other cephalopods (*Histioteuthis bonnellii*, *Teuthowenia megalops*, *Loligo forbesi*, octopods) attained only 0.2 %. The 13 whales stranded in Germany contained nearly 55000 lower beaks of *G. fabricii*, resembling a cumulative prey biomass of around 12000 kg; the five whales stranded in the Netherlands contained ca. 11000 beaks of *G. fabricii*, resembling a prey biomass of ca. 2100 kg; and the one sperm whale that stranded in France contained nearly 32000 lower beaks of *G. fabricii*, resembling a prey biomass of ca. 5700 kg. The total numbers of beaks were sometimes markedly high; in one whale stranded in Germany mounting up to a number of nearly 40000 lower and upper beaks. The vast majority of cephalopod beaks were dry and eroded with the prey's flesh already digested. This clearly implies that sperm whales accumulate great numbers of beaks in their stomachs before vomiting them. The enormous numbers of *G. fabricii* beaks support the outstanding importance of this squid as an essential food resource for the north eastern Atlantic sperm whale population.

November 13, 1045

Gonatus fabricii and the North Sea sperm whale trap

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The late Chris Smeenk proposed that the North Sea was a sperm whale trap, on the coasts of which sperm whales are known to have become stranded since the mid-17th century. Since most strandings occur when males deviate from their normal route while migrating southwards from the Arctic winter feeding grounds, an obvious question is whether travel through the North Sea is related to feeding behaviour. Past studies suggest that stomach contents of sperm whales stranded in the North Sea consist almost entirely of beaks of *Gonatus fabricii* while recent publications have focused on possible links between solar (sunspot) activity and strandings. In the present study, we present recent stomachs contents data from Scotland and compare these with previous dietary studies. We also analyse Smeenk´s updated strandings time series to re-evaluate possible links with environmental variables. Results from recent strandings from Scotland and other North Sea coasts confirm the continued dominance of *Gonatus* in stomach contents of sperm whales while statistical analysis of the updated time series identified a weak but statistically significant positive relationship with sea surface temperature. Various theories about the relationships between squid distribution and abundance, environmental conditions and sperm whale strandings are discussed.

November 13, 1100

What limits the thermal tolerance of octopus embryos? A study along of latitudinal gradient of some American species

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From a physiological point of view, thermal tolerance is linked with the capacity of ectotherm organisms to obtain enough energy to satisfy the demands of metabolism. In juvenile forms of aquatic organisms, there are physiological mechanisms that allow them enhance the oxygen supply, and with it the energy production, increasing in this form the thermal tolerance. Octopus embryos have several limitations that prevent the increment in thermal tolerance because: 1) they are in development and many of physiological mechanisms are not active; 2) they are enclosed in a capsule that restring the exchange with the exterior environment and 3) they have an energy supply defined by female and that cannot be improved. During the last five years we has been accumulated data obtained of embryos of six octopus species exposed to some different thermal regimes in attempt, in one hand define the thermal limits of each one and in other hand understand the physiological mechanisms involved in thermal tolerance of species from different environments: three tropical species (*O. maya*, *O. briareus* and *O. insularis*), one subtropical species (*O. bimaculoides*), three temperate species *(O. mimus* and O. *vulgaris* type II from Brazil and *O. tehuelchus*) and one sub polar (*E. megalocyathus*). Using different forms to evaluate the physiological condition and the thermal limits, a general model integrating morphology, physiology, and biochemistry, will be proposed to explain what limited the thermal tolerance of embryos of those octopus species.

November 13, 1115

Transition of food habit with increase in body size in Japanese flying squid *Todarodes pacificus* around Hokkaido, Japan

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To reveal the transition of food habit of Japanese flying squid *Todarodes pacificus* as they grow, I examined their stomach contents collected from the Sea of Japan and Pacific Ocean, around Hokkaido. Different prey items were assessed based on appearance ratio. As the mantle length (ML) increased, major prey shifted from crustacean zooplanktons (calanoid copepods, hyperiid amphipods, and Euphausiacea) to squids (probable cannibalism). Beyond 240 mm ML, fishes were the principal prey, especially Japanese anchovy *Engraulis japonicus*. Although *T. pacificus* is a pelagic species, some benthic species were also found in the stomach, likely fed in the neritic area. *T. pacificus* resources in the Pacific Ocean conspicuously decreased after 2015. Before their decline, the *E. japonicus* biomass also decreased. To verify the effect of the decrease *in E. japonicus* on the food habit of *T. pacificus*, I compared the stomach contents and liver weight index before (from 2006 to 2010) and during (from 2015 to 2017) their resource depletion. In addition, I examined stomach contents of other squid species around Hokkaido, e.g. neon flying squid *Ommastrephes bartramii*, smallfin gonate squid *Okutania anonycha*, boreal clubhook squid *Onychoteuthis borealijaponica*, and Japanese *squid Loliolus japonica*. Among pelagic squids, transition of food habit with size were found to be similar to that of *T. pacificus*. On the other hand, in benthopelagic species, major prey items were benthic crustaceans.

November 13, 1130

Ecological roles and trophic diversity of the cephalopod assemblage near Bear Seamount in the Northwest Atlantic Ocean

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Cephalopods comprise a vital component of marine food webs worldwide, yet their trophic roles remain largely unresolved. This study used stable carbon and nitrogen isotopes to describe the trophic structure, ontogeny, and isotopic niche overlap of major cephalopod groups from pelagic and demersal habitats near Bear Seamount in the Northwest Atlantic Ocean. Beak tissues from 225 specimens (13 families; 27 species) were analyzed. The majority of samples were collected during a deep-sea biodiversity cruise conducted in 2012 by the National Marine Fisheries Service. Significant differences in mean δ15N and δ13C values were detected among all families, and among species within the families Ommastrephidae, Histioteuthidae, Mastigoteuthidae, and Argonautoidea. Inferred trophic positions ranged from 2.7-5.0 across all community members, with top positions held by the families Mastigoteuthidae, Joubiniteuthidae, Vampyroteuthidae, Chiroteuthidae, and the species *Illex illecebrosus* and *Histioteuthis reversa*. Cephalopod families exhibiting the greatest isotopic niche widths and most diverse niches overall included Ommastrephidae, Cranchiidae, and Octopoteuthidae. Groups with the narrowest isotopic niches included Joubiniteuthidae, Onychoteuthidae, and Vampyroteuthidae. Trophic position increased significantly with mantle length across all individuals sampled, and ontogenic shifts in δ15N values with increasing body size were detected within the species *Abraliopsis morisii, Octopoteuthis sicula, Taonius pavo, I. illecebrosus, Ornithoteuthis antillarum, Ommastrephes bartramii*, and *Sthenoteuthis pteropus*. The continuous gradient and broad spectrum of isotopic values measured across families, species, and body sizes suggests an unstructured assemblage within the region. Results provide some of the first quantitative trophic metrics for many poorly known species, and advance our understanding of the diversity of cephalopod ecological roles in marine ecosystems.

November 13, 1145

Transitional characters to the benthic lifestyle in juvenile merobenthic octopuses

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Merobenthic octopuses produce numerous small eggs that hatch into planktonic, free-swimming hatchlings with few suckers, simple chromatophores and transparent musculature. After a planktonic period that can range from few weeks to half a year, depending on the species and temperature, a major ontogenetic change occurs in morphology, physiology and behaviour when animals settle on the sea bottom. At settlement, a positive allometric arm growth emerges and their body surface generates chromatophores, iridiophores and leucophores, and skin sculptural components develop. At the same time, they seem to lose the Kölliker organs, whose function is unknown, as well as the lateral line system. These organs are present on its external epithelium from hatching, however, they have never been reported from the skin of subadult and adult benthic octopods. These structures and their possible presence, transformation and/or degeneration are carefully searched here over the skin body surface of recently settled juveniles as well as in subadult merobenthic octopuses Eledone cirrhosa and Octopus vulgaris. Techniques used are Scanning Electron Microscopy (SEM) and Selective Plane Illumination Microscopy (SPIM). All of these morphological changes are discussed in the context of the ontogenetic transition towards a benthic lifestyle.

November 13, 1330

Long-term research data shows migration, habitat and lunar effect of *Ommastrephes bartramii*

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The neon flying squid, *Ommastrephes bartramii*, is widespread in subtropical and temperate regions. This economically important oceanic squid species has been harvested commercially by Japan since 1974, and subsequently by the Republic of Korea, the People's Republic of China, and Taiwan in the North Pacific. Fisheries Research Agency (FRA) studied this species from 1976 to 2009 using commercial squid jigging vessels for expounding on resource ecology. This research was conducted throughout the north Pacific for approximately 9000 fishing days total. A Generalized Linear Model (GLM) to examine neon flying squid habitat indicated that: (1) catch was lowest during the full moon, contrary to prior studies that reported that the catch of swordfish, a main predator of this species, was highest during the full moon (Bigelow); and (2) a temperature difference is observed between the habitat in the eastern and western parts of their range. The temperature boundary was the 170E meridian. To further examine the relationship between habitat and oceanographic condition, a Generalized Additive Model (GAM) was applied and mapped onto the Simple Ocean Data Assimilation ocean/sea ice reanalysis (SODA) data. The resultant habitat map indicates a high abundance area occurring at the Subarctic Frontal Zone (SAFZ) with the abundance increasing from April to August. The SAFZ extends from 40N to 43N latitudes separating the cold, low salinity subarctic water to the north from the North Pacific Transition Zone waters to the south and intersects two epipelagic ecosystems each with characteristic nutrient regimes, productivity cycles, and nektonic faunal compositions. The SAFZ plays an important role in North Pacific ecosystems providing a region of optimal balance between environmental temperature and food density for the neon flying squid.

November 13, 1345

Biodiversity of paralarval cephalopod in the Southeastern Brazilian Bight: richness and spatial-temporal distribution

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Plankton samples (n=882) from historical research cruises (1974–2010) were examined to investigate biodiversity and spatial-temporal distribution patterns of cephalopod paralarvae in the Southeastern Brazilian Bight (SBB, 22−25ºS), based on bongo net (333µm) surveys across the continental shelf (up to 200m depth) during spring, winter and mainly summer. The SBB shelf dynamics is dictated by the Brazil Current (BC) circulation, with a strong north-to-south variability and cross-shelf gradients, influenced by seasonal coastal upwelling, meanders and eddies in the northern portion, and less influenced by these BC mesoscale features in the wider southern shelf. In order to identify richness and abundance of paralarval cephalopod in association with shelf dynamics, 6 subareas were considered: north-south and inner (< 50m), middle (51-100m) and outer shelf (> 100m). A suite of 3438 paralarvae were recorded, belonging to 11 families: Ancistrocheiridae (*Ancistrocheirus sp*), Argonautidae (*Argonauta sp*), Cranchiidae, Enoploteuthidae (*Abralia sp, Abraliopsis sp*), Loliginidae (*Doryteuthis spp* and *Pickfordiateuthis sp*), Lycoteuthidae, Octopodidae (*Octopus spp*), Octopoteuthidae, Ommastrephidae (*Illex sp, Stenoteuthis sp* and *Ommastrephes sp*), Pyroteuthidae and Sepiolidae (*Heteroteuthis sp*). Highest richness and abundance were found during summer in the outer shelf of the northern subarea which was dominated by epipelagic and mesopelagic families Argonautidae, Enoploteuthidae and Ommastrephidae. During winter, richness increased towards the middle shelf, where Argonautidae was dominant. In the inner shelf, the lowest richness was found in both latitudinal subareas, as well as the dominance of coastal Loliginidae in all seasons. These findings suggest an important role of the SBB shelf dynamics regulating spatial-temporal patterns of composition and distribution of cephalopod paralarvae.

Session 4: Reproduction

November 13, 1400

From sneaky to bully: ontogenetic mating tactics lead to ejaculate transition in dimorphic male squid

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The expression of alternative reproductive tactics (ARTs) is often influenced by the environmental cues and intrinsic conditions experienced by each male, and it may be reversible or permanent. In male loliginid squid, ARTs include consorts and sneakers, which differ in behavior, body size, sperm deposition site, and morphology and functioning of ejaculates. Here, we describe intermediate-sized males in the squid *Doryteuthis plei* (Blainville, 1823) that produce sneaker-like, consort-like and intermediate ejaculates. In addition, age estimates show that sneakers and intermediate males are younger than consorts. Our findings suggest that ARTs in this species may be ontogenetically expressed. Such a transition between phenotypes requires dramatic anatomical and physiological reorganization of the male reproductive system, not only to produce different-structured spermatophores, but also to generate sperm with distinct behavior, both of which are compatible with the switch in sperm deposition site. These findings demonstrate that this peculiar mating system may also fit the current predictions of ARTs.

November 13, 1415

Secret in female arms – how female inseminate her eggs with stored sperm

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Many coastal squid species have one or more seminal receptacles on buccal membrane, and store sperm in there until spawning. How stored sperm can reach ova during spawning is one of the biggest mystery in cephalopod's reproduction, as the seminal receptacle is isolated from oviduct. We observed details of spawning behavior and spawned eggs in Japanese pygmy squid *Idiosepius paradoxus*, to understand how sperm move from seminal receptacle to ova. During spawning, female bent her all arms toward ventral side and put funnel into arm crown, probably caught egg jelly from nidamental gland there at the beginning of spawning, and then attach a jelly-coated egg on spawning substrate just after the egg came through funnel into arm crown. We succeeded in filming inside of female arm crown during spawning. Female attached the opening of seminal receptacle to a holding egg when attaching the egg on substrate, and muscle around the seminal receptacle was moving at that time. Spawned eggs were coated with multiple layers of egg jelly from the oviducal gland, like a cross section of onion, and there was a tunnel-like space through the jelly-layers. Sperm cells in the jelly-layers were observed by nuclear staining with Hoechst. Sperm cells were mostly distributed around the tunnel of egg jelly. All observed eggs were fertilized, and the number of sperm cells per egg was varied from 11 to 586 (134 ± 131 cells, mean ± SD). The structure of egg jelly and sperm distribution pattern of eggs collected from the field were similar with those from captive experiments, and 23 – 283 sperm cells (72 ± 61 cells) were observed per egg. Our observations suggest that female pygmy squid actively inseminate her eggs one-by-one, and the structure of egg jelly enable efficient sperm allocation for fertilization.

November 13, 1430

Inferred post-spawning reproductive system morphology in female *Octopoteuthis sicula* Rüppell, 1844 (Cephalopoda: Octopoteuthidae)

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Octopoteuthid squids remain a poorly studied group owing to the historic taxonomic disarray of the family, with little know about their biology or ecology. During a recently completed global revision of the family, opportunistic observations were made on variation in the morphology of the reproductive system in female *Octopoteuthis sicula*. Maturity stages of 75 female *O. sicula* were assessed and initially categorised into three stages: immature, maturing, and mature; interpretation midway resulted in the proposition of a fourth stage, resting. Size-at-stage for immature individuals was ML 9.5–92 mm; ML 70–142 mm for maturing; ML 152–206* mm for mature; and ML 151–201 mm for resting specimens. Detailed examinations of the reproductive organs of 22 mature or resting females (ML 142*–206* mm) showed two distinct morphologies among individuals of overlapping size. Mature individuals had large, developed nidamental (length 40–53% ML) and oviducal (length 14–22% ML) glands; oviducts were filled with ovulated ova; ovaries contained additional ova as well as ooctyes in various stages of development. Resting individuals had considerably reduced nidamental (length 15–24% ML) and oviducal (length 7% ML) glands; oviducts were empty; ovaries contained only undeveloped oocytes. At present, differences in appearance of the reproductive glands differentiate resting from maturing individuals; additional analyses including histology are forthcoming. Preliminary evidence suggests *O. sicula* may employ a polycyclic spawning strategy. Alternatively, the resting stage as proposed for *O. sicula* may instead represent spent morphology among octopoteuthids, which would contrast that found in other oegopsid families (e.g., Gonatidae, Onychoteuthidae).

November 13, 1445

Revisiting the squid mating system

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Loliginids offer an interesting model to study sexual selection due to their complex mating system, which includes male intrasexual dimorphism (sneaker vs. consort) associated with two spermatophore deposition sites (buccal membrane vs. mantle cavity) that differ in fertilization timing (late vs. early) and success (low vs. high). Recent studies from our lab, which included behavioral observations, mating experiments and detailed morphological analyses based on *Doryteuthis pleii*, contributed to expand our knowledge of this system. Several lines of evidence indicate that male dimorphism in *D. pleii* is ontogenetic. The sneaker phase is characterized by higher gonadal expenditure that is translated into smaller spermatangia (SPT) delivered in a higher number of copulations. Sperm release from sneaker SPT is slower and incomplete, retaining residual sperm, which is compatible with a larger interval between mating and fertilization (e.g., sneakers may copulate with not fully mature females). Also, sperm competition with other sneakers may be avoided by the use of SPT as plugs that obstruct the female seminal receptacle (SR). The consort phase, in turn, is characterized by higher investment in somatic growth and, hence, female guarding and agonistic contests. Sperm release from consort SPT is faster and more intense, compatible with mating occurring near spawning. Although their SPT deposition site is more privileged, consort's reproductive success apparently depends largely on successful mate guarding. In this context, male intrasexual dimorphism may be a strategy that successfully extends the male mating period. From the female perspective, functional SRs (with stored sperm and even plugs) are already found in maturing females (stage II), which also suggests an extended mating period for females. Moreover, in a mating system where one single consort may sire a large portion of the offspring, we hypothesize that the SR may play an important role in securing genetic diversity in the offspring at the female discretion.

November 13, 1500

Mating tactics, male dimorphism, sperm deposition site and fertilisation patterns within broods of a loliginid squid (*Loligo reynaudii*)

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Polyandry is widespread and influences patterns of sexual selection in many animal groups, with implications for sexual conflict during mating and subsequent fertilisation of eggs. Assessing sperm precedence patterns is a first step towards understanding sperm competition within a female after copulation, and elucidating the roles of male- and female-controlled factors. Previously (Naud et al. 2016) we have shown using microsatellite DNA-based paternity analyses that in the chokka squid *Loligo reynaudi* multiple paternity is the norm, with 79% of broods sired by at least two males, but also that different patterns of paternity are displayed among egg strings that suggest a complex interaction of male-male competition (guarding and "sneaker" male tactics) and potential for cryptic female sperm choice. We have also shown (Iwata et al. 2018) that the different male mating tactics are associated with dimorphism of spermatophores/sperm, and associated with deposition in different female sperm storage sites (oviduct and buccal mass). In the present study, by DNA typing of sperm in the different storage sites of individual females and embryos in their extruded egg strings, we attempt to link male sperm deposition tactic with patterns of paternity within broods.

November 13, 1530

Is the recruitment of the tropical arrow squid *Doryteuthis plei* linked to retention processes? A case study case off southeastern Brazil

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Squid recruitment depends on the survivorship of paralarvae, as the parental stock dies following reproduction. Recruitment strength is modulated by the physical and biological environmental conditions experienced by paralarvae. To benefit from the favourable environments (i.e. nursery grounds) in the dynamic and fluid pelagic domain, squid paralarvae rely on passive retention in favourable areas by currents to some extent. To evaluate the retention conditions off South Brazil Bight (SBB, 22–29°S) for tropical arrow squid (*Doryteuthis plei*) paralarvae, we ran a series of particle-tracking Individual-Based Models (IBM) coupled to a 3D Princeton Ocean Model (POM). The hydrodynamic model was forced with in situ data obtained from January 2000 to December 2010. The IBM-POM models considered two transport scenarios: (1) passive Lagrangian transport and (2) Diel Vertical Migration (DVM). Results suggest that retention on actual and putative nursery grounds is high, and may retain paralarvae in a suitable environment for survival, growth and ultimately to recruit to the adult population. Taking in account a nine months lifespan for de species, plotting of autumn-winter retention indexes against spring-summer industrial and artisanal landings in the following year showed high correlation, and this can hold promise for forthcoming adult biomass prediction.

November 13, 1545

Why do you have so many seminal receptacles? : The sperm storage pattern in the Japanese flying squid *Todarodes pacificus*

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Cephalopods show great variety of the female's sperm storage organ(s) in morphology, location and number. It is widely recognised that post-copulatory sexual selection drives the evolution of structure and function of the sperm storage organ, and therefore these variables could also be related to interspecies diversification of reproductive contexts. In loliginid squids, for example, large consort males and small sneaker ones transfer their spermatangia on the oviduct and buccal membrane around the seminal receptacle (SR), respectively, resulting in simultaneous sperm storing at two separated locations in the same female. Although many cephalopod species including the loliginid squid have no more than two SRs, some species of Ommastrephidae have more than twenty SRs on the buccal membrane. Why do they have so many SRs? We speculate that number of the SR would be associated with male and/or female mating strategies and multiple SRs may be good for securing genetic variation to store sperm transferred from more males. However, little is known about sperm storage pattern, especially the level of promiscuity, in this family. We investigated the relationship between female body size, the number of the spermatangium transferred on buccal membrane, the number of the SR and the pattern of the stored sperm in each SR with *Todarodes pacificus*. We found that the larger the female body size, the more spermatangia they receive. However, neither the female body size nor the spermatangium's number was correlated with the number of the SR. Next, to investigate how many males were involved in sperm storage within a single SR, we conducted in vitro fertilization assay using spermatozoa collected by squeezing each SR and paternity analysis of resulting embryos. Our preliminary results suggested that sperm of same males were observed among SRs. We will discuss what factors would be related with the evolution of multiple SRs.

Session 5: Culture and Welfare, Cephbase

November 13, 1600

Pelleted diet with thermal treatment of ingredients for *Octopus vulgaris* type II ongrowing: zootechnical performance and digestive enzymes

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One of the main factors that limit the commercial octopus aquaculture is the absence of pelleted or extruded diets that promote adequate growth and survival rates. Here, we tested a dry pelleted diet with ingredients processed at 40 °C and a semi-moist diet with frozen ingredients (control diet) to *Octopus vulgaris* Type II subadults ongrowing with respect to the zootechnical performance and activity of digestive enzymes. The diets were formulated using the same ingredients: the pelleted diet was prepared with mussel and crab meal and the semi-moist diet was prepared with frozen mussel and crab, and gelatin was used as a binder. We observed well acceptance of the pelleted diet by the octopuses. The pelleted diet also presented more stability and lower lixiviation rate compared to the control diet. Both diets promoted similar growth, survival, feed efficiency and feed conversion rate although total food intake, protein, and energy intake rates were higher in the octopuses fed on the semi-moist diet. In addition, the pelleted diet stimulated the higher activity of amylase, pepsin-like and chymotrypsin in the caecum as a physiological response to improve the absorption and assimilation of nutrients. We conclude that the pelleted diet with ingredients processed at 40 ° C promotes adequate growth rate, survival and enzymatic activity of the octopuses. Therefore, the pelleted diet can be used as starting point to elaborate formulated diet to octopuses.

Session 6: Genetics and Evolution

November 15, 0800

Genomic signatures of GPCR expansions reveal step-wise functional evolution of cephalopod sensory organs

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The evolution of cephalopod neural and morphological novelties has been attributed at a genomic level to independent gene family expansions. In the *Octopus bimaculoides* genome, one such expansion occurred in the G-protein coupled receptors (GPCRs) repertoire, a subfamily of proteins that primarily mediate signal transduction in sensory systems and regulate homeostasis via endocrine and neuronal functions. Here we assessed the evolutionary history of this GPCR expansion and its relationship with cephalopod novelties. Using phylogenetic analyses, two cephalopod- and two octopus-specific GPCR expansions were identified. Signatures of positive selection were analyzed within the four groups, and the location of these sequences was extracted from the *O. bimaculoides* genome assembly. Additionally, the expression profile of the octopus GPCRs across various tissues was extracted from available transcriptomic data. Our results provide evidence that GPCR subfamilies shared across bilaterians show nervous tissue-specific expression profiles. Octopus GPCRs forming the cephalopod-specific clades differed in their expression patterns, in some cases despite close physical proximity in the genome. In contrast, genes from octopus paralogous groups that were also co-localized in the genome showed mainly conserved expression profiles and signatures of positive selection. These paralogous genes were mostly expressed in the axial nerve cord of the arms and the suckers, structures involved in contact chemoreception; an important ability in octopuses to sense preys and feed. These results help us reconstruct the genomic expansion history of the GPCRs and contribute to our understanding of the molecular basis underlying the evolution of unique sensory-related features in cephalopods.

November 15, 0815

Genome reorganization and the evolution of cephalopod novelties in the Hawaiian bobtail squid, *Euprymna scolopes*

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Many metazoan genomes, from cnidarians to humans, retain large chunks of clustered, or syntenic, genes, but the octopus genome poses an exception to this rule: massive genome rearrangement has led to the loss of a large proportion of otherwise conserved bilaterian synteny. Whether this genome rearrangement is shared across various cephalopod genomes and how it functionally contributed to the evolution of cephalopod innovations remains unknown. Here we report, for the first time, the analysis of the Hawaiian bobtail squid (*Euprymna scolopes*) genome. We find that this genome shows signatures of genomic reorganization previously reported in octopus and the emergence of several new local syntenic gene clusters, uniquely present in cephalopods. We study the evolutionary history of the genes within these syntenic clusters and utilize several available transcriptomes in Euprymna to analyze their expression across tissues, identifying contributions to both cephalopod synapomorphies (such as the complex nervous system), and bobtail squid-specific innovations (e.g., the light organ). To further address the functional aspects of syntenic regions, we apply chromatin conformational capture (Hi-C) and open chromatin profiling (ATAC-seq) approaches to reveal functional co-regulated units within the *Euprymna* genome. We report the association of those units to both retained bilaterian and novel cephalopod synteny, linking genome evolution to the evolution of novel regulatory cascades in the squid. We identify regulatory differences among genes in those syntenic types, which allow us to study the emergence of novel regulatory architecture underlying tissue-specific expression.

November 15, 0830

Genomic "big bang": Chromosomal-scale assemblies reveal fusion- and fission-rich history during cephalopod karyotype formation

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Synteny, or the arrangement of genes along the chromosome, is largely conserved across many metazoan genomes. While local gene clusters ("micro-synteny") can be readily studied across metazoans, analysis of large-scale gene linkages on the chromosome level ("macro-synteny") has been hampered by the limitations of genome assemblies. Coleoid cephalopods, with their highly re-arranged genomes, provide unique model systems to study the plasticity of animal genome architecture and its contribution to evolutionary novelties.

Having obtained a chromosome-scale assembly of a cephalopod, we can, for the first time, reveal the general trends of macro-synteny evolution within this clade. Our analyses of the *Doryteuthis pealeii* (Boston market squid) chromosomes uncover striking preservation of chromosome-level macrosynteny between octopus and squid, segregating the ancestral coleoid genome into at least 40 chromosomal units. We found that these units were formed by fissions of ancestral chromosomes, which may explain the high chromosome complement in extant cephalopods. We also identify several chromosomal fusions in the last common ancestor of coleoids, suggesting a significant reduction in the number of ancestral chromosomes prior to fission.

These data reveal a surprising path of chromosome-scale genome reorganization that predates the radiation of coleoid cephalopods and set the stage for future chromosome-scale comparisons between cephalopods and other metazoans.

November 15, 0845

A Molecular Search for the Giant Squid Continued: Going Nuclear

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Despite being a high-profile example of charismatic megafauna, the giant squid remains a creature shrouded in mystery. Collections of specimens are geographically fragmented and primarily based on dead or moribund specimens. A previous molecular study, based on mitochondrial genomes of such museum specimens from across the world, found that the level of mitochondrial genetic diversity is exceptionally low, adding strong support to the idea that there is only one extant species of giant squid (*Architeuthis dux*, Steenstrup 1857). Furthermore, it showed a complete lack of population structure, indicating complete global panmixia, and that the squid of this species are highly mobile, possibly dispersing over long distances via a drifting paralarval stage. This study follows up on that surprising result, with a data set of ~10.000 genome-wide nuclear SNPs on a subset of 30 samples from those in the previous study, covering the same major regions of the world's oceans. The extent of phylogeographic structure in the global population of the giant squid was once more assessed by phylogenetic tree reconstruction and genetic admixture analyses. The new results support the existence of one single global species, but counter to the previous result, the nuclear SNP-based analyses detect a signal of genetic substructure, dividing populations from the Atlantic, the northern and the southern Pacific into three genetic clusters, indicating either the presence of discrete barriers to dispersal and interbreeding between these regions, or a pattern of isolation by distance stretching from the Atlantic, through the Indian Ocean and into the northern Pacific. The new results reveal a remarkable case of mitochondrial and nuclear marker incongruence, and that in the case of some marine organisms, a very powerful genetic approach may be necessary in order to detect subtle population differentiation. Additionally, they reveal fresh insights into the biology of the giant squid.

November 15, 0900

Phylogeny of Octopods in coastal waters of China by complete mitochondrial genomes and exploration of the optimal method for phylogenetic analysis

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Octopodidae is the largest group in Cephalopoda in terms of species. They are also one of the most confusing groups in international taxonomy and systematics, especially in the tropical waters of China where a large variety of groups remain poorly documented. In addition, the higher-level systematic relationships within the octopod group remain unclear and are difficult to establish based on only morphological data. Hence, mitochondrial genomes of 12 species in Octopodidae were sequenced to investigate their systematic relationships and phylogenetic status in coleoid cephalopods. We found that the mitochodirial genes exhibted different evolutionary rate but shared highly consistent gene arrangement and re-rearrangement. The phylogenetic analysis indicated that Amphioctopus genus and Cistopus genus are two monophyletic groups. *Octopus* genus was divided into two clusters, which was further demonstrated its polyphyletism. *O. minor* showed a very closer relationship to *O. conispadiceus*, but exhibited a distant relationship to the other species in *Octopus* sensu stricto genus. At the same time, we added RNA genes for phylogenetic analysis, which formed five different gene datasets at the first time. According to the phylogenetic tree, current classification of Cephalopoda was basically consistent, which all divided 43 species of Cephalopoda into ten taxons including Oegopsida, Bathyteuthidae, Spirulidae, Myopsida, Sepiolidae, Idiosepiidae, Sepiidae, Octopoda, Vampyromorpha, Nautilida, and contradiction was mainly focused on the taxonomic status of *Idiosepius sp*. and *Semirossia patagonica*. Compared with phylogenetic trees based on different gene datasets, we found that phylogenetic analysis based on P123+RNA was the optimal method for Cephalopoda. In addition, the cascaded RNA gene can only be used to explore the phylogeny among higher taxon, but it has a positive effect on that of Cephalopoda.

November 15, 0915

Gene family expansions in the evolution of cephalopod novelty

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Coleoid cephalopods have a suite of evolutionary innovations, including their elaborate, highly centralized nervous systems and camera-like eyes, which are classic examples of convergent evolution. Coleoids also present a number of true novelties, such as their adaptive coloration system and sucker-lined arm crown, which have no obvious correlates in other animals. To study the genetic bases underlying these morphological innovations, we sequenced the genome of the longfin inshore squid, *Doryteuthis pealeii*. We find that the *D. pealeii* genome is substantially larger than that of *Octopus bimaculoides*. The massive increase in C2H2-zinc finger genes we observed in the *O. bimaculoides* genome is absent in *D. pealeii*. However, the expansion of protocadherins, a family of cell adhesion molecules important for wiring vertebrate brains, described in octopus appears to be even larger in squid. Most importantly, our chromosome-scale assembly identifies many tandem expansions of genes expressed in novel cephalopod structures, including the iridophores and the suckers. Some of these gene clusters are cephalopod-, or even squid-specific, while others appear to be local expansions of genes also found in more distantly related animals. These data highlight a major role for the acquisition of novel genes and the selective expansion of gene families in the evolution of cephalopod morphological innovations.

November 15, 0930

Disentangling the decapodiforms: can currently available genome-scale data resolve squid and cuttlefish phylogeny?

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Relationships among the six major extant lineages of Decapodiformes—Idiosepiida, Myopsida, Oegopsida (including Bathyteuthoidea), Sepiida, Sepiolida and Spirulida—have puzzled cephalopod biologists for over a century. There are 2,752 possible patterns of relationships among six lineages, and a surprising number of these possibilities have been proposed based on analyses of morphological, paleontological and molecular data or combinations thereof. High-throughput sequencing methods and genome-scale data have successfully resolved several recalcitrant phylogenetic questions, but recent studies of mitochondrial proteome and nuclear genome/transcriptome data have supported differing patterns of relationships within Decapodiformes. The standard response to this dilemma (including from us) has been that broader taxon sampling and even more data will be needed to clarify relationships, but can we do better than that? To squeeze as much phylogenetic insight as possible from currently available genome-scale data, we inferred relationships within Decapodiformes using all publicly available decapodiform transcriptome and genome data as of July 2018 and evaluated the impact of numerous factors known to influence phylogenetic inference, including taxon/data sampling, orthology inference, outgroup choice, missing data, conflicting signals among loci and compositional and substitution rate heterogeneity among lineages. Our findings clarify which aspects of decapodiform phylogeny are robustly supported, eliminate some proposed hypotheses from serious consideration and highlight the remaining problematic nodes.

November 15, 0945

Divergence and convergence in evolution of sensory organs in cephalopods and fish

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Cephalopods and fish have been co-existing for about 500 million years in close competition to dominate the world oceans. Both groups originated in Late Cambrian from demersal ancestors by evolving the means to lift off the bottom and swim or 'soar' in the water column at the adult stage. Early fishes appeared as a result of neoteny of their chordate ancestor, retaining larval features into their adult phase. On the contrary, the first cephalopods appeared due to the development of characteristic enclosed chambers in a modified adult shell that created an effective floating device. In order to live in either near-bottom or pelagic realms, cephalopods and fish first diverged in evolution of their main sensory systems. In Ordovician and Silurian, primitive agnathan fish were mainly scavengers and therefore underwent the 'olfactory' evolution by improving and relying mainly on their olfactory organs. First cephalopods (nautiloids) were predominantly predators and underwent the 'optic evolution' by developing the eyes and relying on vision to capture their prey. In Late Silurian - Devonian, the origin of the jaw in fishes and corresponding formation of predatory life style caused a convergence in structure and function of cameral eyes between fish and cephalopods. Together with other sensory systems to locate the prey distantly (lateral line, electrosensing and auditory organs), the fish became more successful predators and diversified quickly. Conversely, cephalopods relied mainly on improvement of their visual system to identify the prey and tactile chemoreception to taste it after capture. Formation of nektonic life style in fish and cephalopods in Carboniferous/Permian and its further development in Mesozoic led to convergent evolution in vestibular organs to detect both linear and angular accelerations during locomotion in three-dimensional space. Ecological aspects of the formation of sensory complexes in cephalopods and fish are also discussed.

November 15, 1015

Reproduction of coleoid cephalopods from Paleozoic to Recent

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The Bactritida –direct ancestors of coleoids that are known from the early Devonian, produced small-eggs throughout their entire history (embryonic shell ~ egg size, ES 0.8-2.9 mm). The first unambiguous and short-living coleoid order Hematitida (early Carboniferous) spawned pelagic eggs of ~3-4 mm (initial chamber, IC 1.0 mm). Reproductive strategies of archaic phragmocone-bearing coleoids (Aulacocerida, Diplobelida, Phragmoteuthida and Belemnitida) up to the end of the Cretaceous included production of small eggs with initial chamber (IC) size ~0.3-0.7 mm (ES ~ 1 - 2 mm) though some species like the cold-water polar *Oxyteuthis* and *Praeactinocamax* had IC = 1-1.2 mm (ES ~ 3-4 mm). There is no evidence of deposition of large eggs on the bottom until the appearance of Sepiida; eggs size in extinct Paleogene cuttlefishes was possibly similar to that in recent *Sepia*. Diverse Mesozoic neocoleoid Octopodiformes and rare Decapodiformes did not leave paleontological evidence of their egg size. The mode of reproduction cannot be inferred from modern observations as evolution of reproductive strategies occurs very fast with respect to morphological body changes, as exemplified by the sibling octopod species *Octopus bimaculatus* (eggs 2.5-4.0 mm) and *O. bimaculoides* (eggs 12-17 mm). Appearance of the large-egged strategy at the end of the Cretaceous enabled modern coleoids to survive the K/T extinction as it did for Nautiloidea in contrast to Ammonoidea and archaic belemnoid coleoids. The small-egged strategy might have re-evolved later from a large-egged strategy in modern coleoids, as had already happened in the Ordovician when cephalopods evolved small eggs for the first time from the initial large-egged strategy. Modern coleoids exhibit a range of patterns of reproductive investment generally falling into three major categories: a) small eggs released or brooded in pelagic layers b) small eggs laid on the bottom; c) large eggs laid on the bottom.

November 15, 1030

Establishing a novel model organism for cephalopods: Development of genetic tools in pygmy squids

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Cephalopods and humans independently evolved camera-like eyes, a high-pressure cardiovascular system and a complex nervous system. This evolutionary convergence makes cephalopods an ideal comparative model to vertebrates for understanding the complexity of their systems. Publication of the first sequenced cephalopod genome in 2015 has provided researchers with an immense amount of raw data. Focus is now shifting to the development of powerful gene editing tools, such as the CRISPR/Cas9 system, to functionally explore the genomes of diverse cephalopod species. In this context, the pygmy squid (*Idiosepius paradoxus*), with its short generation time, fast development, small size, and transparency (embryos and hatchlings), is a promising candidate as a model for neuroscience and behavior, in particular for studying novel and convergent complex systems. High quality genome assembly for pygmy squid is underway, and we are now developing genetic tools, specifically, CRISPR/Cas9-based loss-of-function manipulations in pygmy squid, targeting 8 genes of interest. Those genes are expressed during embryonic development and are involved in heart (NKX2-5), eye formation and function (PAX6, RHO), cilia biosynthesis (IFT88) and pigmentation (TYR, TDO, KMO). Succesful manipulations should lead to dysfunction, malformation or absence of organs in injected embryos. The research is divided into three phases: (1) Computational identification of orthologous genes in pygmy squid transcriptome and design of CRISPR/Cas9+gRNAs cocktails, (2) injection of cocktails into early embryos and culturing, (3) and phenotyping, behavioral analysis and genotyping. Phylogenetic analyses allowed identification of orthologous genes in pygmy squid, and we are in the process of injecting embryos with CRISPR/Cas9 cocktails. Ultimately, this study will generate a powerful genetic tool which can be optimized for more sophisticated techniques, such as light-based functional imaging or optogenetics.

Session 7: Behavior

November 15, 1045

Rapid adaptive camouflage in cephalopods

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Rapid change in appearance is one of the hallmarks of cephalopods. Camouflage is the primary defense of most cephalopods yet this subject has rarely been studied until the last decade. I will summarize the most informative field studies of camouflage in octopus, cuttlefish and squid, including some very new approaches using HyperSpectral Imaging to quantify color match to backgrounds and determining how cephalopod camouflage patterns look different to different predators. I will address challenging questions such as "How many camouflage patterns are there? What exactly do cuttlefish cue on in the surrounding substrate to decide on the appropriate pattern? How fast can they change their pattern? How and when do they choose crypsis versus masquerade versus mimicry for primary defense? How does secondary defense work?" This presentation will include video sequences and still images to illustrate how to quantify camouflage and test some of the predictions of mechanisms and functions of camouflage.

November 15, 1100

Stalking the stalkers: an integrative approach to understanding octopus movement and predatory response

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Octopuses are one of the most ubiquitous and important predators in benthic coastal ecosystems. Their efficient foraging behaviors can have significant effects on the abundance of small invertebrates. Yet, our understanding of what drives octopus movement and prey discovery behavior is limited. We synthesized the results from three studies with differing methods aimed at addressing this knowledge gap using the California two-spot octopus, *Octopus bimaculatus*. First, octopuses exhibited a rapid response to the introduction of abalone in a stocking experiment, demonstrating their ability to discover and exploit new food resources. Second, we used a passive Vemco Positioning Array (VPS) to triangulate the precise position of acoustically-tagged octopuses as they moved through the habitat. Octopuses were highly mobile within and outside the array boundaries, demonstrating the large area individuals of this species can inhabit. Third, we tested the effects of a diurnal and a nocturnal predator on octopus exploratory behavior, assessing the impact of predation risk on octopus movement. Collectively, these results demonstrate that *O. bimaculatus* is capable of assessing and integrating multiple environmental variables to inform and motivate movement decisions. This integrative approach allows for a more comprehensive understanding of octopus behavior and provides opportunities to apply this knowledge to current conservation challenges.

November 15, 1115

Decision-making, communication, and cognition in interspecific cooperative hunting between octopus (*Octopus cyanea*) and fishes

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Cephalopod cognitive prowess, i.e. broadly defined as "intelligence", is still a matter of intense debate. Brain evolution is often associated with the cognitive demands and challenges of social life, since cooperative partners rely on being able to understand non-immediate rewards, constantly re-evaluate costs and benefits, and correctly use partner control mechanisms (Social/Machiavellian intelligence hypothesis). Notwithstanding their solitary lifestyle, cooperative hunting between octopus and fish occurs through recently-described complex communicative mechanisms. However, in multi-partner contexts, a network of interactions occurs and the consequent existence of multiple sources of information has the potential to shape cognition and decision-making, by eliciting the emergence of alternative non-cooperative strategies (e.g. dynamic leader-follower status, information manipulation). We analysed footage of interspecific cooperative hunting events between *O. cyanea* and multiple partners (i.e. various fish species) in Eilat (Israel). To describe the underlying dynamics of these events, multi-level quantitative and qualitative approaches were used, analysing: 1) Motor and chromatic patterns of *O. cyanea*, and fish partners, 2) Leadership during collective movements, 3) Relative orientation of interacting partners (e.g. facing towards octopus, towards fish, towards prey), 4) Aggressive interactions (fish biting octopus, octopus chasing fish), 5) Kleptoparasitism (i.e. stealing prey from one another), and 6) Overall/individual prey capture. We found that these events follow game-theory principles and that, within the theoretical framework of cooperative hunting, are described as highly social, communicative, with great interdependence and member specialization. The mechanisms underpinning these interspecific interactions can potentially further our understanding of cephalopod cognition, interspecific communication, and collective decision-making in nature.

November 15, 1130

Effects of viewing conditions on camouflage in *Sepia officinalis*

Kerry Perkins

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Camouflage is highly reliant on visual information and therefore it is important to understand how reduction of this information may affect camouflage. In situations such as low light and high turbidity visual information is reduced to the eye due to the reduction of light or high scatter in the latter. The influence of these on camouflage has only been tested in a couple of experiments Allen et al 2010, Cartron et al 2013 and has yet to determine if the reduction of information results in similar camouflage of a low contrast environment. Through testing of varying conditions, we found that reaction to turbid and low light levels show similar responses in camouflage, suggesting similar mechanisms are employed when there is reduced light and high scatter information.

November 15, 1145

"Model" Behavior? Temperamental differences in *Octopus vulgaris*

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The marked behavioral differences between individual octopuses has been long been known anecdotally, but has only been studied formally in three species of octopus (*Octopus rubescens* in 1993, *O. bimaculoides* in 2001 and *O. tetricus* in 2010). In two of these species, behavioral responses could be reduced to three or four principal components (Activity, Reactivity, Aggression and Avoidance) that explain about half of the variation between individuals. On the other hand, responses within individuals were inconsistent over time, conflicting with the strong human impression of strong temperamental differences between individual octopuses. This inconsistency may be partially explained by the fact that there are currently no published data concerning behavioral stability in the most commonly-studied species of octopus, *O. vulgaris*—the de facto group model and likely origin of the impression of distinct octopus "personalities". Here, we present the results of two unpublished studies concerning behavioral consistency in individual *O. vulgaris* (N = 51 and 20) over the course of several days. These preliminary data support the notion of distinct individual temperaments in this species. We hope that these data will prompt a discussion of the evolutionary and ecological effects of temperamental differences in *O. vulgaris* and other cephalopods. Most critically, the research community must contend with how this phenomenon could affect interpretations of the large body of existing behavioral data for this species. For example, the standard metric of measuring behavior using treatment group means may obscure the fitness of an animal's response by blurring the disparate behavioral strategies of multiple individuals. This potential problem and others presented by individual temperamental differences are worth close examination and discussion by the cephalopod research community.

November 15, 1330

Octopus mirror recognition

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Researchers in the study of animal cognition recognize that cephalopods, particularly octopuses, are highly intelligent. Yet they debate about their intelligence compared to that of mammals and birds. One test used for assessment of comparative intellectual abilities is the mirror test, particularly the mark aspect. If an animal sees the image of itself in a mirror, especially if some aspect has been changed by a mark on the animal's surface that is not directly visible, does it recognize itself? This has been used to test 'self-awareness', particularly of mammals and birds. Does the mark test measure self-awareness in octopuses? Two species, *Octopus rubescens* and *O. vulgaris*, were evaluated for their visual recognition of self in a mirror. *O. rubescens* behaved differently to their image than to a non-reflective surface, but similarly to the view of a conspecific. *O. vulgaris* again showed different reactions to the mirror than a non-reflective surface. However, they also behaved differently to the view of themselves versus a conspecific, making more 'passing cloud' skin displays to the mirror and more agonistic mantle-up displays to the conspecifics. Thus, they did not see the image as a conspecific, but likely did not recognize it as self. This suggests that vision is not used by octopuses similarly to its function in mammals, and that visual recognition is not a test of self-awareness in cephalopods.

November 15, 1345

Diversity in body pattern development within the species complex of juvenile Bigfin reef squid, *Sepiotheuthis lessoniana* from Okinawa archipelagos Japan

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Body pattern behavior of the species complex of the Bigfin reef squid, *Sepiotheuthis lessoniana* was observed over 90 days. *S. lessoniana* is a widely distributed from the northern island of Hokkaido in Japan to the Indo-Pacific. Previous studies indicate that there are at least three different biological species identified in the Ryukyu Archipelago, which local fishers describe as shiro-ika or Aori-ika (Sp.1), aka-ika (Sp.2), and kuwa-ika (Sp.3), forming a species complex. In this study, we collected egg masses of all three species from each respective spawning beds in the coastal waters of Okinawa Island during each respected time of the year. The egg masses were then aqua-cultured separately using the identical method. Paralarvae were video recorded for one hour starting 0 days from hatching and recorded every 7 days until 91 days. From 14 hours of video, 1260 freeze frames were extracted for body pattern analysis. 12,225 individual animals (Sp. 1=3385, Sp.2=4776 and Sp.3=4064) were identified from the frames, which were separated into 7 distinctive chronic (lasting for minutes or hours) body patterns found in all three species and counted. The data collected during the 90 days, we have observed distinctive patterns in body pattern development, variances between pattern appearances and overall body pattern display. Sp.1 remained pale (65%) with least amount of variances between the patterns. Sp.2 displayed similar total body pattern appearances and configuration as Sp.1 and displayed pale body pattern (47%) with a moderate variance. Sp.3 stayed predominately in dark body pattern (65%) with a moderate variance.

Session 8: Anthropogenic Effects

November 15, 1400

Stress responses and variability in hatchling squid, *Doryteuthis pealeii*, reared under acidification and warming

Casey Zakroff, Aran Mooney

Ocean acidification (OA) and warming due to increased anthropogenic CO2 is a significant concern for coastal systems and species. The Atlantic longfin squid, *Doryteuthis pealeii*, a keystone of the Northwest Atlantic trophic web, has demonstrated impacts (decreased mantle length, delayed hatching, and degraded statoliths) under high chronic exposure to acidification (2200 ppm), but the combined effects of OA and warming have not been explored. In this study, *D. pealeii* egg capsules were reared under a combination of acidification levels (400, 2200, & 3500 ppm) and temperatures (20 & 27 ºC). Hatchlings were measured for a range of morphological and physiological metrics in three trials over the 2016 breeding season (May - Oct). Although notable resistance to stressors was seen, highlighting variability between clutches, reduced mantle lengths and malformation of the embryos occurred at the highest OA exposure. Surprisingly, increased temperatures did not appear to exacerbate OA impacts, although responses were variable. Rather, high OA-exposed hatchlings from the warmer conditions often showed reduced impacts compared to those reared in ambient temperatures. This may be due to the increased developmental rate and subsequently reduced OA exposure time of embryos in the higher temperature treatment. The viability and survivability of these high OA, high temperature hatchlings could not be observed, however. Therefore, these results indicate a substantive potential plasticity to multiple stressors during the embryonic development of this species of squid, but do not predict how this species would fare in the future ocean.

November 15, 1415

A meta-analysis of physiological responses of cephalopods to ocean acidification

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Ocean acidification has been identified as a major contributor to ocean ecosystem decline, impacting bio-calcification, behavior, physiology, and survival of many marine organisms. The impact on cephalopods, which form aragonite cuttlebones and statoliths, is of concern because of the central role they play in many ocean ecosystems and because of their importance to global fisheries. We used meta-analytical techniques to explore the biological responses of cephalopods to ocean acidification and found negative effects on their calcification, acid-base regulation, survival, and growth. However, the degree of sensitivity varied greatly depending on their ontogenetic stage, with the highest sensitivities often found during the early life stages. Extreme hypercapnia (excessive CO2) may induce metabolic depression in the early stages, because the ion regulatory mechanisms may respond to changes in seawater carbonate chemistry induced by elevated CO2 and lowered pH. While the acid-base regulation capabilities of adults are well developed, it is still not known how their body buffering capacity change under the impact of ocean acidification, which is supposed to cost extra energy to cope with the acidic stress even for large organisms. Although higher hypercapnia levels have the potential to trigger acute negative developmental, metabolic, and calcification effects, the ongoing ocean acidification parallel with other ocean global changes (such as deoxygenation) can directly or indirectly affect marine animals, including the well-studied cuttlefish species *Sepia officinalis* that showed tolerance against the acidic stress. Their relative tolerance may be a result of living in benthic coastal habitats, pre-adapting *S. officinalis* to natural environmental pCO2 fluctuations. Our analyses suggest generally large and negative physiological effects on cephalopods from ocean acidification, but the sensitivity variation among different ontogenetic stages holds important implications for the overall ecosystem response.

November 15, 1430

The One-Two Punch: A tale of CO2, temperature and octopus physiology

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There has been exceptionally few investigations into the impact of future climate conditions on octopuses. To assess the responses of *Octopus rubescens* to such conditions, I maintained octopuses in either control conditions (pCO2 = ~700 µatm, Temperature = 11.3C), elevated temperature (13.7 C), elevated pCO2 (~1500 µatm) or both elevated temperature and pCO2 for 5-weeks. During treatments octopus food consumption, growth and feces production was measured. At the conclusion of the 5 weeks, metabolic rate, critical oxygen pressure (Pcrit), and ventilation efficiency was measured in each octopus. Octopuses showed no change in metabolic rate at elevated pCO2 or elevated temperature but showed a marked increase in metabolic rate in combined treatments. A similar trend was observed in Pcrit with only small changes at elevated pCO2 or temperature alone, but showed a marked increase in combined conditions. Despite metabolic rates being relatively unaffected by elevated pCO2, ventilations rates rose and ventilations efficiencies dropped at elevated CO2. In addition, to determine changes in mRNA expression in response to elevated CO2, 3 octopuses were maintained in control condition and 3 octopuses maintained in elevated pCO2 for two weeks. At the conclusion of this time a sample of gill was collected, mRNA extracted and RNA-sequencing performed. Expression of mRNAs related to aerobic metabolic pathways appears to be down-regulated at elevated CO2 and anaerobic pathways appear to be up-regulated. Together these data suggest that despite negligible effects on total metabolic rate of octopuses as a result of elevated CO2 alone, there may nevertheless be aerobic impairment in these conditions. Also, the combined stressors of increased pCO2 and temperature have a greater than additive impact on the physiology responses in *Octopus rubescens*.

November 15, 1445

Biodiversity, distribution and ecological role of cephalopods in the Arctic and Antarctic marine ecosystems under a changing ocean

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Cephalopods are known to play an important role, both as prey and predators, in polar marine ecosystems. In this review, we compare the biodiversity, distribution and ecological role of cephalopods in the Arctic and in the Antarctic. Sixty-one species have been reported from the Arctic (including the Pacific Subarctic) while 54 species are known from the Antarctic. The only confirmed species known to occur in both poles is the giant squid *Architeuthis dux*. No cuttlefish species occur in either polar region. The cephalopod fauna of the Arctic and Antarctic are different and have different origins: the Arctic fauna colonized the Arctic relatively recently (<12 000 years ago), whereas some of the Antarctic fauna has evolved *in situ* over the past 33 Ma (e.g., most octopods). Polar cephalopods prey on crustaceans, fish, and other cephalopods (including cannibalism), whereas predators include fish, other cephalopods, seabirds, seals and whales. Cephalopods from these regions are likely to be influenced by climate change: Arctic fauna is more subjected to increasing temperatures *per se*, with these changes increasing species ranges and probably their abundance. Antarctic species are likely to be influenced by changes in mesoscale oceanography, changes of position of oceanic fronts, changes of sea ice and increase of ocean acidification. Polar cephalopods may have the capacity to adapt to changes in their environment but more studies are needed. Indeed, considerable work is needed with respect to taxonomy, distribution and ecology of this group.

November 15, 1500

Which environmental variables have the most impact on South Africa's squid fishery in a changing climate?

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Loligo reynaudii or "Chokka Squid" supports a commercial jig fishery and is the 3rd largest fishery in South Africa. As a fast-growing species with a rapid population turnover, it is able to quickly respond to a changing environment. The fishery occurs mainly off the Agulhas Bank shelf— one of the most diverse, complex and highly variable environments with changing sea conditions expected to directly impact the resource. In this study, we investigate variables affecting the occurrence of chokka squid, and therefore have the most impact on the resource in a changing climate. The variables investigated included environmental (temperature, dissolved oxygen, turbidity, depth), time (time of day, season), location (longitude, region) and stock (adult and juvenile). Generalized additive models (GAMs) were used to test the effect of these covariates on data collected from routine research trawl surveys. Results show mean Loligo catches were highest in autumn in shallow waters and lowest in autumn in deep waters. GAM results showed that for all years, depth, total trawl catch, and most importantly, turbidity were significant covariates. Location, in terms of region was important for all categories but explained very little of the variation, while oceanographic province was important for all squid and adults but not juveniles. Temperature was a significant covariate for adult squid but not juveniles and oxygen was a significant covariate for juveniles but not adult squid. The final model showed that *Loligo* catches were highest between depths of 60 to 120m, with bottom turbidity of

November 15, 1530

Validation of cephalopod beaks to monitor Hg in the Ocean

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Although generally short-lived species, coleoid cephalopods bioaccumulate Hg in their tissues, reflecting the degree of contamination of their habitats. Therefore, they can serve as bioindicators for assessing and monitoring Hg contamination. Most studies on Hg concentrations in cephalopods are however limited to soft tissues of species targeted by commercial fisheries. Furthermore very few studies investigated Hg in cephalopod beaks. As beaks resist to digestion, stomach contents of cephalopod predators offer an access to a wide range of species from various habitats, including remote and deep ones, allowing to monitor Hg in the ocean at large spatial scales. In this context, we assessed the potential of cephalopod beaks as monitoring tissue for Hg contamination. To this end, beaks from several squid, cuttlefish, and octopus species (84 individuals from 6 species from temperate and tropical environments) caught from the wild were used. The results from beaks were compared to those from soft tissues (i.e., mantle muscle and digestive gland). Hg concentrations in beaks were always far lower than in soft tissues, likely as a consequence of their chitinous composition. Using HR-XANES spectroscopy, we showed that Hg in mantle muscles was mainly organic, which is bound to cysteine amino acids. In beaks, both inorganic and organic forms coexists, with iHg binding to sulphur residues but also to carboxyl and/or amine/amide groups. For all the species, there was no significant correlation between Hg concentrations in beaks and mantle size. In all species but *L. vulgaris*, Hg concentrations in beaks correlate with those in the mantle. In conclusion, even though Hg accumulation potential of beaks seems limited compare to soft tissues in cephalopods, they can constitute relevant index of Hg contamination as they well reflect Hg concentrations accumulated along by other tissues.

November 15, 1545

Global climate changes over time shaping the ecological niche of *Octopus insularis* Leite and Haimovici, 2008 in the Atlantic Ocean

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Species distribution modelling (SDM) is an important tool to describe ecological patterns and make future predictions as it identifies the effects of environmental variables on the species ranges and hence suitable habitat for its settlement. The Maximum Entropy approach (Maxent) was used to estimate a suitable climatic niche for *Octopus insularis* (88 presence data) and its potential geographical distribution under five different scenarios of global climate changes (LGM, MH, modern, 2100 and 2200). Six environmental layers (bathymetry, slope, sea surface temperature, salinity, chlorophyll, and potential of hydrogen) were chosen to model the modern suitable climatic niche of *O. insularis* and four variables (bathymetry, slope, SST, and salinity) were used to past and future scenarios. The modeling of the species distribution in different climatic scenarios showed good validation (AUC = 0.994) and pointed out an increase of the suitable niche for its establishment, from LGM up to future projections. The climatic niche dynamics over time was mainly influenced by the temperature and bathymetry. In the future projections, the availability of species suitable niche will potentially increase in Tropical Atlantic compared to the current distribution. In addition, the modeling showed the possibility of an expansion from the species actual range to Temperate Northern Atlantic, Temperate South America, and Temperate South Africa, which may cause potential threats, such as possible extinction of endemic species, habitat displacement of native octopuses, reorganizations in the trophic chain.

November 15, 1600

Metals in the pelagic squid *Nototodarus sloanii* (Ommastrephidae) from the Chatham Rise, New Zealand

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Ommastrephid squids are important within marine food webs as predators of fish and diverse invertebrates and as prey for marine mammals, fishes, birds and humans. They are also an important vector for both nutrients and contaminants. Evidence shows that squids can accumulate metals to concentrations that are hazardous to human health, and other predators. This study reports concentrations of As, Cd, Cu, Cr, Co, Ni, U, V and Zn determined by MP-AES in 11 tissues of the endemic ommastrephid squid *Nototodarus sloanii* from the Chatham Rise. The Chatham Rise is an elongated submarine platform East of New Zealand and one of the areas where this species is most heavily targeted by commercial fisheries. We have developed a model of metal localization which allows us to accurately estimate total body burdens of metals from measurements upon only two tissues. This allows us to make ecologically relevant inferences about trophic transfer of metals to predators, including humans. We discuss the implications for food security of this important fishery.

November 15, 1615

Recent decline in winter stock of Japanese flying squid, *Todarodes pacificus* related to climate change during winter-spring

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Japanese flying squid, *Todarodes pacificus* is a nerito-oceanic squid, which is distributed in the west of Japan in the Sea of Japan and the East China Sea as well as in the east of Japan in the Oyashio and Kuroshio current systems. There are 3 subpopulations with different peak spawning seasons and areas; summer, autumn and winter, of which the last two are the largest and commercially most important. Annual catches of *T. pacificus*, especially of the winter spawning population decreased during the cool regime period from the late-1970s to late-1980s, while Japanese sardine, *Sardinopsis japonicus* increased exponentially. After the late-1980s warm regime period, squid catch had increased and sustained the Japanese squid markets. Catch fluctuations of *T. pacificus* are similar with those of Jack mackerel, *Trachurus japonicus* and the Japanese anchovy, *Engraulis japonicus*. This phenomenon is called "species replacement with climate regime shifts between warm and cool period". Recently, after 2016 the catch of winter population has extremely declined while after 2010 the catch of Japanese sardine has increased, which is very similar to "species replacement phenomenon". We can map the inferred spawning grounds defined using the SST areas between 18-24ºC, especially between 19.5-23ºC and within a specific range of bottom topography (100-500m depth). Here we used the relative status of inferred spawning grounds to explain the recent winter stock decline and the possibility of species replacement to cool adaptive sardine. After the winter of 2016, the inferred spawning areas of the winter stock in the East China Sea shrank and reduced to the continental edge off the Kyushu Island and the Nansei Islands. The climate change after 2016 introduced anomalous cold water induced by a strong winter monsoon. Hence the recent winter stock decline is thought to have occurred because the inferred spawning areas in winter were covered by cold sea surface water below 18ºC, which severely affects the survival of hatchlings.

November 15, 1630

Ocean warming-induced range-shifting of potential habitat for jumbo flying squid *Dosidicus gigas* in the Southeast Pacific Ocean off Peru

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Climate-induced ocean warming may have significant influences on abundance and geographic distribution of fish species and cause range shifts and/or expansions in their habitats. The jumbo flying squid *Dosidicus gigas* in the Eastern Pacific Ocean is an ecologically and commercially important species. With 1-year short lifespan, *D. gigas* is significantly affected by climatic and environmental variability. This study used the logbook data of the 2011-2015 Chinese squid-jigging fishery off Peruvian waters, coupled with sea surface temperature (SST) data, to explore the variations in seasonal habitat suitability and habitat distribution pattern for *D. gigas* in the Southeast Pacific Ocean under five scenarios: SST in recent years (2011-2015) and with 0.5oC, 1.0oC, 2.0oC and 4.0oC increases in relation to the climate variability. A fishing effort-based habitat suitability index (HSI) model was developed to estimate habitat quality of *D. gigas* and spatial distribution of suitable and optimal habitats in relation to ocean warming. Results indicated that obvious seasonal variations were observed in the SST on the fishing ground of *D. gigas* with spatial variability. HSI modeling approach in this study essentially captured habitat characteristics of *D. gigas* from spring to winter during 2011-2015. SST increase scenarios revealed that seasonal habitat suitability of *D. gigas* significantly reduced, and the percentages of suitable (HSI≥0.6) and optimal (HSI≥0.8) habitats occupying the fishing ground dramatically decreased due to the rising SST. Moreover, an obvious southeastward movement was observed in gravity centers of *D. gigas* habitat under the above-mentioned five scenarios. Our findings suggested that ocean warming was likely to result in the shrinkage and southeastward range-shift in the potential high-quality habitats of *D. gigas* in the Southeast Pacific Ocean off Peruvian waters.

November 15, 1645

Is there a Risk to Humans from Consuming Octopus Species from Sites Polluted with Metals?

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Octopuses are a highly-demand resource for human consumption in the world. Although they can bioaccumulate high concentrations of metals (such as Cd, Co, Cu and Pb), the risk to human health associated to its consumption is poorly understood. Here we compiled literature data on metal levels in octopuses from eight locations in Europe and North America, each with different sources and levels of metal pollution, to evaluate: 1) the risk to human health through consumption of muscle tissue (mantle and/or arms) and digestive glands of *Octopus hubbsorum, O. vulgaris* and *Eledone cirrhosa*, and 2) the maximum allowable consumption rates of each tissue by species and location. The metal risk assessment was done according the guidelines of the United States Environmental Protection Agency, calculating the estimated daily intake, the target hazard quotient, the hazard index and the maximum allowable consumption rate per person (CRlim). The risk for muscle consumption was estimated using the average ingestion rate of cephalopods in each location, while the risk for digestive gland consumption was estimated assuming an intake of one gland per week, due to lack of official records. The results indicate the absence of human health risks from the consumption of muscle tissues. However, consumption of digestive glands of almost all species indicated a high risk to human health, which can be attributed to its high Cd levels, and is reflected in its low CRlim values, especially from octopuses captured at the most polluted sites (e.g., Santa Rosalia mining port in Mexico). In conclusion, even in contaminated sites the consumption of octopus muscle tissue can be consider as an issue of low concern, while the intake of digestive glands should be carefully monitored and its real rates of consumption must be studied. Additionally, this study presents maximum consumption data, which can be used as a guide to avoid health problems due to the consumption of octopuses.

November 15, 1700

Ocean warming along the coast of the Gulf of Alaska evidenced and tracked by a persistent range expansion of market squid, *Doryteuthis opalescens*

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Recent reports in the Gulf of Alaska (GOA) have shown anomalous sea surface warming with the onset of the "warm blob" in 2013 and continuing with a marine heat wave through 2015. Historically, the presence of market squid (*Doryteuthis opalescens*) in the Gulf associates with basin-scale oceanographic change. In 2015, market squid arrived in Southeast Alaska likely through waters advected by El Niño; this temporary range extension has been observed in the past. Market squid have been captured in low numbers and frequencies in NOAA Fisheries' periodic summer bottom trawl surveys, and in 2017 NOAA detected the highest frequency of occurrence (1.7% of stations) since the survey began in 1984. Other recent and persistent observations of market squid in the GOA is exceptional not only regarding its duration but also in its spatial extent. Their spawning distribution (e.g., inferred by eggs) accurately track warm hydrographic conditions for nearshore GOA ecosystems. Spawning events have been continuously reported in the Southeast Alaska since 2015 and have extended far into the GOA reaching Kodiak Island in late 2016. This extension is a key step towards their permanent residence in the GOA and highlights the effects of environmental forcing on the composition of forage species within the GOA coastal ecosystem.

Lightning Talk Introduction

November 15, 1730

The Quick and the Dead: Measuring the hydrodynamic cost of extinct ammonite shells

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When dinosaurs ruled the earth, ammonites ruled the seas. Despite their exquisite fossil record and central role in marine food webs, the basic swimming speeds and metabolic demands of these ectocochleate cephalopods remain controversial. We present experimental and simulation measurements of fluid drag incurred by different ammonoid shell shapes, to better constrain their ecology. We built shell models in open-source 3D software Blender: laser scans of specimens; idealized coils; and hybrids. Physical experiments placed printed models (medical resin, 5 cm) in a chamber with 10-50 cm/s flow. Simulations in Ansys FLUENT reproduced these conditions and extended the velocities to 1 cm/s. We tested five items to compare with published benchmarks: a sphere; *Nautilus pompilius*; and three fossil ammonites. Experimental drag measurements are noisy, but reproduce the rank and order of magnitude of published data. Simulated drag measurements are more refined, and in most cases matched the rank, slope, and value range of published data. We tested additional shell shapes and specimens to contour drag across the empirical fixed-frame Westermann Morphospace. We use drag coefficients to compare locomotion costs for a variety of scenarios, particularly the large simply-coiled shells that were abundant after the Triassic-Jurassic mass extinction (201 Ma). Results refine and expand previous interpretations. Drag at Reynolds numbers (Re) >1000 is most responsive to shell inflation. At lower Re, streamlined shells with high whorl expansion rates trade lower pressure drag for greater viscous drag. Exposure of central whorls in simply coiled serpenticones is negligible at low Re but causes linear drag increase at Re >2000. Large serpenticones (>10 cm) would be most efficient at very low speeds (<0.5 shell lengths/second). We conclude that metabolic efficiency, at the expense of a high maximum jet speed, was the most parsimonious selective pressure for these post-extinction species.

November 15, 1735

Adaptive Infrared Camouflage Inspired by Cephalopods

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Cephalopods (e.g. squid, octopuses, and cuttlefish) represents an exciting source of inspiration due to their visually stunning camouflage capabilities. By drawing inspiration from these unique marine invertebrates, we have developed and validated a new class of adaptive infrared camouflage platforms with an unprecedented combination of properties, including a simple actuation mechanism, low working temperature, tunable spectral range, weak angular dependence, fast response, stability to repeated cycling, amenability to patterning and multiplexing, autonomous operation, robust mechanical properties, and straightforward manufacturability. These findings may open opportunities for next-generation camouflage applications and other technologies that regulate infrared radiation.

November 15, 1740

How to expand your invertebrate brain: are stem cells key to solve the cephalopod enigma?

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Within the clade of mollusks, cephalopods have developed an unusually large and complex nervous system. The increased complexity of the cephalopod centralized "brain" parallels an amazing amount of seemingly intelligent behaviors that culminate in one class, the octopods. The mechanisms that enable evolution of expanded brains in invertebrates remain enigmatic. While expression mapping of known molecular pathways demonstrated the conservation of major neurogenesis pathways and revealed neurogenic territories, it did not explain why cephalopods could massively increase their brain size compared to other mollusks. Such an increase is reminiscent of the expansion of the cerebral cortex in mammalians, which have enlarged their number and variety of neurogenic stem cells. We hypothesize that similar mechanisms might be at play in cephalopods and that focusing on the stem cell biology during cephalopod neurogenesis might be a smarter strategy to uncover the mechanism that has driven cephalopod brain expansion.

November 15, 1745

Chromatophores in *Octopus vulgaris* paralarvae from the eastern Campeche Bank: intraspecific variation in their spatial arrangement

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Chromatophores patterns in paralarvae are considered an accurate taxonomic feature and their number and spatial arrangement have been regarded as species-specific. Hence, the aim of this work was to determine the presence of intraspecific variation in the number and spatial arrangement of *Octopus vulgaris* paralarvae chromatophores. Plankton samples were collected in the shallow sublittoral zone of the eastern Campeche Bank during 2016 and 2017. In the laboratory, paralarvae were identified to the species category and only those pertaining to *O. vulgaris* were retained for analysis (n=35). Using a stereomicroscope with a ZEISS Axiocam ERc 5s digital camera integrated, photographs of each paralarva were taken dorsally and ventrally, paying attention to the chromatophores. Using ZEN lite software, morphometric parameters of each paralarvae where taken. To display the size structure of the paralarvae population, only the mantle length (ML) was hereafter considered, obtaining as a result 7 size classes. Employing the Image-Pro Plus Software 7.0, ventral photographs were used to carry on Digital Image Processing and Analysis, determining the spatial arrangement of chromatophores in the ventral mantle, as well as the area, perimeter, and mean diameter of each one of them. The analysis was carried out for each ML size class. Paralarvae found were between 985 and 1893 μm in ML. The number of chromatophores in the ventral mantle ranged from 20 to 9. In the ventral mantel of paralarvae distances among chromatophore centroids in the size classes 984 to1124, 1405 to 1544 and 1545 to1684 μm differed significantly [$F(1, 28) = 8.36$, $p=0.0073$], [$F(7,129) = 3.00$, $p=0.0058$], [$F(10,150) = 2.98$, $p=0.0018$]. In conclusion, for at least the ventral mantle of *O. vulgaris*, significant intraspecific variation in the chromatophore spatial arrangement was observed, questioning if this spatial trait may be considered actually as an accurate taxonomic feature.

November 15, 1750

Construction of the brain atlas in the long arm octopus, *Octopus minor*

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Cephalopods have the most complex nervous system of all invertebrates, with large and specialized brain structures. They offer an important comparative insight into the understanding of human intelligence. To investigate the molecular and network mechanisms underlying cephalopod intelligence, the construction of a brain atlas that contains information on the organization of each brain region is fundamental. Here, we constructed sagittal and coronal serial sections covering the entire brain of adult *Octopus minor* (Sasaki). Sections were stained by Hematoxylin and Eosin (H&E) to visualize the cellular nucleus and subregions. Images of the serial sections were taken at 30–70-μm intervals for the sagittal plain and 40–80-μm intervals for the coronal plain. More than 70 subregions were identified on the delineation of representative H&E images. To establish the coordinates of each plate in this atlas, the midline point of the posterior end was selected as the fiducial point. We found that the brain represents the typical brain structure of the Octopodidae. A number of lobes are discriminated by the Hematoxylin-positive layer, of which the thickness and cell density are diverse depending upon the region. Although the individual difference in brain size according to the body weight should be considered by the readers, this atlas is the first step towards investigating the brain of *O. minor*, and we hope that cephalopod research is facilitated by this atlas.

November 15, 1755

Cephalopod Community Trophic Ecology in Relation to Body-size in an Oceanic Ecosystem

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Cephalopods are a critical component of marine ecosystems, globally abundant in coastal, oceanic and deep-sea ecosystems. Many aspects of their biology and ecology, such as voracious predation, rapid growth, and short life spans, suggest they are influential in the transfer of energy through marine food webs. However, we lack a detailed understanding of cephalopod food web dynamics, and it is vital to resolve cephalopod trophic connections in order to understand how this key group influences the structure and function of marine ecosystems. We used nitrogen stable isotope analysis of a mesopelagic cephalopod community, sampled in the southern Tasman Sea using pelagic trawls from 0 - 500m depth, to assess their trophic ecology. We found a wide diversity of trophic positions held by the with groupings roughly aligned by morphological characteristics. The relationship between trophic position and body-size varied between species, particularly those with distinct morphological differences. These results will provide the first steps to including a trait-based cephalopod community in trait and size-structured ecosystem models. These models are increasing in complexity and power, offering a powerful means to predict abundance and biomass, which will enable us to better understand the role of cephalopods in the structure and function of marine ecosystems.

November 15, 1800

Clinal descent into the deep sea by octopuses of Graneledone

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Octopuses of Graneledone are known from nearly every ocean basin in the world. In general, a single species of the genus is recognized from each main ocean area. Recent work has supported the hypothesis that species can best be distinguished using the distribution of what can be the prominent skin warts or tubercles. However, variation in the number and prominence of those warts is amply apparent. Do they contain biologically significant information? To test the hypothesis that they do, we counted the skin warts in a line across head & mantle, the number of points in each wart, gill lamellae & arm suckers in 50 specimens from the northeast Pacific Ocean from 1116 to 2780 m depth. We analyzed these counts with head width as an indicator of size using Principal Components Analysis (PCA) to explore variation in the data & Canonical Correlation Analysis (CCA) to test the strength of resultant patterns. We also compared sequences of 12S, 16S & COI genes to test for divergence. Deep specimens had fewer gill lamellae and suckers and more, more prominent warts than did shallow specimens. The depth groups were genetically different, but not by very much; they appear conspecific. We thus discovered a depth cline. Why does the cline exist? We suggest that food scarcity at depth may limit size & sucker number; more gill lamellae in the shallowest octopods may help them survive near the lower limit of the oxygen minimum zone; under higher hydrostatic pressure, embryos may consume more energy; smaller hatchlings grow into smaller adults with fewer suckers & gill lamellae.

November 15, 1805

Proposal for a protocol to document cephalopod new records

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I believe that all teuthologists involved in the preparation of cephalopod species lists – either general or limited to a particular taxon, either worldwide or regional – have incurred in problems such as implausible geographical and depth distributions (e.g. an Arctic species reported in the Mediterranean Sea; a coastal one allegedly collected at 700 m depth) and/or nominal species impossible to recognize as established specific entities. Unfortunately, still today it is customary, especially in fishery papers, to produce lists of specific names without providing any documentation to back the animals identification, not even in case of new records for a given area and/or problematic species. Hence, the need to set a system of rules to report cephalopod "first" records, which include designation and deposit of voucher specimens in curated collections with proper information; inclusion of photographs, meristic and morphometric characters, and, whenever possible, DNA sequences (Barcoding) of the specimens; reference to literature used to identify specimens. Additional rules will concern the occurrence of cephalopod remains in the stomach contents of teuthophagous predators and the stranding of cuttlebones and ram's horn shells. This protocol – especially when endorsed by CIAC which has always shown an interest in teuthogeography – will take care of the appropriateness of future cephalopod records. As for puzzling records from the past, i.e. implausible distributions and unresolved nominal species, the production of regional reference catalogues where all pending matters are cleared out is recommended. In this respect, it may be pointed out that accurate catalogues play an important role to trace the teuthogeographical changes caused by bioinvasions and ocean warming.

November 15, 1810

A new species of dumbo octopod from the North Pacific

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During the deep sea expedition SO-249 BERING in 2016, several adult octopodiform specimens were collected from the North Pacific, including from the Bering Sea. One of these specimens was an excellently preserved cirrate individual collected on Tenji seamount, which forms part of the Emperor Seamount Chain. The sample was photographed and measured on deck and DNA sampling was carried out prior to fixation. The specimen was tentatively identified as belonging to the genus *Grimpoteuthis*, while further analyses on land showed that the specimen can actually be assigned to a new species of *Grimpoteuthis*. Due to the scarcity of this particular material, a three-dimensional imaging technique (magnetic resonance imaging) was used to extract as many morphological characters as possible without the need to dissect the specimen. The reconstructions of the internal anatomy in combination with molecular barcoding data unequivocally show that this single male specimen is the first representative of a new species of dumbo octopus in the North Pacific. The characters used to erect this new species are presented and compared with characters of other cirrate taxa known to occur in the North Pacific.

November 15, 1815

Valuing information for sustainable squid fisheries

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Thereliance on fishery-dependent data can lead to biased or very imprecise estimates of fish populations. This can lead to management measures that are overly cautions or overly risky. Independently-gathered data is crucial to reducing bias and imprecision in population estimates. Before managers can move forward with fishery-independent data gathering, they need some mechanism in which to assess the risks, rewards, and costs of investing in a scientific population survey.

This project develops anestimator for which managers can assess the value of fishery-independent data for California market squid (*Doryteuthis opalsecens*). Current harvest of market squid is limited to a fixed annual maximum catch limit. There is no adaptive management of market squid, which are known to exhibit large annual fluctuations in biomass (eg. Dorval et. al, 2013), and are sensitive to environmental variation, performing poorly in El Niño years (eg. Koslow and Allen, 2011).

The methods examine the precision different survey types would add to population estimates through simulation. The value of fishery-independent information is assessed via a comparison of the cost to implement each survey type versus potential increase in fishery revenue from more precise population estimates. The risk of over-exploitation by maintaining the status quo versus improvements to long-term sustainability measures due to improved population estimates are also presented.

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November 15, 1820

Recruitment failure of the tropical arrow squid *Doryteuthis plei* linked to anomalous oceanographic conditions during the spawning season

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Recruitment failure is a remarkable phenomenon in most cephalopod fisheries. This is due their short life cycle associated to semelparity, and the reliance on paralarval survivorship to ensure spatial and temporal continuity of cephalopod populations. Recruitment failures have been detected at least four times over the last 17 years for the tropical arrow squid (*Doryteuthis plei*) off southern Brazil (22–29°S). Bearing in mind the vulnerability of paralarvae to unsuitable environmental conditions, this study aims to investigate the possible causes of such failures using publicly available catches (tonnes landed) and satellite products data (namely, sea surface temperature, sea surface salinity, sea surface chlorophyll and wind fields) recorded on shelf waters off southern Brazil. Based on a 9-month life cycle, it is hypothesized that anomalous abiotic conditions during austral autumn-winter may lead to recruitment failure in the following spring-summer (the main harvest season for the species). Results will allow a better understanding on the causes of natural population fluctuations for the species.

November 15, 1825

Octopus genome provides insights into evolution for physiological adaptations and heart development

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Octopus minor also known as the common long-arm octopus, is a benthic littoral species and their habitats are mudflat of coastal regions and is relatively small and possesses a shorter life cycle (approximately 1 year), thinner arms, and a lower ratio between head size and arm length compared to those of other octopus species. The species is widely distributed in Northeast Asia, particularly in coastal regions of South Korea, China, and Japan. The ability to adapt its morphology and behavioural repertoire to diverse environmental conditions makes the species a promising model to understand genomic adaptation and evolution in cephalopods. Especially, cephalopods are the only mollusks with a closed circulatory system and have two hearts, called branchial hearts, pump blood through each of the two gills, and the third heart, called system heart, pumps blood through the rest of the body. However, the evolutionary background for this is unknown. To understand how the genome of *O. minor* evolves in response to harsh environmental conditions and evolution-development of three hearts, we sequenced its genome. We found that the molecular factors of heart development is likely regulated by expanding the number and compositions of some genes, such as Gata, Hand, Mef, Nkx, and Tbx, which are important for heart development. The *O. minor* genome assembly provides a valuable resource for understanding octopus genome evolution and the molecular basis of heart development.

November 15, 1830

Measuring anesthesia effects on the central and peripheral nervous system of cephalopods

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Cephalopod are included in research animal welfare regulations in many countries, and a recent position statement by AAALAC suggests regulatory efforts are occurring in the USA as well. Humane and effective anesthesia is a basic requirement of research animal welfare, however, there has been scant evidence that agents believed to act as anesthetics produce effects beyond immobility. Recently, we have demonstrated that two of the most commonly used agents in cephalopod general anesthesia, magnesium chloride and ethanol, are capable of producing strong and reversible blockade of afferent and efferent neural signal; thus they are genuine anesthetics, rather than simply sedating agents that render animals immobile but not insensible. Additionally, we demonstrated that injected magnesium chloride and lidocaine are effective local anesthetic agents. This represents a considerable advance for cephalopod welfare. Using a reversible, minimally invasive recording procedure, we measured activity in the pallial nerve of cuttlefish and octopus during induction and reversal of putative general and local anesthetic agents. We describe the temporal relationship between loss of behavioral responses (immobility), loss of efferent neural signal (loss of "consciousness") and loss of afferent neural signal (anesthesia) for general anesthesia, and loss of afferent signal for local anesthesia. Both ethanol and magnesium chloride were effective as bath-applied general anesthetics, causing immobility, complete loss of behavioral responsiveness and complete loss of afferent and efferent neural signal. Subcutaneous injection of either lidocaine or magnesium chloride blocked behavioral and neural responses to pinch in the injected area. Based on these data, we conclude that both magnesium chloride and ethanol can function as true general anesthetic agents, and lidocaine and magnesium chloride can function as true local anesthetic agents for cephalopod molluscs.

November 15, 1835

Captive breeding of the oval squid (Aori-ika; *Sepioteuthis* sp.)

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Genus *Sepiotheuthis* contains species that are both commercially and scientifically important. Culturing attempts have been ongoing since the mid 1980s. In the present study, we examine possible effects of different tank types and sizes, flow rates, and feeding regimes on survival rates of the second generation oval squids in our laboratory. Squid eggs were initially collected in the tidal zone of Okinawa Island in July and August 2017. Animals were hatched and raised in a flow through salt water system until they reached sexual maturity. They were then housed in large flow through tanks in groups of 7 to 12 animals. In order to induce egg deposition, tree branches were introduced into the home tanks of these squid groups. Before hatching, the eggs were transferred into different tanks ranging from 50 to 700 liters. We raised the animals in tanks with different interiors from bare to enriched. Primary illumination was provided using an automatic timer to control T8 light bars of a daylight emission spectrum on a 12:12 night day cycle. Two different feeding regimes were carried out. Animals were fed live crustaceans and dead fish three times a day (~10:00, ~12:00, and ~17:00) with some being fed once more at ~22:00. Survival rates for each tank were recorded on a daily basis.

November 15, 1840

Can *Sepia pharaonis* be reared on sea roach, *Ligia exotica*, from the first day after hatching?

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The pharaoh cuttlefish, *Sepia pharaonic*, is one of the largest and economically important sepiid cuttlefish in the tropical Pacific region. The species became a focus for aquaculture due to its reproducibility and high tolerability to culture conditions, hence a high feasibility for commercial scale culture. However, innate feeding on specific live prey during the early phase of their life is the bottleneck. In nature, cuttlefish hatchlings prey preferably on live crustacean species than fish. The use of live Pacific white shrimp of *Litopenaeus vannamei* of appropriate size is recommended during the hatchling stage and the weaning to frozen shrimp would be performed after 20–30 day after hatching (DAH) depending on the temperature. Both the availability of live shrimp and their price seriously affect the profitability of cuttlefish farming. Sea roach *Ligia exotica* belong to a marine isopod crustaceans. It is found in various parts of coasts living on rocks and harbor walls just above high water mark. *Ligia exotica* can grow to 4 cm (1.6 in) in length. We observed that *Sepia pharaonic* hatchlings forage *Ligia exotica* ferociously in the cement pools and even some individuals bigger than hatchlings cannot escape. Since *Ligia exotica* is both a grazer and a scavenger on plant remains and detritus, and is easily adaptive to the environment, the cost of being cultured should far less than that of live shrimp. Currently we are evaluating the nutritional values of *Ligia exotica* and is going to conduct a practical feeding experiment in the coming spring from the first DAH of cuttlefish hatchlings. The content of crude protein of *Ligia exotica* is 43.14% (51.64% of krill meal), while crude lipid is 8.83% (7.81% of krill meal). According to the amino acids score and chemical score, the first limiting amino acid of *Ligia exotica* is isoleucine. The Essential Amino Acids Index (EAAI) is 39.61 while EAAI of krill meal is 49.89. The ligia culture method is developing at the same time.

November 15, 1845

An insight into the Chinese traditional seafood market: Species characterization of cephalopod products by DNA barcoding and phylogenetic analysis

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Squids, cuttlefish and octopus are used for the preparation of traditional products sold on the Chinese market without a specific denomination. In this study DNA barcoding and phylogenetic distance analysis of COI and 16S rRNA genes' fragments were used to characterize the most commonly processed species in dried whole, grilled shredded and salted cephalopod preparations. Ninety-five products (23 sold as cuttlefish, 4 as octopus and 68 as squid) purchased in Chinese local markets were analyzed. Overall, the study identified 10 different species: *Sepia pharaonis, S. esculenta, S. recurvirostra, S. lycidas* in cuttlefish; *Amphioctopus marginatus* in octopus; *Uroteuthis chinensis, U. edulis, Ommastrephes bartramii, Illex argentinus* and *Dosidicus gigas* in squids. This latter species, characterized by a low commercial value, was found in the majority of the samples (50.5%) and in all the shredded products. By comparing the molecular results with the declared macrocategory (cuttlefish, octopus and squid), two cases of misdescription were pointed out, involving shredded cuttlefish and octopus which were identified as *D. gigas*. Our results are of particular interest in the light of the scarcity of data regarding the identification of cephalopods on international markets and considering that China is one of the leading cephalopod producing countries.

Session 9: Physiology

November 16, 0800

Keynote Speaker: Gretta Pecl

November 16, 0830

Octopamine in octopus brain

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Octopamine has been originally identified in the 1948 in the salivary glands of the cephalopod mollusc *Octopus vulgaris*. It is a monoamine found in plants, invertebrates and vertebrates. While it only occurs as a trace amine in vertebrates, octopamine (OA) is one of the most abundant biogenic amines occurring in the nervous system of invertebrates. It is considered the invertebrate counterpart of noradrenaline. OA and/or its receptor have been identified in a large number of invertebrate species, and play a role in several physiological and behavioral processes acting as neuromodulator, neurotransmitter, or neurohormone by prompting the whole organism to a "dynamic action". Interestingly, while scientists largely explored OA presence and functioning in many taxa, the study of its role in octopus, where it has been originally discovered, and in other cephalopods has been neglected, and a detailed account of its distribution has been missing. After more than 60 years, we explored the octopus brain and characterized, for the first time, octopaminergic neuronal populations in the brain lobes of O. vulgaris by immunofluorescence labeling. Selected areas are identified of positive cells, and an intricate network of OA-positive fibers. These are mapped onto TH positive neurons we identified providing for the first time a detailed overview of OA distribution in the octopus brain. A possible role of OA for the visual and chemo-tactile sensory-motor processing is also provided by functional comparison with other invertebrates.

November 16, 0845

Comparative growth increments of gladius surface and cross-sections to estimate *Sthenoteuthis oualaniensis* age

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The age of the purpleback flying squid (*Sthenoteuthis oualaniensis*) has been widely studied over its geographic range based on hard structure analysis, especially the statolith. In this study, we propose an additional method for estimating the age of *S. oualaniensis* using growth increments in the gladius. Our results show regular growth increments in the proostracum dorsal surface and stem cross-sections. Linear regression analysis of the relationship between statolith and gladius readings showed the formation of increments in stem cross-sections to be daily, assuming that the formation of increments is daily in statoliths. A progressively more faint microstructure in the half posterior of the gladius possibly explains why the number of growth increments in the proostracum dorsal surface is less than the statolith-determined age. The low average percentage error (APE) and coefficient of variation (CV) indicate that our modified processing and counting methods are reliable. We believe that using growth increments in the gladius stem cross-section is a feasible alternative tool for determining the age of *S. oualaniensis*, whereas using growth increments in the proostracum dorsal surface is more informative for reconstructing ontogenetic life history.

November 16, 0900

Imaging regeneration: exploring the unseen in the adult common octopus, *Octopus vulgaris*

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We studied nerve regeneration in *Octopus vulgaris* with a focus on the pallial nerve which is involved in the control of mantle muscle contraction and body patterning. Complete transection of one of the pair of nerves is followed by full regeneration and functional recovery, both achieved in relatively short time. We applied for the first time to any cephalopod a combination of imaging approaches including multiphoton microscopy and neural tracing method to identify regenerative tissues and pathways involved in regeneration

Transected pallial nerves and contralateral nerve as control, were processed following Imperadore et al (2017) and imaged for multiphoton microscopy (MP) or used to trace neural connections. MP allowed label-free identification of structures, overcoming the lack of commercial markers designed for this taxon. In particular various optical contrast methods have been utilized: coherent anti-Stokes Raman scattering (CARS), endogenous two-photon excited fluorescence (TPEF), and second harmonic generation (SHG). We detected cells and structures often not revealed with classical staining methods. CARS highlighted the involvement of hemocytes in building up scar tissue; CARS and TPEF facilitated the identification of degenerating fibers; SHG allowed visualization of fibrillary collagen, revealing the formation of a connective tissue bridge between the nerve stumps, involved in axons guidance. Trough tracing methods, injured fibers were tracked and seen to cross the injury site, to re-innervate target tissues eventually resulting in functional recovery. The combination of the two approaches provided an unprecedented set of information on neural pathways allowing to identify selected cellular components involved in degeneration and regeneration.

November 16, 0915

Identification, Characterization, and Expression Analysis of a FMRFamide-Like Peptide Gene in the Common Chinese Cuttlefish (*Sepiella japonica*)

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The peptide FMRFamide is one of the well-known peptides involved in multiple physiological processes in the phylum Mollusca. In this study, a FMRFamide gene (GenBank accession No. KJ933411) was identified in a cuttlefish species called *Sepiella japonica* and was designated as SjFMRFamide. The total length of the SjFMRFamide sequence was found to be 1880 bp while the open reading frame contained 996 bp encoding a protein of 331 amino acid residues with a predicted isoelectric point (pI) and molecular weight (MW) of 9.18 and 38.8 kDa along with a 333 bp 5'-untranslated region (UTR) and 551 bp 3'-UTR. The deduced SjFMRFamide precursor protein contains one signal peptide and expresses four kinds FMRFamide-related peptides including a single copy of FLRFamide, ALSGDAFLRFamide, and FIRFamide and multiple copies of FMRFamide. Results of phylogenetic relation analysis strongly indicated that the sequence of this gene shares high identity with the genes of known FMRFamides. Spatial expression analysis indicated the highest mRNA expression of SjFMRFamide in the brain of male and female cuttlefishes among the eight tissues analyzed. An in situ hybridization assay of the brain indicated that SjFMRFamide was transcribed in several functional lobes, which suggests that it might be related to many physiological regulatory mechanisms. This is the first study describing FMRFamide in S. japonica and the results may contribute to future studies of neuropeptide evolution or may prove useful for the development of aquaculture methods for this cuttlefish species.

November 16, 0930

Embryonic Development of the Central Nervous System of Green Octopus *Octopus hubbsorum* (Berry, 1953)

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The nervous system of cephalopods is considered the most complex within the invertebrate groups, although there are few studies focused on the embryonic development of the central nervous system (CNS) of octopods. On the other hand, octopuses display two types of life strategies known as merobenthic and holobenthic, which show some differences during the embryonic development in general, as well as of the CNS. *Octopus hubbsorum* is in important marine resource in the northern Pacific, but there is a gap of information regarding embryonic development. That is why the object of this study is to histologically describe the development of the CNS of Octopus hubbsorum during each embryonic stage. Twenty-two series of *O. hubbsorum* egg clutches were characterized according to their embryonic stage and processed with the paraffin embbeding protocol, applying hematoxylin-eosin, nissl and Cajal's silver nitrate stains. *O. hubbsorum* presents 20 stages of embryonic development, which are described, and the development of the nervous system can reportedly be seen since stage 9, but in this study its described from stage 11 due to the absence of stage 9 embryos. The central nervous system (CNS) is divided into four principal domains: the supra and subesophageal masses, the optic lobes, and the optic gland. It is derived from the ectoderm and presents three phases of development: the ganglionic accumulation, lobe differentiation and the neuropil increment. The ganglionic masses assemble into a cluster that encircle the inner yolk and the foregut anlage which gradually forms the four domains. The paralarvae that are ready to hatch show almost the same arrangement reported in the adults, except the lobes are much smaller.

November 16, 0945

A novel approach to age estimation of *Sepia officinalis* (Linnaeus 1758)-analysis of beak microstructure

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Precise and reliable estimation of age, growth and maturation is a very important part of the stock assessment. Management of *Sepia officinalis* stocks is not an exception. At present, different methods of cuttlefish ageing are used, however some of them provide rather unreliable results, while others are not suitable for mass studies. To support the stock assessment of the common cuttlefish a new method was tested. Preliminary studies showed that beaks could be a promising tool for age estimation of cuttlefish. Two basic methodological problems were addressed in the present work: periodicity of increment deposition and the time of the first increment formation. Cuttlefishes of known age sacrificed at 1, 10, 20, 30, 75, 100, 115, 130, 145, 160 and 175 days old. Their beaks were extracted, measured and preserved in ethanol. Lateral wall surfaces (LWS) and rostral sagittal sections (RSS) of beaks were analyzed, following the standard methods previously developed and validated for *Octopus vulgaris*. It was found that preservation in alcohol lowers readability of beak sections. Nevertheless, the majority (over 70%) of specimens from 10 to 175 days the RSS were successfully prepared and read. RSS of 1 day-old cuttlefishes were damaged and unsuitable for reading, although some increments were observed on the LWS. The study showed that both LWS and RSS provide a precise and accurate estimation in animals from 10 to 30 days-old (hatchling stage). The readability of increments on LWS and upper RSS decreased with age and in specimens older than 30 days usability of LWS become limited. The rostrum tip in the oldest animals undergoes the erosion in the axis area. However, more increments could be counted in the hood (above) and crest (below) areas to complete the reading. Thus, the hypothesis of 1 day - 1 increment was preliminarily confirmed in animals from 10 to 175 days old, although additional studies on tip erosion, increment formation at time of hatching, and coverage of older ages are still necessary.

November 16, 1015

Exploration of statolith shape variation in jumbo flying squid, *Dosidicus gigas*, based on wavelet analysis and machine learning methods for stock class

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The statolith in cephalopods has a stable morphology and contains important ecological information. Influenced by genetic structures and environmental variability, statolith shapes often vary among different stocks and are ideal indices for stock discrimination. A wavelet analysis is used to explore the statolith shape variations in *Dosidicus gigas* among four geographic stocks obtained by Chinese jigging fleets in the eastern tropical Pacific Ocean (ETP). In addition, machine learning methods are compared with traditional classification methods to improve the stock classification results of *Dosidicus gigas*. According to our analyses, statolith shapes of *D. gigas* differed significantly among the four stocks. Wavelet coefficients extracted from the statolith images by computer software were used to reconstruct the mean statolith shape for every stock. The rostrum and wing of the statolith are two main components determining the variances among stocks. Canonical analysis of principal coordinates (CAP) clearly separated Costa Rican (CR) from other stocks. Machine learning methods performed better than the traditional method of statolith shape classification. The results in this study supported the geographical separation of *D. gigas* stocks (Costa Rican and equatorial stock in the northern hemisphere and Peruvian and Chilean stock in the southern hemisphere) reported in previous studies. Wavelet analysis is an appropriate method for stock classification and machine learning methods can effectively improve the classification accuracy and is a promising method for determining the stock structure.

November 16, 1030

Do squids breathe through their skin?

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In 1988, a hypothesis was put forth that squids obtain a fairly large portion of their oxygen via simple diffusion across the skin rather than across the gills. Although this idea has support from indirect evidence and has been generally regarded as valid for the three decades since, no empirical examinations have been conducted to assess the validity of this hypothesis. In this study, we examined this claim via manipulative experiments on two squid species, *Doryteuthis pealeii* and *Lolliguncula brevis*, by mechanically separating the oxygen uptake across the gill and skin of an intact animal using respirometry in a divided chamber. We found that squids at rest obtain no more than 12% of their oxygen cutaneously, sufficient to provide some oxygen to the skin tissue locally, but with the majority of oxygen entering via the traditional branchial pathway. We furthermore re-examined the indirect evidence that has supported the cutaneous respiration hypothesis and discuss its implications in a non-cutaneous context.

November 16, 1045

Unlocking the cephalopod brain

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Cephalopods have the most complicated central nervous system of all invertebrates at both anatomical and functional levels as demonstrated by the pioneering neuroanatomical work of Cajal and Young decades or indeed more than a century ago. Here using the new technique of diffusion magnetic resonance imagery (dMRI) focused on the squid brain and validated with the same traditional Golgi techniques used by Cajal and Young, we shed new light on this remarkable structure and uncovered a far more detailed and complete view of the squid brain. This first squid tractography, leading to a squid connectome combined with better visualisation of individual lobes and the associated structural connections, allows us to propose new structural and functional circuits and examine previously described ones. As with vertebrate connectomes, this 'circuit diagram' for the squid suggests a set of functional morphology interactions and leads to new predictions at the behavioural level. The brains of all cephalopod groups, octopus, cuttlefish, squid and nautilus, are built around a circum-oesophageal set of ganglia or lobes that have expanded dramatically along with the visual and limb-based tactile capability that sets cephalopods apart from other molluscs. These advances have allowed the astonishing feats of camouflage, colour change, manipulation and intelligence in the cognitive realm that cephalopods are capable of and that continue to fascinate both scientists and the public.

November 16, 1100

Behavioural Study of Contrast Sensitivity in Octopus: Evidence of Lateral Inhibition

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Cephalopods, like vertebrates, have a camera-type eye which provides them with a highly developed sense of vision. However, the optic nerve of cephalopods is made up by the axons of the photoreceptors. Because the optic nerve limits the amount of information that can be sent at any given time, processing of visual information in vertebrate retina is thought to be aimed at decreasing redundancy of the message sent via the optic nerve from eye to brain in an effort to increase efficiency of visual processing. This reduction of redundancy is achieved by lateral inhibition in vertebrate retinal ganglion cells and is manifested in decreased contrast sensitivity to low spatial frequencies. In octopus, this lateral inhibition would not be expected to occur because its photoreceptors are directly connected to the optic lobe. We presented octopus (*Octopus tetricus*) with sinusoidal gratings of different spatial frequencies ranging from 0.05 to 12 cycles per degree (cpd) and measured behavioural contrast sensitivity using a fixation reflex. We show that the contrast sensitivity is minimum at 12 cpd, reaches its maximum at 0.3 cpd, and decreases to approximately half of the maximum value at the lowest spatial frequency. This decrease in contrast sensitivity to low spatial frequencies indicates that lateral inhibition is present in octopus. Because octopus does not have retinal ganglion cells, we infer that the lateral inhibition occurs in the octopus brain and therefore is not explained by the reduction of redundancy hypothesis. Alternatively, the lateral inhibition may occur by direct interaction between photoreceptor cells in octopus eye.

November 16, 1115

Molecular dissection of Octopus memory circuits- thinking outside of the skull

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Cephalopods (Octopus, Squid, Cuttlefish, Nautilus) exhibit behavioral flexibility that rivals that of many mammals. The Vertical Lobe (VL), a cephalopod-specific memory circuit, parallels mammalian analogues (hippocampus) in cell number and function, but evolved independently in molluscs. We integrated Next-gen sequencing technology and bioinformatic analyses, followed by anatomical validation, to identify molecular maps of signaling molecules implemented in cephalopod memory circuits. We produced transcriptomes of various *Octopus* neural tissues (VL, CNS, SFL, Arms), and individual cells from sub-populations within the VL circuit: Amacrine (Am) Interneurons and Large Efferent (LE) Neurons. We mapped these transcriptomes to a publically available *Octopus* genome and our gastropod transcriptomes including Aplysia. We identified 16,194 transcripts in the VL and found ~25.5% appear to be cephalopod-specific. Next, we used targeted and unbiased computational predictions and manual annotation to identify putative signaling molecules form the VL transcriptomes. We have cloned and localized 30 neuropeptides (NP) to components of the VL circuit. NPX3 is abundantly expressed in the cell bodies of the SFL, where the VL circuit originates and NPX1 & 2 localize to the AM cell bodies of each VL gyrus. Among the 7 NPs that localize to the VL LE neurons, 2 unique cephalopod NPs, NPX4 & 5, are abundant in *Octopus*, but absent in the ancestrally branching Nautilus (which lacks a VL). This expansion of signaling molecules in the VL circuit may be a key feature of unique memory systems of cephalopods, implying extensive parallel evolution of cephalopod brains and memory circuits.

November 16, 1130

Neuronal conversations – The brain of *Octopus vulgaris*

Ruth Styfhals[1], Giovanna Ponte[1], Oleg Simakov[2], Remo Sanges[1], Eve Seuntjens[3], Graziano Fiorito[1]

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The creation of a functional neural network that consists out of more than 500 million neural cells is an impressive feat for a mollusc. However, the mechanism of how *Octopus vulgaris* can generate such a high level of complexity remains largely unexplored. Vertebrates and invertebrates were presumed to utilize diverse molecular mechanisms for neuronal recognition, respectively the protocadherin gene family (Pcdh) and the Down syndrome cell adhesion molecules (Dscam). Recently, 168 putative Pcdh were found within the genome of *O. bimaculoides*, which lead to the hypothesis that cephalopods use a vertebrate-like system to create a complex brain. To study the role of these molecules in brain complexity and neural plasticity, we carried out an in silico analysis of the *O. vulgaris* transcriptome to identify Ov-Dscam and Ov-Pcdh. A significant number of Ov-Pcdh (>50) and one Ov-Dscam were identified within the transcriptome, which supports the hypothesis that *O. vulgaris* developed the same system as vertebrates. Our data support the hypothesis that the expansion of the Pcdh gene family occurred before speciation in octopods. By analysing the expression patterns of these molecules in brain areas where given neuromodulators have been identified, we were able to characterize neuronal populations. The expression patterns of these molecules within the octopus brain seems to be in accordance with the expression observed in vertebrates; some Ov-Pcdh exhibit localized expression patterns while others are more broadly expressed throughout the nervous system. Neuronal recognition is crucial during nervous system development, but the continued expression of these molecules within various parts of the adult brain suggests that protocadherins play an important role in neuronal plasticity.

November 16, 1145

Catch and Release: differential hemocyte binding in the squid-vibrio symbiosis

Sarah J. McAnulty, Spencer V. Nyholm

The Hawaiian bobtail squid, *Euprymna scolopes*, engages in a specific symbiosis with *Vibrio fischeri*, a bioluminescent bacterium that lives in a specialized light organ. The cellular immune system of *E. scolopes* consists of one type of blood cell, the hemocyte. Previous work showed that hemocytes from wild-caught squid bind *V. fischeri* and non-symbiotic *Vibrio harveyi*, at low and high levels respectively. Treating the adult light organ with antibiotics to remove the symbiont significantly increased hemocyte binding to *V. fischeri*, suggesting that the presence of *V. fischeri* influences hemocyte recognition of symbiotic bacteria. In order to better understand hemocyte-bacteria binding dynamics, hemocytes from wild squid were isolated and co-incubated with symbiotic and non-symbiotic strains of bacteria and polystyrene beads. Live cell imaging by confocal microscopy was used to quantify the binding and release of bacteria. These experiments revealed that over a period of 30 minutes, all tested bacterial species were bound, but hemocytes released a significantly higher percentage of a symbiotic strain of *V. fischeri* ES114 (60%) compared to non-symbiotic *V. fischeri* strain MJ11 (24%), *V. harveyi* (12%) and polystyrene beads (2.7%). To better understand whether long-term colonization by *V. fischeri* influences hemocyte-bacteria binding dynamics, squid were raised to sexual maturity in the presence (symbiotic, n=3) or absence (aposymbiotic, n=4) of *V. fischeri*. Hemocytes from symbiotic raised adults bound and released *V. fischeri* and *V. harveyi* similarly to wild-caught squid (72%), whereas hemocytes from aposymbiotic raised animals released significantly fewer *V. fischeri* (28%). Overall, these results suggest that long-term colonization influences hemocyte recognition of *V. fischeri* and that both host and symbiont mechanisms ensure that the light organ symbiont evades clearance by the host's cellular innate immune system.

November 16, 1330

Comparative evaluations of acid/ammonium transport machineries in branchial epithelium of cephalopods

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Advanced neural systems and athletic swimming mode enable cephalopods behave as apex predators in ocean system. According to their habitats and life styles, they could be divided into crawl and jet-propulsion behavior patterns; however, those locomotion patterns are inefficient and highly energy-consuming appearances. And energy source in cephalopods primarily arises from protein catabolism and may cause metabolic ammonia accumulation and acid-base disturbances. In this study, we observed that common octopus (*Octopus vulgaris*) would accumulate ammonia in blood 10 times higher than bigfin reef squid (*Sepioteuthis lessoniana*). Moreover, ammonia transport rate (circulating from ctenidial veins to body tissue) in *S. lessoniana* is two times faster than that of *O. vulgaris*. Inhibition of apical and basolateral Na+/H+ exchangers (NHEs) by ethylisopropyl amiloride (EIPA) showed significant disruption of H+ and ammonia excretory processes in gills of common octopus but not in squid. However, inhibition of vacuolar-type H+-ATPase (VHA) by KH7 significantly disrupts H+ and ammonia excretion in gills of both animals. Accordingly, jet-propulsion squids prefer active transport for acid/NH4+ transport, while crawling octopus utilize both passive and active transport for homeostasis. Consequently, squid may have developed more efficient ammonia-excretory machinery than octopus to avoid nitrogenous toxic effects in blood.

November 16, 1345

The cardiac and metabolic effects of taurine in the cuttlefish, *Sepia officinalis*

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Taurine is the most abundant amino acid in the blood of the cuttlefish, *Sepia officinalis*, where levels can exceed 200 mmol/L. In mammals, intracellular taurine modulates cardiac Ca2+ handling and carbohydrate metabolism at much lower concentrations but it is not clear if it exerts similar actions in cephalopods. Blood Ca2+ levels are high in cephalopods and we hypothesized that taurine would depress cardiac Ca2+ flux and modulate contractility in systemic and branchial hearts of cuttlefish. Heart performance was assessed with an in situ perfused systemic heart preparation and contractility was evaluated using isometrically contracting systemic and branchial heart muscle rings. Stroke volume, cardiac output, and Ca2+ sensitivity were significantly lower in systemic hearts perfused with supplemental taurine (100 mmol/L) than in controls. In muscle ring preparations, taurine impaired relaxation at high contraction frequencies, an effect abolished by supraphysiological Ca2+ levels. Taurine did not affect oxygen consumption in non-contracting systemic heart muscle, but extracellular glucose utilization was twice that of control preparations. Collectively, our results suggest that extracellular taurine depresses cardiac Ca2+ flux and potentiates glucose utilization in cuttlefish. Variations in taurine levels may represent an important mechanism for regulating cardiovascular function and metabolism in cephalopods.

November 16, 1400

Ontogenetic changes of *O. maya*: morphology, respiratory metabolism and antioxidant defense mechanisms

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Octopus maya is an endemic species from the Yucatan peninsula, Mexico, which presents holobenthic embryonic development. Characterizing the biology and biochemistry of embryonic forms provides information on how organs develop and activates the physiological mechanisms that will prevail for the rest of their lives. The importance of aerobic respiration lies in the efficiency and production of energy, translated into ATP which is traditionally measured as oxygen consumption (MO2). During respiration, reactive oxygen species (ROS) are produced, which react with biological macromolecules, producing structural cellular damage. Its effects are counteracted by the antioxidant defense system (ANTIOX). The objective of this work was to establish a solid baseline of the ANTIOX system, morphological changes and MO2 throughout the embryonic development of *O. maya* in order to have enough information to adequately evaluate, in the case of an environmental disturbance, the physiological changes that could occurs. It was observed that there is a very close relationship between organogenesis and the metabolism of embryos: at the end of the XV of development the enzymatic activity, the consumption of yolk, and the MO2 showed an evident increase, just when the activity of the systemic heart started. This dynamic reached its maximum just before the embryos hatching. The inverse relationship between oxidative damage indicators and development is evidenced. Results suggest that there is a direct relationship between the antioxidant response and the metabolic rate, indicating that the embryos have the capacity to control the effects of ROS, specially at the end of the organogenesis.

November 16, 1415

Transposable elements in *Octopus vulgaris* neural transcriptome

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Cephalopods neural complexity and plasticity is comparable to that of vertebrates as recognized in the relative brain size, number of neuronal cells and anatomical organization. In *Octopus vulgaris* the nervous system reaches the greatest level of complexity. By sequencing octopus transcriptome we found high number of transcribed long non coding RNAs (lncRNAs) and high frequency of retroelements embedded in transcripts, similar to that reported in mammals. Short interspersed elements (SINEs) are significantly more abundant in lncRNAs than in protein coding genes, and both lncRNAs and SINEs are enriched in transcripts expressed in the central nervous system of octopus. We also found a long interspersed element (LINE), fully competent for retrotransposition expressed in nervous tissue and abundant in the octopus brain regions known to be associated to learning and cognitive abilities. In situ hybridization support a diffuse expression of LINE mRNA in the great majority of amacrine cells (posterior buccal and frontal and vertical lobes) and in large cells in the sub-esophageal mass and arm cord, putatively considered large motor neurons. Transposable elements are known to generate germinal and somatic genomic heterogeneity in mammals and favour neural mosaicism in mammalian brain areas such as the hippocampus and the cortex. Our findings suggest that a convergent evolutionary process, driven by transposable elements, has led to the evolution of mammalian-like molecular traits in *Octopus* nervous system, possibly contributing to develop its cognitive abilities.

November 16, 1430

Octopus Hormones: A New Noninvasive Technique to Measure Stress Hormones

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Octopuses are highly intelligent animals, however in the wild they spend a majority of their time in their den as a reclusive predator. In captivity, enrichment may make these animals more active than they are in the wild which may be stressful. Therefore, we have created a technique to noninvasively measure and track stress in captive octopuses. The goal is to combine behavioral and hormone swabs to better understand what makes octopuses stressed. Commonly in zoos, caretakers track hormones of captive animals to mediate and reduce distress. Previously hormones in octopuses have been measured invasively through blood or noninvasively methods through fecal samples. Fecal sample collection, however can be opportunistic and smaller animals may not produce enough material to analyze. We created a technique that collects mucous from the surface of the octopus's skin and can be collected at any time from any size animal on a researcher's or caregiver's timeline, allowing for strenuous studies to be completed. Swabs were tested on *Octopus mercatoris* and *Octopus bimaculoides*. Results showed that cortisol can be easily measured with swabs and provide measurements of changes in cortisol over time. A stress test, where an octopus was swabbed every ten minutes after a stressor for 2 hours, showed a peak of cortisol at 90 minutes. Therefore, it takes about 90 minutes for cortisol molecules to reach the mucous on the octopus's skin after a stressor. This technique can provide a greater understanding of how octopuses react to changes in captivity and how caregivers may improve octopus welfare.

November 16, 1445

Mapping putative nociceptors in *Octopus vulgaris* arm

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The existence of nociception in cephalopod molluscs has been based on their behavioural responses to putative negative stimuli. Despite the extensive use of cephalopods in physiological and behavioural studies aimed at understanding neural and behavioural plasticity, Young and co-workers considered the contribution of pain signalling questionable in cephalopods. Most recently, Robin Crook and coworkers have provided the first experimental evidences for the presence of high threshold mechanoreceptors (putative nociceptors) in two species of cephalopods, but a morphological characterization and a precise mapping of these cells are still missing. To contribute to this aim we first explored in silico the *O. vulgaris* transcriptome searching for pain related genes. We focussed on 32 transcripts coding for transient receptor potential channels (e.g. TRPA1, TRPV1), peptides (e.g. Substance P, CGRP), G-protein coupled receptors (e.g. ADORA2a, CALCRL) and other proteins involved in nociceptive pathways. We estimated their expression in central and peripheral nervous system. A μ-type opioid receptor has been also identified in different areas of octopus' nervous system. Subsequently, we validated through RT qPCR a subset of 9 genes and determined their relative expression to evaluate possible functional difference between proximal and distal areas in octopus' arm. We also provided a tentative mapping of putative nociceptors in the arm by localizing fibres and cellular structures expressing proteins involved in nociceptive pathways. We identified TRPA1+-like positive cells in the suckers and ganglia of the arm, Substance P+ fibres running along the axial nerve of the arm, TRPV1+ structures diffuse in the whole arm and also IB4+, Nav1.8+, and CGRP+ structures positive to the nociceptors marker Isolectin B4. Our data contribute in providing evidences of the existence of a nociceptive system in *Octopus vulgaris,* nevertheless functional studies are necessary to validate them.

November 16, 1500

Neural coding in the octopus visual system

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Cephalopods have remarkable eyes that instruct a rich repertoire of visually guided behaviors. Although superficially similar, the camera-type eyes of cephalopods and vertebrates emerged independently, resulting in one of the most stunning examples of convergent evolution. Cephalopods therefore have a vertebrate-like sensor, their eye, connected to an invertebrate-like processor, their brain. Despite the enticing complexity and tremendous capability of this visual system, relatively little is known about visual coding and neural computations in cephalopods. Anatomical studies have delineated the morphology and structural connectivity of neurons in the retina and optic lobe, and identified connections from the optic lobe to other areas of the brain. Likewise, traditional electrophysiology has characterized the initial steps of visual processing, through recordings of photoreceptor and optic nerve activity. However, there has been no direct measurement of the visual response properties of neurons in the cephalopod central visual system.

Our lab has been focused on understanding how neurons in the octopus optic lobe represent information about the visual scene. We have developed a calcium imaging system that allows us to measure activity in hundreds of individual neurons, while presenting parameterized visual stimuli. Our studies have revealed features of octopus visual processing that are shared with vertebrates, such as retinotopy and orientation selectivity, as well as aspects that appear to be distinct from vertebrates, including a patchy organization for the representation of luminance. We also expect that this system will allow for future studies exploring neural computations underlying specialized aspects of cephalopod vision, such as polarization detection and the signals that drive camouflage.

November 16, 1530

Protein content of the statocyst endolymph in common cuttlefish (*Sepia officinalis*): an assessment of acoustic trauma after exposure to sound

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Recent studies have demonstrated damage after sound exposure in the cephalopod statocyst sensory epithelium, which secretes endolymph protein. Proteomic analysis of the endolymph was performed before and after sound exposure to assess the effects of exposure to low intensity, low frequency sounds on the statocyst endolymph of the Mediterranean common cuttlefish (*Sepia officinalis*), determining changes in the protein composition of the statocyst endolymph at different times after sound exposure. Significant differences in protein expression were observed, especially 24 h after exposure. A total of 37 proteins were significantly different in exposed specimens, 17 of which were mostly related to stress and cytoskeletal structure. Thus, endolymph analysis in the context of acoustic stress allowed us to establish the effects at the proteome level and identify the proteins that are particularly sensitive to this type of trauma. The observed changes are known to affect the physiology and functioning of the statocyst in *S. officinalis* and alter the sensory information of this species, compromising their survival and their role in the oceanic ecosystem.

November 16, 1545

Stress-related parameters in plasma and muscle of three species of octopus after fishing

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Several Octopodidae species have a great potential for the diversification of worldwide aquaculture. Unfortunately, the lack of stress-related biomarkers in this taxon results an obstacle for its maintenance in conditions where animal welfare is of paramount relevance. In this study, we made a first approach to uncover physiological responses related to fishing capture in *Octopus vulgaris*, *Eledone moschata* and *E. cirrhosa*. Captured octopus from all three species were individually maintained in a recirculated aquaculture system on board of oceanographic vessel in South-Western waters of Europe. Haemolymph plasma and muscle were collected in animals at the moment of capture, and after a period of 24 h of recovery. Physiological parameters such as plasma peroxidase activity, osmolality and glucose levels, as well as muscle water percentage and glucose values were analysed. The immune system appears to be compromised in these species due to capture processes; while energy metabolites were mobilized to face the stressful fishing situation with higher plasma glucose levels respect to specimens recovered for 24 h. Moreover, this situation exerts hydric balance changes, as observed in the muscle water, being these responses depending on the species assessed. In conclusion, three Octopodidae species from South-Western waters of Europe have been evaluated for stress-related biomarkers resulting in differentiated mechanisms between species. This study may pave the way to further study the physiology of stress in adult octopuses.

Poster presentations

Session: Anthropogenic Effects

P1

What can squids tell us about marine animal sensitivity to ocean acidification?

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Ocean acidification is believed to limit the performance of squids due to their exceptional oxygen demand and pH-sensitivity of blood-oxygen binding, which may reduce oxygen supply in acidified waters. The critical oxygen partial pressure (Pcrit), defined as the PO2 below which oxygen supply cannot match basal demand, is a commonly reported index of hypoxia tolerance. Any CO2-induced reduction in oxygen supply should be apparent as an increase in Pcrit. In this study, we assessed the effects of CO2 (40 to 140 Pa) on the metabolic rate and Pcrit of two squid species: *Dosidicus gigas* and *Doryteuthis pealeii*. Carbon dioxide had no effect on metabolic rate or hypoxia tolerance in either species. Furthermore, considering oxygen transport parameters (e.g. Bohr coefficient, blood P50) and blood PCO2 values from the literature, we estimated an increase in seawater PCO2 to 100 Pa (1000 μatm/ppmv) would result in a maximum drop in hemocyanin-O2 saturation of 4% at normoxia and a Pcrit increase of 0.7 kPa in the absence of active extracellular pH compensation. Such changes are unlikely given the capacity for acid-base regulation in many cephalopods. Moreover, this estimated change is within the 95% confidence intervals of the Pcrit measurements reported here. Squid blood-O2 binding is more sensitive to pH than most other marine animals measured to date. Therefore, the lack of effect in squids suggests that ocean acidification is unlikely to have a limiting effect on blood-O2 supply in most marine animals.

P2

Physiological and behavioral responses of *Octopus maya* juveniles to environmental temperature regimes

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As ectotherms, body temperature of cephalopods depends on environmental temperature, constraining a variety of organismal processes with profound implications on fitness and large scale patterns. In the wild, temperature is frequently fluctuating, exposing organisms to thermal conditions not always into the optimum, especially in marine habitats. Considering that thermal fluctuations may be wider in the global warming scenario, the aim of this study was to measure the effects of thermal regimes on phenotypic expression and plasticity of physiological and behavioral traits related to thermal tolerance in *Octopus maya*, an endemic and commercially important species of the Yucatan Peninsula (YP). Juvenile octopuses were acclimated during 30 days to constant (22 and 30 ºC) and fluctuating (22-26 and 22-30 ºC in 24 h cycles) regimes taking into account that temperatures around 30 ºC are sub-optimal. The study included evaluations of survival, thermoregulatory behavior (preference and thermal window), the thermal aerobic scope (TAS), the specific growth rate (SGR) and some muscle metabolites. Results obtained indicated that thermoregulatory behavior (preferred temperature ranged between 21 and 24 ºC) was not modified by thermal regimes, suggesting that *O. maya* juveniles' physiology lacks of the capacity to alter thermal preferences in order to broaden the thermal niche selection in a changing environment. In regards with energy utilization, SGR and survival were higher at 30 ºC (4.18 ± 0.97 % day-1 and 79 %, respectively) than in the rest of the acclimation treatments. However, this regime induced metabolic stress as it was reflected by lactate levels (50 % higher than observed in animals maintained at 22 °C and 22-26 °C) and the TAS (50 % less than observed at 22 °C and 22-26 °C). Juveniles' physiological performance was optimized by acclimation to relatively low temperature in constant regime, suggesting their high thermal sensitivity.

P3

How do you identify marine fisheries most vulnerable to climate change? The squid resource and lessons from South Africa

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The South African Department of Agriculture, Forestry and Fisheries has a comprehensive Climate Change Adaptation and Mitigation Sector Plan which was recently revised to include the Fisheries Sector. Only the most vulnerable of South Africa's twenty-two marine capture fisheries and the marine aquaculture sector were included in the National Plan. The first task was to evaluate the vulnerability to climate change of all of South Africa's marine fisheries and the marine aquaculture sector, including the squid resource. This was attempted by indexing the sensitivity to climate change of the resources themselves, the sensitivity of the fisheries to climate-induced changes in the resources, the impact of potential climate-induced changes on the socio-economics of the fishery, and the ability of each fishery to adapt to such changes. These indices were then used to derive a Vulnerability Index for each fishery including squid, and the marine aquaculture sector, allowing comparison between the different sectors and resources. The indices, methods used to derive them, and rankings were discussed at a Workshop in October 2015 and are presented here. All marine fisheries and the marine aquaculture sector were judged to be vulnerable to climate change to some extent, for reasons that will be presented. Three resources identified as the most vulnerable to climate change were included in the National Sector Plan. The foremost was the linefish fishery (specifically the small-scale, commercial small-boat and net fisheries), followed by the fishery for small pelagic fish (i.e. sardine, anchovy and round-herring), and the marine aquaculture industry. The latter sector was selected because of its unique challenges compared to wild capture fisheries, and more importantly, its potential for growth. Lessons learned in this assessment, and current follow-up steps to build resilience to climate change in all of South Africa's marine fisheries, including the squid resource and the marine aquaculture sector, are discussed.

P4

Cephalopods as vectors of harmful algal bloom toxins

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Harmful algal blooms (HABs) are natural occurrences that can severely impact marine communities. Depending on the species, they can produce a wide array of toxins, which may elicit devastating effects on marine life. Paralytic shellfish toxins (PSTs) and domoic acid (DA) are both water-soluble neurotoxins that frequently accumulate in bivalve molluscs along the Portuguese coast. It is established that cephalopods accumulate these toxins in their tissues, mostly in the digestive gland, through ingestion of contaminated prey. Here, we describe the uptake and transfer kinetics of PSTs between octopus (*Octopus vulgaris*) body tissues. It was shown that octopuses present low toxin conversion, depuration and excretion rates. PSTs were found to be present in kidney tissue at much lower concentration than in the digestive gland. Throughout the experimental period (15 days) the specimens did not display obvious outward effect of such high dosages of a paralytic toxin. In order to determine if these HAB-toxins enter cephalopod's central nervous system, wild octopuses and cuttlefish were collected and brain tissue was analysed for DA. This neurotoxin causes amnesic shellfish poisoning in vertebrates and it is known to accumulate in cephalopods' digestive gland. Here, we also show that octopus and cuttlefish accumulate this potent toxin in their brain tissue for at least four months (in octopus), suggesting that the can selectively retain and possibly tolerate this toxin. In conclusion, out studies show that cephalopods have very high potential for HAB-toxin accumulation and may have developed mechanisms that can protect them against these potent neurotoxins.

P5

Effect of temperature in growth and antioxidant response during embryonic development of *Octopus bimaculoides*

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The life cycle of ectotherms is affected by temperature due to its role as a governor factor of the biochemical reactions involved in the maintenance of cellular homeostasis. Temperature accelerates embryos metabolic rate, and below or above optimal thermal range, this factor stimulates accumulation of oxygen reactive species and free radicals inside the cell, which can produce damages affecting the embryo development. In the present study, the effect of experimental temperatures on the embryo growth and antioxidant activity were evaluated in an attempt to obtain information about its thermal limits and how physiological processes could explain these limitations. Groups of embryos were incubated at 16, 18, 20 and 22 °C, until hatch. Morphological changes (mantle, head and arm length and eye diameter) as well as antioxidant enzymes activity (ANTIOX; superoxide dismutase, catalase, total glutathione, and glutathione S-transferase) were measured throughout the embryo development. ANTIOX system efficiency was evaluated measuring lipid peroxidation levels. In addition, physiological state of embryos was evaluated through enzymatic activity of acetylcholinesterase and carboxylesterase, both indicators of lipid and nervous system development. The greatest growth was obtained in embryos incubated at 18 and 20 °C, with significant development in arm's length and eye diameter. Also, in those temperatures, ANTIOX mechanisms, involved in lipid peroxidation regulation, were more effective. The high survival percentage of non-fed juveniles over 10 days, used as an indicator of the thermal consequences in hatchlings, showed that internal yolk accumulation was not affected by the experimental conditions, allowing the 100% survival of juveniles obtained from all thermal regimes. These results indicate that the thermal limit of embryos is linked with the ANTIOX mechanisms capacity, and not by the energy derived from the yolk.

P6

Cognitive impairments in future oceans: the case of cephalopods and its potential universality

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Global climate is facing exacerbated change, mainly due to the continuous rise in atmospheric CO2 concentrations (nowadays ≃400 CO2 μatm). CO2 is dissolved in the ocean, increasing H+ ion concentration and dropping mean ocean pH levels around 0.14-0.35 units (≃1000 CO2 μatm), the so-called ocean acidification (OA). It is already established that OA prompts severe acid-base deregulation and physiological damage in most marine organisms, including cephalopods. Cephalopods and vertebrates are phylogenetically distant taxa, however they are a case of convergent evolution, exhibiting similar cognitive/behavioral complexity and performance. Furthermore, deleterious behavioral modifications (e.g. alteration of defensive and predatory behaviors) are reported not only in fish, but also in cephalopod mollusks, including the squids *Idiosepius pygmaeus* and *Sepioteuthis lessoniana*, and the cuttlefish *Sepia officinalis*. As key factors in the adaptive capabilities of cephalopods, behavioral plasticity and cognitive performance are crucial for coping with fluctuations in the environment. Consequently, OA-induced neural functioning disruptions may lead to pronounced negative consequences in decision-making processes, higher vulnerability and decreased foraging success, making cephalopods a prime model for these studies. We have begun breaking down this issue, by systematically testing the effects of OA on a variety of cognitive skills, such as S. officinalis social learning. The existence of similar OA effects in vertebrates and cephalopods suggests ionic alterations (either associated to H+ or CO2 derivates) in conserved neural and physiological pathways. Despite the potential universality of acquired knowledge, no studies have focused on how cephalopod neural correlates of behavior alterations are affected by future ocean conditions. Thus, we aim to fill this gap with state-of-the-art techniques and use cephalopods as a model for assessing potential cognitive impairments in the oceans of tomorrow.

P7

The effects of elevated CO_2 on the behaviours and physiology of two cephalopod species

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There is increasing evidence that projected near-future carbon dioxide (CO2) levels can alter behaviours and physiology in marine invertebrates. However, little is known about the possible effects on cephalopods. Here, we tested for the first time, the effects of elevated CO2 on the predatory behaviours, activity levels, and aerobic scope of two ecologically distinct cephalopod species, the pygmy squid, *Idiosepius pygmaeus*, and the bigfin reef squid, *Sepioteuthis lessoniana*. Both species exhibited an increased latency to attack and altered body-pattern choice during the attack sequence at elevated CO2. I. pygmaeus also exhibited a 20% decrease in predation rate, an increased striking distance, and reduced preference for attacking the posterior end of prey at elevated CO2. Elevated CO2 increased the activity levels of both species, which could adversely affect their energy budget and increase their potential to be preyed upon. However, no changes in the aerobic scope of either species was found at elevated CO2 when compared to control. This indicates that changes in metabolic performance as a result of elevated CO2, as has been shown in other taxa, is not responsible for the changes in behaviour seen here and other physiological mechanisms will be responsible for altered behaviour. The effects of elevated CO2 on predatory behaviours, predation strategies, and activity levels of cephalopods reported here could have far-reaching consequences in marine ecosystems due to the ecological importance of cephalopods in the marine food web.

P8

Effect of temperature on growth and sexual maturation in the young Japanese flying squid, *Todarodes pacificus*

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In recent years, cephalopod growth has been found to change greatly within the same species depending on environmental conditions such as water temperature and food resources, which indicates high plasticity. However, due to the difficulties in rearing the Ommastrephid squid *Todarodes pacificus*, it is unknown if they also exhibit such plasticity. In this study, we investigated the effect of water temperature on growth and sexual maturation in *T. pacificus* by rearing young (4–5 months after hatching) obtained from the wild (Wakasa Bay in the southern Sea of Japan) to adult squid of ripe stage at four different temperatures (13 °C, n = 26; 17 °C, n = 23; 20 °C, n = 36; and 23 °C, n = 36) during April and June. At the onset of the experiment, the mantle lengths of the young ranged from 57.2 to 132.4mm. The squid were fed a fillets of anchovy and saury once or twice daily, and the daily food intake for each individual was recorded. In addition, the growth rate of body weight was calculated for each water-temperature group. Day and night cycles were set to 12 hours each. Males reared at 23°C began to show signs of sexual maturation after 10 days, while those at 17°C and 20°C began to mature after 20 days. The squid maintained at 13°C did not begin to mature until after 40 days. The females reared at 23°C began to show signs of sexual maturation after 20 days. The mantle length of mature individuals ranged from 86 to 147mm, which was small and is rarely observed at maturity in the field. Although the daily food intake per individual was highest for the group at 23°C, the maximum growth rate of body weight was seen in those under the 20°C conditions. These results suggest that the life span of *T. pacificus* is also flexible, depending on habitat conditions, and may be reduced to six months under specific conditions. Based on these results, 20 °C appears to be the optimal habitat temperature for young Japanese flying squid.

Session: Behavior

P9

What can social humans tell us about social cuttlefish? Citizen science meets cephalopod behavioral ecology

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Cephalopods, including the cuttlefish (Sepiidae), are complex and intelligent animals who until recently were considered mostly asocial species, except for the squids. Using retroactive requests for citizen science we gathered video footage demonstrating reproductive and non-reproductive sociality in the common European cuttlefish (*Sepia officinalis*). We provide evidence of shoaling (N=6 cuttlefish) during what to appears to be resting periods (~55 minutes) and possible schooling behaviour (N~30), a group size so far un-reported for this species. In addition to these non-reproductive social behaviours, we provide further evidence that reproductive social behaviours may be more common in this genus than previously believed, as may alternative reproductive tactics. Although at times difficult to analyse, interpret and make concrete conclusions we found retroactive requests for citizen science to be a good way to formulate testable novel hypotheses in a family of cephalopods that are well known in the laboratory but understudied in the field.

P10

Behavioral and neurological effects of early life injury in the squid *Euprymna scolopes*

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Noxious or painful experiences occurring in early life are known to result in profound changes to pain and sensory processing that extend into adulthood. These effects have been studied only in mammals, despite strong conservation of nociceptive signaling across a range of vertebrate and invertebrate taxa. In a long-term study in our laboratory, we are examining the effect of early life injury on behavior and neural excitability in adult squid, (*Euprymna scolopes*). This sepiolid is a small, experimentally tractable cephalopod that can be reared through multiple generations in the lab, and as such is ideally suited to life-time studies of behavior and neurobiology. In this study, squid hatchlings receive a single abrasive injury to one fin, given at 2-3 weeks post hatching, when animals are 1-2mm long. Between 14 and 16 weeks of age, the behavior of injured and control squid were measured in a series of behavioral assays. At 16 weeks, animals were euthanized and excitability in the peripheral nervous system was measured using simultaneous extracellular recordings from the fin and pallial nerves. There were no differences in growth rate, general hunting success or time-to-maturity between early-life injured and control groups. Behavioral assays showed that adult squid that experienced early life injury expressed impaired cognitive performance and suppression of startle responses to a model predator, compared with control squid that did not receive injury. Recordings from the peripheral nervous system indicated that early-life injury produced life-long sensitization of nociceptive sensory afferents in the fin nerve, and changes to the way sensory information was relayed to the CNS from the stellate ganglion, which appeared to modulate non-nociceptive signal in control animals, but amplify it in early-life injured animals. These data suggest life-long effects of early life injury occur in cephalopods in similar patterns to those known in mammals.

P11

The effects of injuries on associative learning in *Euprymna scolopes* under predation threat

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Since the first "prawn-in-a-tube" experiments, associative learning has been demonstrated in many cephalopod species. Recently, *Euprymna scolopes* was the first squid to show associative learning by the same methods. The relative ease of rearing specimens in the lab, combined with the highly observable "prawn-in-a-tube" technique, makes this species a strong candidate for studying cognitive effects of a wide range of developmental and experience-dependent variables. Here, we examine the effects of early-life (chronic) injury, acute injury and predation threat on adult *E. scolopes* during associative learning. We counted the total number of strikes made per 10-minute trial for each condition and compared the results across treatment groups to identify differences in learning rates. We also counted the number of strikes in the first and last 5 minutes of each trial and compared strike counts within treatment groups to assess learning. Finally, we compared strike counts in the presence and absence of predator scent. In the absence of predator scent, the total number of strikes did not differ among all experimental groups. However, we observed a significant decrease in the number of strikes in the second half of the trials for uninjured animals, while the injured groups had a similar number of strikes in both halves of their trials. These data suggest that injury, whether acute or chronic, impairs cognitive performance. When a predator's scent was present, all groups made fewer strikes compared with the no-scent condition, although the decline was significant only for uninjured animals. There were no differences in total strikes made among the different treatment groups in the presence of predator scent. These results indicate that uninjured squid are more risk-averse than injured squid, which was unexpected. Thus, both chronic and acute injuries affect associative learning and risk assessment, and these effects persist for very long periods after injury.

P12

Description of sleep and learning behavior in two octopus species: *Octopus* cf. *vulgaris* and *Octopus insularis*

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Sleep is a behavior that can be seen in several taxa of the animal kingdom, suggesting that its origin occurred primitively in the evolutionary chain of the metazoa. Although sleep is a well studied behavior in vertebrates, mainly in mammals, birds and reptiles, it is known that some invertebrates also show this state of quiescence. Octopus is one of the invertebrates in which this behavior is expressed in a complex way, with behavioral and electrophysiological records that suggest the existence of at least two sleep stages. In addition, octopuses have the most complex nervous system among invertebrates, having a brain with specific lobes for learning. In mammals, post-learning sleep has been shown to benefit memory consolidation. We set out to investigate the relationship between sleep and learning in the octopus. We aim to investigate and describe in detail, through comprehensive behavioral quantification, the sleep of *Octopus cf. vulgaris* and *Octopus insularis*. In addition, we also aim to evaluate how young adults of these species learn a novel task named "Russian dolls", which requires animals to open up to three jars in sequence, one inside the other, one smaller than the other, with a reward (fish, mussel, crab or shrimp) inside the smallest jar. Preliminary results with 5 *O. cf. vulgaris* showed conspicuous behavioral changes during the quiescent state. Some of these changes have already been cited in the literature, such as the "half and half" body pattern, or the narrow or completely closed eye pupils, but other changes have not been described yet, such as body pattern alteration to dark color and movement of the eyes. With regard to learning, we observed preliminarily that the octopuses were able to open the three jar types and open the same jar in different manners, which emphasizes the behavioral and cognitive versatility of these animals.

P13

Social learning in *Sepia officinalis*: Testing inhibition of predatory behavior and primed goal directed behavior

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Learning can occur through self, asocial experiences with the surrounding environment, allowing for adaptive behavior towards changes in the environment and maximizing the individual's success. In fact, many studies have shown that cuttlefish species (in particular, *Sepia officinalis*) are capable of learning through unimodal or multimodal sensorial integration. In the widely used "prawn-in-the-tube" procedure, cuttlefish inhibit predatory behavior over trials by learning that prey is unreachable, avoiding futile energy expenditure. Accordingly, if prey is available within a fixed maze with two possible entries, cuttlefish are expected to increase prey capture success over time, by choosing the open entry. Despite their demonstrated cognitive prowess, cuttlefish have rarely been tested under observational learning-enabling scenarios. Here we tested 40 pairs of recently-hatched S. officinalis individuals, divided equally between two tasks assessing distinct reward-linked learning: "prawn-in-the-tube" and maze solving. One of the animals undertook the task as a standard individual learning procedure, while another was allowed to observe all trials performed (20 observers and 20 demonstrators per task), before being tested himself. In the observational "prawn-in-the-tube" procedure, only 1 experimental pair reported higher attack number for the observer, having the latter outperformed or equaled demonstrators in the remaining pairs. As for learning the maze side which would yield access to prey by watching a conspecific (i.e. goal directed behavior), results are currently being analyzed. Nonetheless, our preliminary findings show that *S. officinalis* individuals are able to socially learn through conspecific observation, specifically by inhibiting predatory behaviors. Despite the ongoing changes on neural organization during these early ontogenic stages, this complex form of learning is already present in *S. officinalis* individuals with only 5 days post-hatching.

P14

An *Octopus minor* characterization of predation behavior adaptability with a novel signal display

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Although little-known cephalopod behaviors gradually are revealed to mankind while predation behaviors of octopus are rarely recognized either in the field setting or laboratory, especially the manipulation of arms when an octopus gropes blindly into crevices for preys. Our objective was to construct a general predation ethogram of *Octopus minor* (Sasaki, 1920) and evaluate the body's "infinite scalability" and cognition of predation adaptability. To achieve this, we designed a "certification test" (level 1) and a "prey choice test" (level 2, 3) to 1) record routine hunt strategies, 2) determine whether octopods could discriminate predation difficulty to make an appropriate choice and 3) discover if octopods could modify predation tactics to retrieve preys from different obstacles. We found that *O. minor* utilized several predation strategies, which included ambushing, stalking, pursuit and speculative hunting. Additionally, *O. minor* preferred to feed on crustaceans (*Hemigrapsus sanguineus*, accounted for 55.99±3.21% with a 21.77±0.58 min average consumption time) rather than bivalves (*Plicatula philippinarum,* 34.49±3.08%, 18.52±0.76 min) or small fishes (*Ctenogobius giurinus*, 9.52±2.40%, 23.55±1.80 min). We observed that *O. minor* consciously chose the least difficult obstacle when selecting prey, even exerting extreme "infinite scalability" for a high-difficulty hunt. We used the three levels of tests to investigate the predation behavior adaptability of octopods that ranged from using arms to squeeze through a small opening to the cooperative use of tactile, chemical, and visual cues. We also describe a novel predator-related signal (NPS) used in solving the predation puzzles and is discussed in detail.

Session: Conservation and Biodiversity

P15

A new species of pygmy octopus of the genus *Paroctopus* (Octopodidae) found in antropic debris in southeastern Brazil

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A new species of a pygmy octopus is described from subtropical shallow water, in southeastern Brazil. *Paroctopus* n. sp., is small (20-30 mm Ml), head as large as the mantle, prominent eyes, enlarged suckers from 1st to 3th arms, usually at 5th row in both sexes. Small ligula, with transverse laminae and a distinct calamus, almost half of ligula length. Branchia typically with six lamellae. We found a spawned female with a brood of eggs around 4.5 mm long, attached inside the valve of sunk snorkel. Colors in life from red to brown. The genetic analyses using the mitochondrial gene COI showed 8.9% of genetic distance (K2P model) between the *Paroctopus* n. sp. and both *P. joubini* (AY377732) and *P. cf mercatoris* (GQ900743, Florida) COI sequences deposited in GenBank. The 100% similarity between the samples of *P. joubini* and *P. cf mercatoris* suggest a misidentification problem or that both species are synonymous. In any case, the genetic distance is large enough to claim that *Paroctopus* n. sp is a different undescribed species. The Western Atlantic *Paroctopus* spp have a close relationship with *Paroctopus digueti* from East Pacific and may have shared a common ancestor before the uplift of the Isthmus of Panama. *Paroctopus* n. sp may have moved southward via shallow bathymetry of the continental shelf linking South and Central America. Most of the collected specimens were found on a sandy bottom less than 10 m deep inside cans, bottles and other debris. This strong use of anthropogenic materials as dens could be a signal of deterioration at its natural habitat or its expansion to soft bottoms with few available small molluscan shells.

Session: Culture and Welfare

P16

Improving keeping and welfare for captive *Octopus cyanea* trough environmental enrichment

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Octopus cyanea has a wide range of natural distribution and is potentially very interesting for comparative studies. However, unlike its temrate climate cousin, *Octopus vulgaris*, the species is poorly studied and little data exists on best practices for keeping them. Adult *Octopus cyanea* (n = 16) were locally collected in Okinawa trough out the entire year. Once the fisherman caught an octopus they were transferred to the laboratory facilities at the Marine Station of the Okinawa institute of Science and Technology. Animals were kept in a flow through salt water system and fed a diet of live or dead crustacean prey. They were then housed either individually in tanks ranging from 500l to 750l or in 2000l tanks with other animals found in the same habitat. All tanks had a semi natural enriched environment consisting of natural substrate (sand or crushed coral) and either clay pots or natural rocks as dens. Also, we tested different cover types to asses an optimal tank cover to prevent escapes of the animals. In order to assess different keeping conditions, we compared escape attempts and non-natural deaths during the animals' time in human care.

P17

Development of a system for the maintenance of octopus on board of oceanographic vessels: evaluation of the survival rates after bottom-trawling

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By the year 2019 Europe will adopt the policy of no-discards. This entails the landing obligation of all catches of cephalopods unless their survival and recovery are guaranteed after catch and release. By the development of a recirculating aquaculture system (RAS) on board of oceanographic vessels we provide new methodologies to evaluate survival of octopus and further investigate their physiological recovery after capture. The system includes a set of tanks where octopus are maintained individually, offering a physical, chemical and visual independence respect to other specimens. Octopus from three species (*Octopus vulgaris*, *Eledone moschata* and *E. cirrhosa*) were captured in South-Western waters of Europe by bottom-trawling. After on board selection of commercial individuals, discards were maintained in single RAS tanks. Their survival rates after 24 h of recovery were calculated for all three species. Moreover, haemolymph was collected from octopuses before being introduced into the system and after 24 h of recovery. Haemolymph plasma was analysed looking for stress-related biomarkers. Amongst them, free amino acids showed differentiated concentrations between recently-captured and recovered groups, with higher levels in the first group for all three species. This information points out at an animal recovery after the stressful situation induced by capture. Due to its easy handling and proven utility, this system may be employed to evaluate survival rates in other cephalopods worldwide.

P18

Post-mortem: The National Resource Center for Cephalopods (NRCC), 30 years of cephalopod aquaculture and research

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Eight years ago, one of the most influential laboratories for cephalopod research closed its doors. The National Resource Center for Cephalopods (NRCC), located in Galveston, Texas, USA, was created in 1976 with funding from the National Institute of Health to supply cephalopods to researchers and educational institutions. At a time when the knowledge of cephalopod husbandry was sparse, the NRCC was truly a unique facility that significantly advanced our understanding of cephalopod captive care and aquaculture. Over 39 years, the NRCC developed culture and husbandry techniques of squid, cuttlefish, nautilus, and octopus now commonly used throughout the world. The NRCC itself published over 100 papers and has been acknowledged in hundreds more. Here, we will provide a brief history of the NRCC and the contributions it has made to cephalopod research.

P19

Towards a laboratory suited cephalopod: laboratory culture of Indo-Pacific Bobtail Squids

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Since the discovery of the symbiosis between bobtail squids from the *Euprymna* and *Sepiola* genera and the bioluminescent bacteria *Vibrio fischeri*, much effort has been made in understanding this relationship. During this time, several groups were successful in culturing multiple generations of several bobtail squid species. Now at the age of omics and CRISPR, traditional cell and molecular biology technologies are becoming increasingly available to non-traditional model organisms. Thus, there has been a renaissance in the research surrounding cephalopods and a push has been made to establish a reliable easily culturable cephalopod species. Bobtail squids prove to be an especially laboratory hardy animal thanks to their small size at maturity, benthic nature, non-cannibalistic tendencies, short life span, and easy accessibility. We will discuss the successes and challenges we've encountered in culturing multiple species of bobtail squids of the Sepiolidae family from the Indo-Pacific, and will present the technical details of our closed systems and some downstream applications our laboratory has benefited from our cephalopod culture.

Session: Fisheries, stock assessment and management

P20

Changes in catch trends and distribution type of the common octopus *Octopus vulgaris* in the continental shelf of the Yucatan Peninsula

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Octopus resource in Mexican Atlantic waters comprises two species *Octopus vulgaris* and O. *maya*. This resource has been exploited since 1949 in Campeche and since 1970 in Yucatan and Quintana Roo. The *O. vulgaris* exploitation is carried out without an adequate knowledge of many aspects of its biology and ecology in these areas. This scenario can have negative consequences for a good management of the exploited stocks, and, ultimately, unwanted effects on the ecosystem. In this study we aimed to identify those zones with higher concentrations of the species per unit area, and to settle the type of spatial distribution of the common octopus (*O. vulgaris*) in the area. Four research cruises were undertaken in May-June, July and December 2016 and January 2017, respectively. Sampling was done with the fishing gear used by fishermen targeting this species and in 30 stations monthly visited. Total number of octopus, total catch weight and individual sex, mantle length (ML) and body weight (BW) was estimated for each station. Relative abundance in terms of catch per unit area (CPUA, specimens / km2) of 30 stations per month was calculated. The distribution type was figured by estimating the averages of each variable (how many octopuses of each species on average per station were caught) and the percentages of probability distribution. From these values, parameters of the negative binomial distribution p, k1 and k2 were estimated. Results showed that: i) the average of CPUA during the months of study was lower in May (9.6 spec. / km2) and higher in December (49.8 spec. / km2), ii) the zone that showed higher concentrations of the species throughout the months of sampling is located in the eastern part of the area sampled, near the continental slope (between 15 and 60 meters depth), and iii) common octopus spatial distribution within the Yucatan continental shelf is clumped.

P21

Development of the first, managed nautilus fishery to save Nautiluses

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In less than 50 years, 500 million years of nautiloid history has been significantly wiped away. In some areas, the populations are already locally extinct. In January 2017, the first international regulation of the nautilus shell trade took effect with the adoption of nautiluses (Family Nautilidae) into Appendix II of the Convention on International Trade in Endangered Species (CITES). These regulations require a permit to accompany any nautilus export, whether it be a live animal, whole shell, or jewelry/curio item made from the shell. The permits must include non-detrimental findings data, or NDFs, showing that the export will not negatively impact wild populations. However, implementing these regulations can be difficult. If not done correctly, an illegal, black-market trade could develop, thereby destroying the years of research and progress to save nautiluses. To support CITES and provide resources for nautilus range countries, the Nautilus Strong Initiative (NSI) was created. Through the NSI, monitoring networks of nautilus populations will be implemented across nautilus range states. The data from these networks will be used to track nautilus populations and provide the NDFs required to manage nautilus exports and determine sustainability levels. These data will also address knowledge gaps in many different areas including population demography, phylogenetics, behavior, diet, and perhaps most significantly, reproduction, and the deep-sea ecosystem. At the time of abstract submission, countries selected for these networks include the Philippines, Fiji, American Samoa, and Palau. By September 2018, data from these initial networks will be available as the first post-regulation nautilus surveys and exports will be conducted between June and August 2018. Successfully implementing these surveys will open new fields of inquiry into conservation of the deep-sea nautilus habitat, an area closely connected to coastal cities but vastly understudied and misunderstood.

P22

Summer-spawning *Loligo vulgaris* in the North Sea: the story of a disappeared stock or a reverse of reproductive patterns?

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The common squid, *Loligo vulgaris*, is widely distributed in the Northeast Atlantic including the Mediterranean and North Seas. In 1930-ies, the species was reproducing in the North Sea in the late spring and summer, with the highest abundance of pre-spawning mature females in late April - May, occurrence of freshly laid egg masses and spent females in May – August, and spent males in July- August (Tinbergen; Vervey, 1945). In the last decade only a few small immature squids if any were present in catches of the summer surveys, and only at the southwestern entry into the North Sea. Meanwhile, low numbers of mature L. vulgaris were present in winter catches (Quarter 1) in the southern and central parts of the sea though whether they reproduce on its shores, or are coming from the winter-spawning population of the English Channel, remains unclear. Thus, the current catch information indicates some major changes in the biology, distribution range and/or reproductive structure of *L. vulgaris* in the North Sea. We present a comprehensive analysis of its biological traits in this area and discuss the interactions with congeneric *L. forbesi* as well as other possible drivers of observed changes.

P23

Evidencing hurricanes effect on octopus catches in the Campeche Bank, Mexico

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The red octopus (*Octopus maya*) is an endemic species of the Yucatan peninsula, being its fishery one of the most important activities in the southeast of Mexico. This region is exposed to a hurricane season during the months of July to November, coinciding with the octopus fishing season. This synchronicity supposes a possible effect on habitat and fishing operations, for which it is assumed hurricanes are a significant source of variation of catches (C). The present study evaluates the relationship between C and hurricanes (H) and tropical storms (TS) events recorded during 1964-2014. A total of 64 events were obtained (21 TS and 43 H), from which a hurricane index (HI) was constructed. For this, H and TS some attributes were considered, but only during their transit over the continental shelf of the Campeche Bank; such as maximum wind speed, minimum atmospheric pressure, hurricane direction, transfer speed, time and distance traveled. To measure the cumulative effect for a given year in which more than one event was recorded, the value of each variable was added to obtain an annual value. A principal components analysis was applied, where the first component explained 67.9% of the variance, having a relatively similar contribution from the different variables. In this context, the eigenvector was used as the annual HI. The HI was correlated with catches to test the hypothesis using a reduced major axes linear regression, which suggests that the HI explains 38% of variation of catches ($r2 = 0.3865$); being a statistically significant relatonship ($F = 18.26$, $p = <0.001$). The model ($Ct = 11731.4 - 10457.2\ HIt$) suggests an inverse relationship between the magnitude of the catches and the intensity of the hurricane's effects.

P24

Cephalopods in multispecies small-scale fisheries: Case study of the Canary Islands

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As other multispecies small-scale fisheries (SSFs), cephalopods are mainly a by-catch group in the Canary Islands (Central-East Atlantic). It is an oceanic archipelago, with oligotrophic waters and mixed temperate, tropical and subtropical species, where marine ecosystems are very sensitive to imbalances. Cephalopod landings are analysed for the 2007-2017 period. Relative importance of cephalopods is low, with less than 1% of total catches in the Canary SSF, but it also means a relevant complement to the global catches and fishermen income. The most important catches were registered for common octopus (*Octopus vulgaris*), ommastrephid squids (*Illex coindetii, Sthenoteuthis pteropus, Ommastrephes bartramii, Todarodes sagittatus* and *Todaropsis eblanae*), cuttlefish (*Sepia officinalis*) and loliginid squids (*Loligo vulgaris, L. forbesii*). They are fished by small vessels of 5-14 m long performing daily trips with traditional gears: fish-traps (octopuses, cuttlefishes) and handlines with jigging (loliginid and ommastrephid squids). A seasonal effort is directed to ommastrephids and loliginids in specific seasons, resulting in a cost-effective activity due to their high spatial-temporal concentration. In other cases, the absence of directed effort to high value species such as octopus and cuttlefish make them highly profitable. Preliminary results indicate a high seasonal and inter-annual variability, which could be related with changes in the environmental conditions. Fishery data were compared with sea surface temperature, thermal anomalies and chlorophyll a concentration. The problem of species misidentification during landings registration procedure was addressed, evidencing that the improvement of the species identification is necessary in some cases (i.e. ommastrephid-loliginid squids). Moreover, landings of scarce but high demanded species (i.e. loliginid squids) could be underestimated due to illegal/direct sales and undeclared recreational catches.

P25

Cephs and Chefs - Octopus, squid, cuttlefish, sustainable fisheries and chefs

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Plus consortium members

Food security, sustainable development and diversification of fisheries are strategically important across the EU. Atlantic Area fleets are over-reliant on a few 'high-value' fish species, some of which are becoming economically unviable due to reduced abundance and strict regulation. We can address this by broadening the appeal of less-developed and non-quota species such as cephalopods, which have their own challenges, including poor species identification, no stock assessment, and competition from low-priced international imports. However, very different markets exist: "mature" southern consumer markets (e.g., Spain, Portugal) are well-developed for cephalopods, indeed Spain imports cephalopods from north Europe. Other national markets are under-developed ("emerging") and require investment to promote increased consumption. Thus, the Irish fleet lands cephalopod catches directly into Spain, but this practice may be suboptimal. Low prices for fishery products are a problem across the sector. An informed strategy is needed to add value to cephalopod products (e.g. sustainability certification), while ensuring sustainability and minimizing environmental impact. Objectives of "Cephs and Chefs" are to add value to cephalopod products through multiple strategies. Firstly, by developing and promoting new products, new market initiatives (e.g. certification) and business opportunities for the sector. Secondly, by improving knowledge of the value chain ("from sea to table"), and what affects sustainability in the short term (e.g. low prices, imports, consumer demand) and potential market developments in the long term. Thirdly, by improving knowledge of eating habits and acceptance of new cephalopod food products by consumers in North and South Europe. And finally, by ensuring sustainability of fishing activity by assessing status of stocks, fisheries and ecosystems based on biological and socioeconomic indicators. Partners include research institutions, NGOs, producer organisations, wholesalers and certification bodies.

P26

Cuttlefish *Sepia officinalis* Linnaeus (1758) discard estimates from the main demersal fisheries operating in the Gulf of Cádiz (SW Spain)

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Among different cephalopods commercial species caught by demersal fisheries, cuttlefish (*Sepia officinalis*) is the second in terms of landings in the Gulf of Cádiz. In the last 10 years, the bottom trawl fleet, composed of 133 vessels, landed an annual average of 668 tones with a discard rate of 0,8% and the artisanal fleet reported an average of 200 tonnes per year with almost no discards. However, the hydraulic dredges fleet informs of high levels of cuttlefish discards. This fleet, with 96 vessels and authorization for fishing only striped venus (Chamelea gallina), lands around 3000 tonnes per year discarding the rest of caught species. Dredge fishing grounds are located at depths between 4 and 12 m on sandy-muddy bottoms, along 100 km of coast. The discard composition was monitored on a monthly base through on board samplings of the hydraulic dredges total capture. The surveys were carried out from April 2008 to May 2009, and along 2017. The estimated discarded biomass was 23% of total caught biomass (266 kg/trip on average). A total of 92 discarded species were identified, corresponding 46 species to molluscs, 26 species to fish, 14 species to crustaceans and 6 species belonging to other invertebrate groups. The mollusc species reported 66% in weight, where three cephalopods species contributed with 1.2%, equivalent to 1.9 kg per trip on average. *S. officinalis* was caught in every survey, with 1.6 kg/trip, followed by *Octopus vulgaris* and *Eledone moschata*, with 0.23 kg/trip and 0.07 kg/trip, respectively. The *S. officinalis* size distribution showed a wide range between 2 and 28 cm, with average size of 9 cm. Annual cuttlefish discarded biomass was estimated, resulting in 25.5 tonnes by the hydraulic dredge fleet, 0.13 tonnes by the bottom trawl fleet and almost zero by the artisanal fleet. This high level of cuttlefish discards in the hydraulic dredge fleet suggests the need to establish measure to reduce it.

P27

Small scale fishery for *Sepioteuthis lessoniana* Férussac 1831 in Cabo Deldago, North Mozambique (Indian Ocean)

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The oval squid *Sepiotethis lessoniana* Férussac 1831 is a shallow water species widely distributed in the Indian and Pacific oceans, being especially abundant in coral reefs, bays and beaches with sea grass of Northern Mozambique. Its artisanal fishery constitutes a subsistence activity for local communities, which have been traditionally carried out by men onboard the small artisanal canoes by using jiggings. Also, they are caught as by-catch using hand hook and by beach trawling. In order to know the main biological and fishery aspects of this species, a first preliminary study was carried out in Cabo Delgado, North Mozambique, during April-November 2015. The main fishing areas in the mainland and in the Quirimbas archipelago were visited in order to collect relevant information on the fishery through interviews with fishermen and biological samplings. The oval squid is distributed all over Cabo Delgado, with a potential fishing effort between 400-500 fishermen. The population presented a wide size range (3-27 cm of Dorsal Mantle Length), being caught the smaller individuals by beach trawling in the shallow water with presence of sea grass. The estimated sizes at first maturity (L50%) were 16.2 and 17 cm, for males and females respectively, corresponding to average weights of 218 and 245 g. The reported information showed that the species present a long reproductive period, extended throughout the year, with a spawning peak from April to August. The presence of recruitment throughout most of the year might evidence that fact like in other regions of the Indo-Pacific Ocean where this species appears as an intermittent terminal spawner. Abundances didn't show a clear spatial and temporal pattern, being related to different factors. The possibility of being able to sell it fresh increases the landing in certain beaches and periods, given the fact that fresh squid have more economic value than dried.

P28

Artificial shelters for reproduction of *Octopus maya*: in the road to sustain the fishery

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The *Octopus maya* fishery is one of the biggest in the world with a annual production around 20,000 Tons. The availability of habitats, or the destruction of these, are important factors for effect the reproduction and the persistence of species. In the case of *O. maya* the availability of artificial shelters for spawn is critical in its life cycle. The aim of the study was to evaluate the occupancy of artificial shelters for *O. maya* as spawn sites and analyze its implications in the fishery management. One hundred and twenty artificial shelters were placed in front of Sisal coast, Yucatán, in a depth between 6 and 12 m. Shelters were divided into two types of bottoms: with rocks – with sand and distributed in densities of 5 and 15 refuges/10 m2. Each zone-density combination had three replicates. Revisions of shelters were made in the months of April, September, and November of 2014, and march and April of 2015. In each time was registered if shelters were used for the spawn of females of *O. maya*. When spawners were found, the entire spawns were taken and transported to the laboratory to evaluate fecundity. In both sites, pikes of occupancy were observed in November of 2014 and march of 2015, when a higher quantity of spawners was located in shelters placed in the sand zone ($P < 0.05$). Shelters in higher density were more occupied than in low density. A relationship between temperature and oxygen dissolved with occupancy was observed, with higher use of artificial shelters in the zone where the temperature was between 22 to 26°C and oxygen dissolved levels higher than 5 mg/L. The *O. maya* wild females fecundity was between 1187 to 1769 eggs/spawn, higher than observed in culture conditions. Results obtained in the present study indicate that the use of artificial shelters can be a good strategy to promote the *O. maya* reproduction in attempt to sustain the fisheries of this octopus species.

Session: Genetics and Evolution

P29

Biometric comparisons in the group *"Octopus vulgaris"* complex: Adaptive strategies of cosmopolitan populations

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Molecular studies have shown that several morphotypes of octopus have been under the name of *Octopus vulgaris* Cuvier, 1797, considered as cosmopolitan specie. Due to the significant oceanographical barriers that exist between the geographical distributions of these forms and the absence of any plausible gene flow mechanisms, this species group at present have been split. In order to generate knowledge about the potential selection pressures and other aspects that relate variations in the phenotype and environmental variables between spatially segregate populations of this group of octopods, we gathered morphological information from 14 regions around its distribution area, including specimens of *O. sinensis* and *O. maya*. We used Mantle Length (ML) - Total Weight (WT) relationships measured from specimens caught in the regions analyzed. The parameter α of the ML – TW relationships ($WT = \alpha \times ML\beta$) was considered as relative to the physiological state of the octopus, and the parameter β as the allometric coefficient ($\beta < 3$ negative allometry, $\beta > 3$ positive, and if $\beta = 3$ isometry). Results show that, the growth of species in the Mediterranean depict isometric patterns (from 2.6 to 3.4) with values of the α parameter between 0.3 and 1.5. However, in Mexico and the Northwest Atlantic, a trend towards allometry was observed (2.06 and 2.5), coupled with higher values of α (4.49 and 2.15). The results indicating that the organisms in Mexico and the NW Atlantic, tend to be more elongated and less robust towards adult sizes than in the Mediterranean Sea. This could be due to their phenotype has already been adapting to the environmental conditions, which differ from those present in the Mediterranean. The adaptive strategies of the species are discussed in association with habitat suitability.

P30

A mitochondrial phylogeny of the family Onychoteuthidae Gray, 1847

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The oegopsid squid family Onychoteuthidae was recently revised based on morphology, but sufficient material for a complementary molecular analysis has not been available until now. In the present study, over 250 sequences of cytochrome c oxidase subunit I (COI) and 16S rRNA for 222 individuals were analysed to create a combined phylogeny for the family. Results support monophyly for the family and all seven onychoteuthid genera (including Moroteuthopsis, established herein as the senior genus name for species formerly attributed to Kondakovia); 29 genetically distinct species were recovered, with the BIN analysis for COI showing good congruence overall with morphological species groupings. No sequences were available for five additional known species, making the total family diversity likely to exceed 34 species. Seven of the BINs formed in this study appear to represent undescribed taxa, suggesting that even in this relatively well-studied family, much additional work remains before a comprehensive understanding of the diversity and evolutionary relationships for Onychoteuthidae can be achieved.

P31

Molecular phylogenetic analysis of the squid family Histioteuthidae (Mollusca, Cephalopoda)

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Histioteuthid squids are an important part of marine food webs, being abundant in the diets of many predators. Although they represent a substantial biomass in the deep sea, their systematics are not fully understood; damaged (especially ex-gut-content) and paralarval specimens are difficult to identify morphologically, since most morphological characters presently used to distinguish species involve external photophore patterns. The purpose of this study was to test a morphological hypothesis for the division of the family Histioteuthidae into species groups using two mitochondrial genes (cytochrome c oxidase subunit I [COI] and 16S rRNA). Both the Bayesian and maximum-likelihood analyses supported the division of this family into six genera (formalising previously hypothesised species groups): *Calliteuthis*, *Fragariateuthis* gen. nov. *Histioteuthis*, *Histiothauma*, *Navia* gen. nov., and *Stigmatoteuthis*. Barcode Index Numbers based on COI and 16S rRNA were used to distinguish 17 currently accepted species, and revealed up to nine additional species, including potentially new, unnamed species. A DNA barcode reference library of sequences generated in this study is available on the Barcode of Life Data Systems (BOLD), which can be used to confirm identifications or identify damaged specimens, such as those from gut contents. This study is the largest, most complete phylogenetic analysis of this family to date.

P32

Composite variations in genetic structure, life-history traits, and statolith morphology of *Sepioteuthis lessoniana* populations around Nagasaki prefec

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Population structure studies of the species, bigfin reef squid, *Sepioteuthis lessoniana*, was categorized into two populations around Japan waters by body color, spawning depth and molecular markers, then identified as three lineages (A, B and C) in the Coral Triangle by molecular markers, but related life-history information and morphology description is scarce around these areas. Squid samples were collected from Nagasaki prefecture seas in six different locations (Tameshi, Sasebo, Goto, Omurawan, Nomozaki nishi and Nomozaki higashi) from June, 2016 to September, 2017. In total, we have collected 142 individuals for biology measurement and statolith morphology analysis, aged 134 samples by reading daily rings in statolith and investigated the genetic structure by CO1. The results demonstrated that two cryptic lineages (B, and C) of *Sepioteuthis lessoniana* were found in Nagasaki prefecture, and this study revealed two subpopulations of lineage C (C1 and C2) which are related to different season cohorts. Life-history parameters are different between sex, location and season. The morphology of statoliths also differs between the left and right side, and between locations and season subpopulations of *S. lessoniana*. We considered the subpopulation in different locations as stocks and different seasons as cohorts, and revealed the boundary between lineage B and C. The life-history parameters, statolith shape and DNA markers are useful methods to contribute to the fishery management units.

P33

Molecular tools to improve the knowledge of loliginid paralarvae and their assessment

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The cephalopod family Loliginidae has an important economic value in the small-scale fisheries in Galicia. Early life stages of loliginids are studied off the entire Iberian coast due to their socio-economic importance and to forecast their population abundance appropriately based on the correct identification of the species. Some previous studies carried out in the Ría de Vigo (NW Spain, NE Atlantic) classified the loliginid paralarvae according to morphological characters. Afterwards, molecular analyses using Cytochrome Oxidase I (COI) have shown that most individuals identified as Loligo vulgaris were mislabelled and thus, most of them belonged to the species *Alloteuthis media* and *Alloteutis subulata*. To date, the studies were restricted mainly in the south of Galicia. This study will reveal, for the first time, the composition of loliginid paralarvae in the Northern Galician coast. Fifty-five individuals were caught in the North of Galicia during June on board of R/V Sarmiento de Gamboa. Moreover, 153 loliginid paralarvae were collected in South of Galicia on board of R/V Mytilus between June and November. Among these, 51 were obtained in June. This research aims to know the composition of squid paralarvae in the North and South of Galicia based on COI analyses. The results of this investigation will shed light on the distribution, abundance and diversity of loliginids paralarvae along the whole Galician Coast.

P34

Understanding the evolutionary history of the *Sepia officinalis* species complex

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Understanding the evolutionary history of a species, as well as the contemporary processes of dispersal, adaptation and plasticity, is fundamental to the conservation and sustainable management of fisheries resources. The Sepia officinalis species complex (*Sepia officinalis*, *Sepia hierredda* and *Sepia vermiculata*) occurs in shallow coastal waters throughout the eastern Atlantic Ocean from the southern North Sea to South Africa, including the Mediterranean, and into the South West Indian Ocean. The close evolutionary relationships among species, in combination with the range of potential barriers to gene flow across its wide distribution makes the *S. officinalis* species complex an interesting model in which to test the genomic basis of speciation. Despite its commercial and artisanal fishery importance much of the species range remains relatively uncharacterised in regards to the processes that have shaped spatial patterns of intra- and inter-specific genetic diversity. This research will report the results of genome-wide genotyping-by-sequencing (GBS) of the *Sepia officinalis* complex collected across its Atlantic distribution. Phylogenetic approaches will be used to describe the evolutionary history of the three species with emphasis on the dynamics of historical vicariance imposed by the Benguela current system. In addition, seascape analyses coupled with outlier tests to identify non-neutral (i.e. selected) genetic variation will be used to assess recurrent environmental drivers of neutral and adaptive population structure. This study will provide a novel insight into how the species complex has been affected by historical environmental change as well as providing a basis for improved prediction of future climate change effects. Such a holistic approach is necessary to ensure sustainability given the predicted increase in commercial pressure that cephalopods will face.

P35

Effect of fast temperature shifts and prolonged heat exposure over the metabolic performance and gene expression of *Octopus maya* juveniles

Oscar E. Juárez, Laura López-Galindo, Leonel Pérez, Fernando Díaz, Carlos Rosas, Clara Galindo-Sánchez

Octopus maya juveniles can rapidly find the temperature where their metabolic performance is the best. However, the thermal anomalies in its distribution area could reduce the periods of favorable temperatures for their optimal metabolic performance. In this study, we assessed the metabolic and molecular response to fast temperature shifts and prolonged heat exposure, testing if the juveniles are adapted to this warming scenario. The juveniles were acclimated 60d at different thermic conditions: 24 °C, 30 °C, and a temperature increase (ramp) from 24 to 30 °C (1 °C every 5 days). Then, they were exposed to acute thermal stress (minimum and maximum) for 5 minutes. The energetic balance, survival and expression levels of genes related to oxidative stress, thermal stress and apoptosis were compared at day 30. An additional assessment of growth and survival was performed at day 60. Until day 30, juveniles acclimated at 30 °C showed the highest energy production and growth. However, signs of stress were detected, since repair genes involved in protein re-folding, apoptosis and response to oxidative damage were highly expressed in this condition. Juveniles from ramp treatment showed the same survival (78%) and similar genetic expression values to those acclimated to 30 °C. By contrast, at day 60 the juveniles acclimated to 24 °C showed higher growth and survival (89%). Moreover, genetic expression patterns in this group suggest better mitochondrial performance and an effective protection against oxidative stress, in response to a metabolic rate increment after the acute heat exposure. In conclusion, high temperature (30 °C) favors the energetic balance in the first 30d. Nevertheless, the physiological performance reduces if juveniles persist in this temperature, suggesting that they are not adapted to prolonged heat exposure.

P36

Transcriptomic analysis of *Octopus maya* white bodies show sex-specific signaling processes during reproduction

Oscar E. Juárez, Laura López-Galindo, Leonel Pérez, Carlos Rosas, Clara Galindo-Sánchez

White bodies (WB), soft multilobulated tissue that connects each eye with the optic lobes, are the hematopoietic organ of the cephalopods. Its glandular appearance and its lobular morphology suggest that different parts of the WB may perform different functions, but a detailed functional analysis of the octopus WB is lacking. The aim of this study is to describe the transcriptomic profile of WB to better understand its functions. In addition, we compared these profiles between sexes before and after reproductive events, to test if such events modify gene expression in this organ. Then, validation via qPCR was performed using different tissues (systemic heart and gonad) to find out possible sex and tissue-specific transcripts. Sex-related differences were observed in the WB transcriptomic profiles. In this regard, males showed higher expression of genes from the androgen receptor-signaling pathway, and genes involved in microtubule-based process and fertilization. Moreover, key genes involved in the immune response and the defense against pathogens showed higher expression in males. On the other hand, females presented higher expression of genes involved in the glycoprotein catabolic process, fat pad development and response to glucocorticoids. Additionally, the neuropeptide, Notch, and integrin-mediated signaling pathways were among the best-represented categories in females. Remarkably, transcripts with similar sequences to FMRF-amide neuropeptide gene, which is involved in reproductive regulation, were only detected in the WB with significant higher expression in females. These results suggest that the WB of adult *O. maya* not only controls hematopoiesis, but also regulates signaling processes and the immune response in a sex-specific way.

P37

Assessment of cephalopod biodiversity and distribution around the Cape Verde Archipelago using environmental DNA

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Traditional monitoring techniques (e.g. nets, in situ observations) are often expensive, invasive and ineffective at detecting rare or elusive species. The analysis of DNA directly from environmental samples (eDNA) is an increasingly used technique for biodiversity assessment and unravelling marine ecological processes. The technique is non-invasive, very sensitive, and only small amounts of DNA from water or sediment samples are necessary. eDNA could be a promising tool for the detection of cephalopod species that can escape approaching nets and cameras or that are not very abundant, but so far it has not been used in any cephalopod studies. The aim of this study is to test and optimize eDNA analysis for biodiversity and distribution analysis of cephalopods and other pelagic invertebrates in oceanic ecosystems. During a recent R/V Poseidon cruise (journey POS520) in the eastern tropical Atlantic around the Cape Verde Islands, we isolated eDNA from seawater samples, collected at a series of three stations in depths between 50 and 1000 m, at three different stations (off Fogo, Santa Antão and north of São Vicente). Water was filtered and the filter with eDNA was then frozen at -80°C until PCR amplification of marker genes. We used next-generation DNA sequencing to sequence the PCR amplicons that were present in the samples. As a reference DNA barcode library we used tissues obtained from specimens captured in nets during the same cruise and from previous cruises, as well as sequences already available in GenBank. We investigated local cephalopod community compositions, and also looked at the distribution patterns of elusive species such as *Vampyroteuthis infernalis, Architeuthis* spp. and *Taningia danae.*

P38

De novo assembly of cuttlefish (*Sepiella japonica* Sasaki) transcriptome during different embryogenesis period based on RNA-Seq data

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Coleoid cephalopods, such as cuttlefish, are active, resourceful predators with a rich nutrition and medicinal value. They present striking morphological innovations including camera-like eyes, prehensile arms, and a highly derived early embryogenesis etc. However, less is investigated about the transcriptome during embryogenesis of cuttlefish. In this study, cuttlefish (*Sepiella japonica* Sasaki) eggs were collected on day one. The growth and development of eggs were observed each day. Eggs of cuttlefish at the three stages of eye primordium formation (SJ1) stage, arms, gills and fins formation (SJ2) stage, and larval (SJ3) stage were collected and used for RNA-Seq analysis. 24 million quality-filtered and trimmed cDNA sequence reads were obtained using the Illumina HiSeqTM 4000 platform. A de novo transcriptome reconstruction based on the RNA-Seq data was made, resulting in 45181 contigs and 142053 transcripts, which resulted in 57160 unigenes with an average length of 3137 bp and maximum length of 15,471bp. The total different genes between SJ1 stage and SJ2 stage were 4341, which were characterised by having approximately 3 times the number of different genes (1441) between SJ1 stage and SJ3 stage. The different genes in biological process were the most frequent categories, followed by cellular component between SJ1 and SJ2 stage, and also between SJ1 and SJ3 stage. However, the total different genes between SJ2 stage and SJ3 stage were 88, in which, the different genes in biological process were the most frequent categories, followed by molecular function.

P39

New frontiers: The potential applications of eDNA in cephalopod science

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With eDNA analysis, DNA is isolated directly from an environmental sample, such as soil or water, without first isolating any type of identifiable organism. Because eDNA does not persist in the marine environment for long, the results are in real time, and the methods can be applied on any time-scale, from the monitoring of daily migration patterns to whole population range shifts in response to variations in climate. It can also be adapted further than collection of free-floating DNA, by using nets with different mesh sizes, to target single cells or planktonic organisms of a specific size, such as elusive cephalopod paralarvae. Application of eDNA methods have been shown to work for detecting and monitoring not only common species, but also those that are endangered, invasive, or elusive, and it could therefore be an especially potent tool for presence/absence monitoring of cephalopod species that are hard to observe, such as the roughly 45 percent of all known cephalopod species, which are non-commercially important open-ocean or deep-sea squids and octopuses. Eventually, further development and application of these tools will open up an entirely new avenue for the study of population and community dynamics of these cephalopods. Furthermore, it may not remain restricted only to presence/absence or relative abundance estimates. Remote population genetic analyses may also be possible, as was recently demonstrated for an aggregation of whale sharks in the Arabian Gulf. Lastly, the impact of eDNA methods may be felt by researchers working on development of aquaculture and breeding of captive cephalopods as well. The problem of collecting sufficient DNA for genotyping without causing lethal injury to the larvae has recently been solved by genotyping of free-swimming, early fish larvae in a non-lethal and non-invasive way, by collecting and characterizing their eDNA, in a way that could quickly be adapted for cephalopods.

P40

Reacquisition of Argonaute outer shells on the octopus genetic background

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Argonaute octopuses, also known as the paper nautiluses, form own outer shells and harvest the eggs in the shells. It is clear that the argonautes acquired the shell again since the ancestral octopods have lost the outer shell. This is contrary to Dollo's law, proposed by paleontologist Louis Dollo, that is "an organism never returns exactly to a former state". The mechanism that the argonautes were able to form the shell, especially the genetic background, has not yet been elucidated. Regarding the responsible genes of shell formation, the argonautes possibly 1) lost the shell-forming genes, but developed novel type of shell-forming genes or 2) recruited the shell-forming genes through the shell-less octopuses keep the gene sets in the genome. Argonauta argo and A. hians are often found around the Oki Islands, Shimane, Japan. We clarified the shell-forming genes by using bioinformatics and transcriptome analysis of the two species. The transcripts were reconstructed from the RNA-Seqs from the first arm, the second arm, and mantle, and compared the gene expression among those tissues. Homology searches found 45 shell-forming genes, showing homology to that of bivalves, snails, and *Nautilus*, in the two argonautes in common. It was also revealed that the shell-forming genes are present not only in the first arm, which was believed to secrete the shell but in the other sites. Phylogenetic analysis revealed that the homologs of shell-forming genes were also kept genome of California-two-spot octopus (*Octopus bimaculoides*) as well. From these results, our data support hypothesis 2), the shell-forming genes are preserved even after the octopus has lost the outer shell and are not used in the shell formation process in common octopus.

P41

PEPTRAQ: a new data analysis tool for searching through –omics databases

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New high-throughput sequencing technologies allow for the sequencing, assembly, and annotation of transcriptomes and/or genomes regardless of the organism of origin. However, in so-called "unconventional" animal models and more particularly invertebrates, in silico analysis of the data remains tedious for lack of sufficient functional annotations and adapted computer tools. The PEPTRAQ software tool was developed by both biologists and computer scientists of the Normandy University of Caen (France) to improve these in silico analyses. This software tool searches databases for specific data on the basis of multiple criteria such as transcript, protein precursor, protein or peptide length, peptide hydrophobicity, occurrence of a signal peptide, conserved subsequences, amino acid patterns, etc. PEPTRAQ has to be considered as a tool dedicated to the investigation of available genomic, transcriptomic, and proteomic databases issued from conventional and unconventional models such as cephalopods. Also designed to translate and re-annotate databases, PEPTRAQ is a multipurpose and user-friendly software tool that allows users to create personalized workflows adapted to answer most of the questions in the field of data mining. As PEPTRAQ saves filtered data in fasta files, it is a powerful tool that generates targeted and reduced databases for mass spectrometry analysis in peptidomic or proteomic approaches.

Session: Life History, Ecosystem Roles

P42

The links between the bacterial profiles of seawater, feces, and egg masses of *Todarodes pacificus* in an aquarium

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Background: The egg mass of the Oegopsida squid species, *Todarodes pacificus*, plays a crucial role in development, is at least 80 cm in diameter, and is mainly composed of jelly-like components secreted from nidamental/oviducal glands. The egg mass cumulatively contains around 200,000 oval-shaped eggs, and it takes approximately 1 week for eggs to hatch after spawning in the egg mass. On hatching, the egg mass is slowly broken down and the paralarvae are released into the ocean. In the present study, we successfully obtained the egg mass from an aquarium environment, as wild egg mass is difficult to obtain. The egg mass is transparent immediately following spawning, yet it appears clouded in the late stage of hatching (4-5 days after spawning). This presumably occurs by microorganisms adhering to the egg mass; however, the details of this remain unknown. The purpose of this study was to analyze the bacterial profile of these adhering microorganisms by comparing with the profiles of seawater, feces, and symbiotic bacteria of squid using a metagenomic approach based on 16S rDNA.

Materials and Methods: Samples were mainly obtained from the aquarium of the Hakodate Research Center for Fisheries. The DNA was extracted from the adhering microorganisms of the egg mass, feces, seawater, nidamental glands of the adult, as well as from the whole body of wild and aquarium (n=8) paralarvae. Following PCR on the v1-3 region of 16S rDNA, metagenomic analysis was performed using MiSeq.

Results: At the genus level, the population of bacteria adhering to the egg mass primarily comprised Vibrionaceae, Oceanospirillaceae, and Flavobacteriaceae. The principal component and tree analysis showed that the egg mass profile was variable, but was most similar to those of the nidamental gland. These results suggested that the adhering bacteria might have originated from indigenous or symbiotic bacteria of the nidamental gland (the secretary organ of adult), rather than from seawater or feces.

P43

Relationship of the relative abundance of *Octopus maya* and its environmental suitability in the Yucatan Peninsula, Mexico by a maximum entropy model

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The ecological niche models (ENM) were developed with the objective of characterizing the spatio-temporal patterns of suitability of different species (ecological niche). However, their effectiveness in predicting population elements such as abundance have been scarcely evaluated. The few studies that have compared the suitability of the environment with the abundance of species usually obtain inconclusive results. In this sense, the present study was directed to evaluate if there is a relationship between catch per unit of effort, (CPUE) of the *Octopus maya* and the environmental suitability obtained by an ENM. Data of two years (2012-2014) of CPUE of *O. maya* from the artisanal coastal fleet of the Yucatan Peninsula, Mexico were georeferenced. An analysis of maximum entropy (MAXENT) was performed with the presence data using as environmental variables, the oceanic layers of "marspec" at a pixel size of 1 km2, generating a map of environmental suitability. Subsequently, the values of environmental suitability for octopus were extracted and contrasted with the CPUE values. The results indicated that there is a positive association between CPUE and environmental suitability. The ability to predict CPUE using a MAXENT model suggests that ENM can be potential tools for fisheries evaluation. It is important to note that this association should be evaluated with more marine species and a variety of other techniques. If the results continue to be positive, we are faced with new tools for the evaluation of marine resources.

P44

How do mesopelagic octopods breathe in oxygen minimum zones?

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The pelagic octopod, *Japetella diaphana*, is known to inhabit intermediate depths worldwide. Across its range, individuals encounter oxygen levels ranging from nearly air-saturated to nearly anoxic. In this study, we compared the physiological acclimations of individuals from the relatively oxygen-rich Gulf of Mexico and Hawaii in contrast with individuals from the California coast and eastern tropical Pacific (ETP) where oxygen is extremely low. Ship-board measurements of metabolic rate and hypoxia tolerance were conducted and a metabolic index was constructed to model suitable habitat. Surprisingly, we found that animals from the ETP had a higher metabolic rate than animals from more oxygen-rich habitats. Despite their higher oxygen demand, they maintained better hypoxia tolerance than animals from oxygen-rich Hawaiian waters. Furthermore, we found that hypoxia tolerance in *Japetella* has a reverse temperature dependence from most marine ectotherms, a characteristic that uniquely suits the physical characteristics of its oxygen-poor environment.

P45

Cephalopod paralarval community of the Northwest Mexican Pacific

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The paralarval community of three areas of the Northwest Mexican Pacific was identified and analyzed: The Western Coast of the Baja California Peninsula (WC), the South West Coast of Baja California Sur (SWC), and the Gulf of California (GC) and its relationship with the seasonal and temporal flow of the water masses. We analyzed 14 oceanographic cruises (1997-2007) with a total of 566 stations obtained from surface and oblique trawls. There were 3 suborders, 14 families, 26 genera, 28 species, 24 morphotypes and the SD Complex (*Sthenoteuthis oualaniensis-Dosidicus gigas*). The abundance of paralarvae in both types of trawls was higher in the CG, where the highest number of taxa at species level was also recorded (33) than in the SWC and WC (both 24). The main families on surface collections were the Ommastrephidae and Enoploteuthidae in the SWC and GC. In the obliques were the Ommastrephidae and Onychoteuthidae (WC), Ommastrephidae and Enoploteuthidae (SWC), and Pyroteuthidae and Argonautidae (GC). The canonical correspondence analysis for surface sampling showed paralarvae of spring in the SWC in a productive environment (high zooplankton volumes) related to Subarctic Water, and a more tropical community during the fall and summer influenced by Tropical Surface Water both in the SWC as in the GC. For the oblique trawls, the separation of the winter and summer WC samples from 1998 and 1999 (El Niño and transition to La Niña) was influenced by the advance of Transitional-Subtropical Surface Water and its confluence with Subarctic Water in chlorophyll-a concentrations higher than the rest of the seasons and areas. In these lasts, the paralarvae were related to the temperature gradient modulated by the seasonal and spatial domain of the water masses: Subarctic, Transitional, and Tropical Surface waters in the WC and SWC, and of Gulf of California and Tropical Surface waters in the GC.

P46

Effect of semi-moist and dry food in the nutritive, digestive and respiratory response of juveniles of the red octopus *Octopus maya*

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The effect of a food formulated and presented in semi-moist and dry form through the responses in the proteolytic activity and content of soluble protein and cholesterol of the digestive gland, as well as growth, survival and respiratory activity was evaluated in juveniles (0.45 g) of *O. maya* during 45 d. The food formulation (Martinez et al, 2014) was elaborated in the form of a semi-moist paste and in a pelleted form, the latter being dried at 45ºC and made with lyophilized ingredients. Both foods were added with a mixture of vitamins, minerals and agglutinated with gelatin. At the end of the experimental period, no differences were observed in weight gain () or SGR () ($p > 0.05$), but in the survival and yield index, where the octopuses fed the pelleted diet had the highest values (70% and 2.87 g d-1 respectively) compared to those maintained with the diet in the form of pasta (43% and 1.98 g d-1) ($p < 0.05$). The highest activity of the alkaline proteases is observed in the octopuses subjected to the pelleted diet ($p < 0.05$). There were no differences in the content of soluble protein and cholesterol in GD ($p > 0.05$). Finally, a greater increase of apparent heat evaluated through oxygen consumption is observed in the octopus of the pelleted diet (0.3979 ± 0.0003) in relation to those maintained with the semi-wet paste (0.1812 ± 0.002) ($p < 0.05$). The results obtained show that the pelleted feed was beneficial for the cultivation of early juveniles of *O. maya* and is a viable alternative for the development of their aquaculture.

P47

First approach to the population structure and reproductive dynamics of *Octopus insularis* in the Veracruz Reef System (Mexico)

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To date, all Mexican Atlantic fishery management policies are based on *O. maya* or *O. vulgaris*. However, *Octopus insularis* has been recently identified as the main species targeted by the artisanal fishery operating in the Veracruz Reef System (VRS). Therefore, the aim of this study was to describe the population structure and reproductive dynamics of *O. insularis* to provide basic information for future management. As part of a long-term study, so far we have sampled 338 octopuses from the artisanal fishery in the VRS during November and December 2017 and in March 2018 (excluding closures). Specimens ranged 56.8–172 mm in mantle length (ML) and 127.5–2,028.3 g in body weight (BW). The 58% of the females were immature, while 26% were maturing, 13% mature and 3% spent. The female size at maturity was 743.3 g BW (C.I. 673.8–810.2 g) and 97.7 mm ML (C.I. 94.3–100.7 mm), estimated by Bayesian methods. November and December were dominated by immature females (82 and 71% respectively) whereas in March their number dropped to 28%. Mean female octopus BW was significantly higher in March, coinciding with a greater percentage of mature specimens (28%). Regarding males, mature specimens dominated the catches (88%), followed by maturing (9%) and immature individuals (2%). Mature males dominated catches in all months, and reached a maximum value of 97% in March. The overall sex ratio did not differ from 1:1 but males were more frequent in December. Our preliminary results show that female size at maturity was estimated as being 30% smaller than the current minimum catch size established for octopus females in the VRS (140 mm ML), hence, we suggest that a revision of local fishing policies should be conducted in order to properly assess the management of this commercially valuable species.

P48

Prey-handling and preference of octopus on red abalone (*Haliotis rufescens*) and potential impacts on recovering abalone populations

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Stock enhancement using captive reared juveniles is a common method used to restore wild populations of endangered and threatened marine animals, such as the white abalone (*Haliotis sorenseni*). However, these stocked juveniles are met with high predation mortality that could limit the success of these efforts. Octopuses (*Octopus bimaculatus*) are primary predators on juvenile abalone, and will sometimes drill through the abalone shell to access their prey. This study aimed to 1) learn more about the placement of these drill holes, 2) determine if a common abalone tagging method increases susceptibility to octopus predation, and 3) test a shell coating as an octopus deterrent. Octopus strongly prefer to drill over the central part of the abalone shell, where the adductor muscle is located, and this placement appears to be an innate rather than learned behavior. Octopuses showed no preference between tagged and untagged abalone, supporting that this method of marking abalone does not increase vulnerability to octopus predation. Finally, octopuses strongly avoided consuming abalone with a wax shell coating. These results will inform future abalone stocking efforts to both reduce initial predation mortality of stocked juveniles and enhance our understanding of octopuses as predators in rocky reef ecosystems.

P49

The trophic role of *Octopus insularis* in the food web of Rocas Atoll, Southwest Atlantic

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Octopuses play an important role in marine trophic webs. The species *Octopus insularis* is frequent in shallow reef areas of Brazilian coast and oceanic islands. As a time-minimizer predator, it feeds mostly on small and abundant organisms, thus reducing foraging trip duration and its own predation risk. However, the occasional consumption of larger prey such as marine birds, newborn turtles and other cephalopods shows a notable level of opportunism that hampers understanding octopuses' place in ecosystems. Therefore, this work aimed to identify the trophic position (TP) of *O. insularis* in order to assess its importance as prey and predator in the food web of Rocas Atoll, a pristine Marine Conservation Area. Samples of octopuses and all other organisms in the food web were analyzed thought stable isotope data (d13C and d15N) and their trophic positions were calculated. The food web exhibited at least three trophic levels, with *O. insularis* showing some trophic fluctuation and occupying a TP between the 2nd and 3rd levels (mean TP=3.16), characterizing as a top consumer of the benthic web alongside morays. The high consumption of small crustaceans, gastropods and benthic fishes corroborated the foraging strategy described for this cephalopod, based on den remains prey. Moreover, there was a significant relationship between TP and size of octopuses (R2=0.37; p=0.005), indicating ontogenetic changes in their diet. Marine birds, nurse sharks, serranid and lutjanid fishes were the main predators of *O. insularis*, which made up 11-14% of their diets. This relatively small contribution was probably a consequence of the cryptic behavior of these animals and their ability to escape and avoid attacks. Finally, the trophic plasticity and dominance of *O. insularis* in Rocas Atoll support a potential role of keystone species for the benthos and its removal from the environment would result in serious changes in the community structure.

P50

The spawning and hatch of juvenile of the deep-sea cephalopod, *Opisthoteuthis depressa*

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Opisthoteuthis depressa is a spicies of cirrate octopoda belonging Opisthoteuthidae, which are found at the depth from 200-1000m in the sea off Japan, and there are scarce knowledge about its ecology. Recently, in the captive environment, the spawning of *O. depressa* was observed in the tank, and gave hatchling from the egg extracted from another dead animal. Observed individuals were collected from the deep sea about 180 m deep off Heda, Numazu City, Shizuoka prefecture, Suruga Bay by commercial trawl fishery. A collected individual was transported immediately to Tokyo Sea Life Park and kept in oval tank. Spawning was observed on 31st day (May 20, 2016) from keeping start. Spawned egg sac was white, opaque, about 27 mm in length, covered by a sticky material, and adhesion matter up in tank bottom. Two eggs were recognized in egg case. The egg diameter was 9mm in this time. The egg sheath turn the color brown as time advances. Spawned egg was observed no any change in 199 days (December 5, 2016) after spawning, and determined to be the non-fertilized eggs. Another extracted egg, 10mm in length, from the funnel of fresh dead animal were incubated. The egg was artificially glued on stone using Cyanoacrylate resin. The hatch out was observed 147 days. The hatchling was 15mm in total length, and characteristic in proportionally broad fins, minute and numerous chromatophores, opened eye lid, and crawled the bottom of tank. It was significant that the fins are relatively larger to its body size in the juvenile's body shape than that of adults. When a thawed-out frozen rotifers was dropped on the hatchling on the hatching date, the reaction carrying it under the web, which is similar behavior in adult. The hatchling was survived 5 days after hatching out.

P51

Cephalopods in the Aegean Sea food web

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Towards a future ecosystem-based management of fisheries, it is essential to determine component keystone species in a certain marine ecosystem and understand their role in food-web structure and functioning. This study aims to the investigation of cephalopd species role in the food web of the Aegean Sea (northeastern Mediterranean) through the review of information concerning cephalopod species occurrence and size structure derived from marine species dietary studies published up to now, including also additional information resulted from re-examination of cephalopod remains not fully explored and compilation of unpublished relevant data. A total of 45 species preying upon cephalopods have been reported in the Aegean Sea, including 21 bony fish, 15 elasmobranchs, 3 shrimps, 5 cetaceans and 1 marine mammal. Identified cephalopod prey species belonged to 17 families, including 18 oegopsid squids, 3 myopsid squids, 8 incirrate octopods, 3 cuttlefishes and 5 sepiolids. Mantle length of preyed specimen ranged between 11 and 540 mm, with larger individuals of pelagic Ommastrephids noted among preys of sperm whales, where as, small sized juveniles of Enopoloteuthids and sepiolids were more frequently encountered among preys of shrimps and demersal fish.

P52

Effect of temperature in embryonic development of *O. vulgaris* type II in laboratory

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Temperature is a key factor for octopus embryos development, because it governs the physiological mechanisms involved in organogenesis. In several octopus species there are evidences that indicate that beyond the thermal limits reactive oxygen species (ROS) provoke thermal stress, causing morphological and physiological damage, affecting the survival of paralarvae. The present study was directed to evaluate how temperature modulates the antioxidant defense mechanisms (ANTIOX) during embryonic development of *Octopus vulgaris* Type II from Brazil. Embryos were exposed at 1) 18°C constant and 2) a thermal ramp, increasing 1°C every 5 days until hatch.

Wild *O. vulgaris* Type II adults were kept in the laboratory for spawning. After spawning (stage I-IV), strings of eggs were randomly distributed in artificial incubators with filtered and UV sterilized recirculatory seawater system, and temperature control of ± 0.5°C in triplicate. Every 5 days, from experimental units were collected to analyze: ANTIOX biomarkers: Superoxide Dismutase (SOD), Catalase (CAT), Carboxylase (CBE), Total Glutathione (GSH), Glutathione-S-Transferase (GST), Lipid Peroxidation (LPO), Protein Peroxidation (PO) and also Acetylcholinesterase (AChE).

Embryos kept at constant temperature of 18°C reached stage XX after 68 days with a hatching rate of 23%. Embryos in Ramp reached stage XIX after 48 days of incubation with a hatching rate of 33%. Results demonstrated that in the early stages of development and the two experimental treatments the ANTIOX defense mechanisms were not active, elevating the levels of LPO, probably of maternal origin. After, a reduction on LPO indicated that ANTIOX mechanisms were activated, when organogenesis ended. Also, it was observed that both lipidic enzyme activities and ANTIOX mechanisms were reduced after hatch in organisms maintained in ramp condition, suggesting that 24°C could be the thermal limit for embryos of this species.

P53

Role of cephalopods in ecosystem functioning and evolution

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Ecosystem structure and functioning are influenced by many biotic and abiotic factors. However, their evolution is often driven by key components or species. Cephalopods play a key role in the transfer of nutrients through trophic levels as they interact with numerous elements of the food web and have high productivity. In the context of climate change and increasing fishing pressure, understanding the role of cephalopods is important as their fast life-cycle allows them to respond quickly to external pressure. Their abundance has already been seen to increase worldwide, while a combination of factors caused an increase of interest from fisheries. The development of ecosystem models is a great step towards a global understanding of marine systems functioning, however, they still suffer from limitations. We reviewed ecosystem models containing cephalopods as a functional group and observed a need for improvement as the group is often coarsely defined and models are often not used to their best capacities, diluting the importance of this ecosystem engineer.

Our objective was to develop an ecosystem model with an adequate representation of cephalopod ecology, in order to identify the key ecological processes involved in their role, and to assess their potential impact on system evolutions in the future. The key processes newly represented in the ecosystem model are the capacity of some cephalopod species to have several cohorts in a single year, opportunistic behavior and ontogenic growth and diet shifts. In an adaptive perspective, these implementations are a valuable step towards the understanding of cephalopods capacity to respond to climate change and fisheries impacts, and their potential to drive system evolutions. The sensitivity of the ecosystem represented to various scenarios of climate change and management strategies will provide a useful tool to assess the optimization of human exploitation while mitigating impacts on marine systems.

P54

Squid *Gonatus fabricii* (Cephalopoda) occupies the trophic level of a top predator in the Arctic: interferences from the beak stable isotope analysis

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Gonatus fabricii is very abundant in Arctic waters, and the only squid with a complete life cycle in the Arctic. In order to understand the ecological role of *G. fabricii* in the Arctic, we conducted a stable isotope analysis of beaks from all ontogenetic groups from West and East Greenland waters and the Barents Sea. The values of both δ13C and δ15N of *G. fabricii* were not related to sex. δ13C showed a low ontogenetic increase, and it was geographically distinct, with highest values found in the western part of the study area. On the other hand, δ15N values showed an intense ontogenetic increase (i.e. 10.0‰ δ15N; 2.6 trophic levels), from epipelagic larval and juvenile forms to large bathypelagic adults, and no significant geographical variation. The observed maximum value of δ15N (14.9‰) is the highest ever recorded in cephalopod beaks. The estimated trophic level (up to 5.1) only compares to vertebrate top predators such as large piscivorous fishes, seals and toothed whales or large benthic scavenging fishes in the Arctic. Thus, *G. fabricii* is an invertebrate top predator in the Arctic, with the widest trophic niche observed to date for any species living there. Its trophic level is among the highest ever recorded in cephalopods, only exceeded by its Antarctic congener, *G. antarcticus,* and by Antarctic colossal squid, *Mesonychoteuthis hamiltoni*. Thus, the polar squids occupy higher trophic positions than squids living in warmer regions. Finally, our study shows *G. fabricii* descents downwards to bathypelagic layers during ontogenesis, continuously increasing its trophic level, by changing prey types and sizes, and avoiding predator pressure.

P55

Global body size-trends in coastal cephalopods

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Understanding patterns of body size variation is a fundamental goal in ecology, but contrarily to happens in the terrestrial biota, the information about broad-scale latitudinal trends of body size in marine fauna is poorly known. Here, we conducted a comprehensive survey of interspecific body size patterns in coastal cephalopod mollusks, covering both hemispheres in the Atlantic, Indian and Pacific Oceans. We hypothesise that latitude and depth range will have a profound effect on maximum body size in each of the major cephalopod groups (cuttlefishes, squids and octopuses), and will consistent among oceans. Concomitantly, and still ongoing, we are investigating the relationship between body size and thermal energy (i.e. sea surface temperature), resource (i.e. primary productivity) and habitat availability (i.e. extension of continental shelves). Last, we also predict unexpected body-size variations between western and eastern margins possibly linked to upwelling systems dynamics.

P56

Paralarvae of the order Octopoda east of Campeche Bank

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Although there is an increasing number of studies on paralarvae (PL) of cephalopods in the Mexican Pacific, the Atlantic coast of Mexico and, in particular, the coast of Yucatan lack information on this life cycle phase of octopuses and squids. The wide (245 km) and shallow (50 m average) continental shelf of Yucatan supports the fishery of two species of octopus: *Octopus maya* and *O. vulgaris* with records of at least seven more species of octopus in the area. Given the relevance of PL to understand the life cycle of octopuses and the management of their fisheries, this study presents the first results of the morphological analysis of PL of the order Octopoda collected during seven oceanographic campaigns (May-June, July, October, and December 2016, and January, September, and November 2017) carried out in the shallow sublittoral zone of Yucatan where part of the octopus fishery is carried out. The samples were obtained by surface trawls with a CalCOFI net (60 cm diameter, 500 μm mesh) during 15 minutes in 30 sampling stations, and were fixed in 7% formalin in seawater or in 96% alcohol. We identified 80 PL of octopus equivalent to a total of 660 PL/1000 m³ constituted by three genera and three species: *Amphioctopus burryi, Macrotritopus defilippi* type A and type B and *O. vulgaris,* plus five morphotypes to the category of species. Highest abundance was found during the "dry" and "nortes" seasons. During the study period, the most abundant taxa *were O. vulgaris* (52%), *Octopus* sp. 2 (17%) and *A. burryi* (13%). *O. vulgaris* dominated during the 2016 cruises but not during 2017 when *A. burryi* was dominant, presenting a distribution pattern located in the northeast region of the Campeche Bank in correspondence with the octopus fishing area in Yucatan.

P57

Assessing the life cycle and longevity of Antarctic incirrate octopods (Cephalopoda: Octopoda)

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Life cycle studies on cephalopods have concentrated on the near shore and commercially exploited forms of squids and octopods. For coastal octopods (*Octopus* spp.), age and growth studies based on quantification of growth increments in beaks revealed that the increments are deposited on a daily basis, and the lifespan is typically less than 2 years. Studies based on brooding times of deep-sea octopods suggest longer lifespans, but data is still scarce. To test the hypothesis that octopods inhabiting cold waters (< 5°C) live longer than coastal and warm water species, this study investigated size-at-age, maturity stages and growth rates in incirrate Antarctic octopods. Age was estimated through the interpretation and quantification of growth marks in their beaks. The studied species included *Pareledone charcoti, Pareledone aequipapillae, Megaleledone setebos* and *Muusoctopus rigbyae* which were all collected during a 27 days cruise on the shelf and slope regions off the Antarctic Peninsula in 2012. The growth increments were counted along the beak's lateral walls using a combination of reflected and transmitted light microscopy under 32-50x magnification. Specimens examined included early juveniles to specimens in advanced maturity stages. Spent females were observed only in *M. rigbyae*. Growth increment number ranged from 192-540 in *P. aequipapillae* (Body Mass 2-90 g), 182-431 in *P. charcoti* (BM 5-124 g), 98-906 in *M. setebos* (BM 1-3858 g) and 208-425 in *M. rigbyae* (BM 24-256 g). After the cruise, eleven specimens of *P. charcoti* were kept alive in captivity for more than 12 months and presented 219-364 growth increments, suggesting that increment formation in this species takes longer than one day. Due the short time duration of the sampling collection and the complex population structure exhibited, our findings suggest that Antarctic octopods live longer than warm water relatives and may have lifespans exceeding 3 years.

P58

Vampire squids are not detritivores

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We once saw a large vampire squid, *Vampyroteuthis infernalis*, ingest a live adult cock-eyed squid, *Histioteuthis heteropsis*, in a shipboard aquarium. This simple observation, though certainly not evidence of a natural predator-prey relationship, casts doubt on the recent suggestion that the vampire squid is a detritavore. We tested the hypothesized trophic status of *V. infernalis* using a large dataset of 13C/12C and 15N/14N isotope ratios from protein extracted from 179 individual midwater animals representing all the major midwater phyla. All samples were captured on the same day by midwater trawling at depths from the surface to 1400 m in the 2000 m water column of San Clemente basin off Southern California. Overall δ13C values ranged from -18.5‰ to -23.5 ‰ and δ15N values ranged from 7‰ to 18.5‰ with neither showing a significant depth trend across the entire dataset. At the same time there appear to be animals of all groups feeding at low trophic levels at all depths in the water column. Values for *V. infernalis* (δ13C ~ -21; δ15N ~14) fell well above gelatinous consumers (δ15N values < 12), many of which are known to be low trophic level carnivores or detritavores, and within the broad range of predatory fishes. We interpret these data to indicate that sedimenting matter is consumed by a large component of the midwater community but that *V. infernalis* feeds predominantly at a higher trophic level. Metabolic measurements for *V. infernalis* and the measured flux of sedimenting material also suggest that a detritus-based diet would not easily support the metabolic requirements of these large animals.

P59

Mode of infection of the Japanese flying squid, *Todarodes pacificus* (Cephalopoda: Ommastrephidae) by anisakid larvae.

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The Japanese flying squid (*Todarodes pacificus*) is generally infected by third-stage larvae of two species of anisakid nematodes. Anisakis simplex s.l., generally appears in an encapsulated form in the outer wall of the stomach and caecum of the squids, whereas *Lappetascaris* sp., is found at the anterior end of the mantle musculature. However, the infection process itself has not been studied experimentally so far. This study has for the first time, experimentally verified the infection process of third-stage larvae of the two species of anisakid nematodes in individuals *T. pacificus*. The larvae were labeled by injecting a 5%FITC-biotin into their caudal end. The labeled larvae of anisakid nematodes were included in the prey to test whether they were infected to squids through prey intake. A. simplex s.l. larvae were infected on the outer walls of the stomach and cecum, but no *Lappetascaris* sp. larvae were found anywhere in the body. Next, to test whether the free-swimming larvae was infected to squid, one squid and several labeled larvae were enclosed in oxygen pack at a time. A. simplex s.l. larvae were not infected, but *Lappetascaris* sp. larvae were attached to the gill. These results indicated that the third-stage larval infection of A. simplex s.l. in the squid happens indirectly when preying on infected euphausiids or fish (e.g. walleye pollock *Gadus (Theragra) chalcogramma*), and that of *Lappetascaris* sp. happens by means of direct exposure to free-living larvae in seawater.

P60

Population structure in two co-occurring cryptic species of big-fin reef squid in Indo-western Pacific Ocean

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Understanding the genetic structure of marine species is important not only for understanding how populations evolve in a changing environment, but also for stock identification of managed species. The latter is particularly important in marine organisms with highly mobile adults or planktonic larval stages because such species are often assumed to have limited stock structure due to high dispersal potential. The big-fin reef squid, *Sepioteuthis cf. lessoniana* (Lesson 1830), is a widely distributed neritic species harvested in Western Pacific and Indian Oceans, the Mediterranean and Red Seas. Genetic studies indicate that the big-fin reef squid is a species complex with multiple unidentified species. Previous work has shown three reciprocally monophyletic lineages within the species complex with no clear geographic delineations or morphological discriminations from throughout the Indian, Indo-Pacific and Pacific Ocean. Moreover, our recent work showed non-concordance of population structure in sibling taxa. Lineage B (called "Red squid" in Japan) is significantly more structured than lineage C (White squid) not only over the Coral Triangle but also Kuroshio Current system in the Northwest Pacific Ocean, despite broad-scale physical processes should result in similar structure in co-distributed species. In contrast, lineage C didn't show strong signals of genetic structure, indicating wide dispersal capacity. We present a follow up study building on this set of samples, using a comparative population genomic approach to examine barriers to gene flow in for *S. cf. lessoniana* over the Indian and Pacific Oceans. This study examines the distribution and population structure of this species complex to determine how many species may exist, where they occur and how they differ using a reduced representation approach called 2b-RAD to sequence single nucleotide polymorphic (SNP) markers from throughout the genome.

P61

Thermal and salinity tolerance limits of *Octopus vulgaris* Type II paralarvae: physiological and ecological implications

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Physiological tolerances limit the distribution of species and have important adaptive significance. Early-life stages usually have lower tolerances to environmental variables than juveniles and adults. Here, we investigate the thermal and salinity tolerance limits of *Octopus vulgaris* Type II paralarvae in laboratory experiments. Paralarvae hatched at 22 ºC, 33-34 salinity were exposed to a broad range of controlled temperatures (4-32 ºC) and salinities (20-50) for 24 h after being acclimated to each condition. Experiments were conducted in replicate units containing 30 paralarvae each, exposed to constant aeration and natural photoperiod. After the end of the experiments surviving paralarvae were sampled for Scanning Electron Microscopy (SEM) to evaluate possible skin damage caused by the tested conditions. Thermal and salinity tolerances were assessed by measuring the lethal temperature and lethal salinity concentration required to kill 50% (LT50, LC 50) and 100% (LT100, LC100) of individuals. Paralarvae were tolerant of a broad range of temperature and salinity conditions, with optimal salinity at 33-34, lower and upper LC50 at 26 and 42 and LC100 at 22 and 50, respectively. Optimal temperature was found between 22-26 ºC, with lower and upper LT50 at 11.4 ºC and 29.2 ºC and LT100 at 4 ºC and 32 ºC. SEM analysis revealed severe skin damage under the extreme conditions tested. These results demonstrate that paralarvae were much more plastic to temperature than to salinity and were more sensitive to lower salinities and higher temperatures. Acclimation prior to testing each condition was important to uncover the physiological tolerances of paralarvae, reflecting their impressive plasticity to environmental conditions and ability to successfully track climatic warming.

P62

Life cycle of the two economic octopods, *Octopus minor* and *Amphioctopus fangsiao* reared in the laboratory

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As important economic octopods, *Octopus minor* and *Amphioctopus fangsiao* have been cultured in the northeast coastal waters of China in recent years. The common culture process mainly includes broodstock capture, acclimatization to captivity, spawning, hatchling culture, ongrowing and harvesting. The embryonic development of *O. minor* lasts for 72–89 days before hatching under the conditions of a seawater temperature of 21–25 ℃ and a salinity of 28–31 psu. Cladocerans, copepods and enriched *Artemia nauplii* are adequate initial feeds for the hatchling rearing. *H. sanguineus* of less than 4 mm body width is also used to feed 10d-old hatchlings. The survival rate is 75 % after 1 month of rearing. Then a mixed fresh diet such as juvenile crab, shellfish and shrimp becomes the main feeds for juveniles. After 6–7 months, O. minor bigger than 100 g can be harvested for marketing. And the longest period of survival for males is 250 days; the female lives 346 days, with a weight of 117.3 g after spawning.
The mature *Amphioctopus fangsiao* spawned in late April. The hatchings (0.08 ±0.02g of weight, 6.51 ±0.94 mm of mantle length) came out after 40 days and were fed with mysids. The juveniles reached 1.71±0.60g of WBW and 18.21±0.67mm of ML 30-day after hatching. Then a clam (1.36±0.26 of weight, 0.89±0.17mm of length), *Potamocorbula laevis*, were provided for the octopuses at a rate 30% of WBW twice a day. When reaching 6.01 ± 0.44g of WBW and 26.43 ±2.38mm of ML the octopuses could be fed with frozen feeds. But the clams, such as *Potamocorbula laevis* were still the major food after this stage until the octopuses reached 10.35±1.46g of WBW and 3.13±0.40mm of ML. The octopuses could reach 65.39±23.77 of WBW 60days after hatching, which is similar to the weight of octopuses (64.20±18.60g of WBW). At this time (middle October to late December, 60-70days after hatching), mature female octopuses spawned. The fertilized eggs finally hatched with 5.03±0.32mm of ML.

Session: Physiology

P63

The contribution of Polycomb Proteins via WNT Signaling Pathway in the octopus (*Octopus vulgaris*) arm growth and regeneration

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Polycomb group (PcG) proteins are evolutionarily conserved gene silencers that determine cell fate during development. In mammals they are mainly involved in muscle differentiation by binding and repressing muscle-specific gene regulatory regions in undifferentiated myoblasts. Recent studies explored the expression of ACHE in *O. vulgaris* arm morphogenesis during development and regeneration, but the machinery involved is still poorly investigated.
Our preliminary data support that PcG-regulated mechanisms involving local de- and re-differentiation could mediate regeneration in the octopus arm. We are also interested to search for a link between PcG protein, Wnt signaling Pathway and muscle regeneration and to investigate the role of epigenetic factors in octopus muscle differentiation. In our experiments the expression levels of 26 genes in arm regenerating tissues at 3-time points have been evaluated. The selected trancripts are *O. vulgaris* orthologs belonging to four subsets of protein families: PcG and Trithorax Group (TrxG) that are the major opposing system of epigenetic regulation; WNT signaling pathway and Fibroplast grow factor (FgFs) that regulate crucial aspects of cell fate including organogenesis, stem cell renewal, angiogenesis and embryonic development. Our results suggest that the genes considered are involved in mechanisms of arm growth and regeneration, showing different pattern of expression. In particular, almost all members of Wnt signaling Pathway and FgFs appeared up-regulated in both arm regeneration and growth. Members of TrxG (Trx, Sbf1, SmarcA4, SmarcC2, Rbp5, Wdr5) resulted to be activated during growth and not in regeneration, as their function of activators of Hox genes (Nurf has opposite trend). With some exception (Tbp, Ezh2, Rnf2 in the first 2-time points), the PcG are also up-regulated in both arm regeneration and growth. This is the first time that the involvement of PcG and WNT in octopus arm growth and regeneration is shown.

P64

Enzymatic characterization and digestive dynamic of *Octopus vulgaris* Type II

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The study of the digestive physiology is a key aspect to promote the commercial octopus aquaculture. The knowledge of the process of digestion, absorption, and assimilation of the nutrients and the factors that module the digestive environment are important to elaborate efficient diets according to the digestive capacity of these animals. Here, we characterized the activity of the total proteases in the gastric juice and digestive gland in *Octopus vulgaris* Type II in the fasting time and in the post prandial period and evaluated their digestive dynamic. In this species, the digestion was predominantly acid with maximal proteolytic activity in pH 3-4 and in temperatures between 40 a 70 °C, confirming their carnivorous habit and higher adaptative capacity of their digestive enzymes. The time of digestion was 400 min at 20 °C when the digestive tract was prepared to start a new meal. The digestion occurred at two phases: the first followed at the beginning of digestion when nutrients passing rapidly through the digestive tract and the second phase was gradually slower, characterized by the digestion, absorption, and assimilation of more complex nutrients. The peak of ingestion occurred at 200min after the food ingestion, when the proteolytic activity was maximal. This species use protein as the main source of energy and mobilize protein before lipids in the intracellular digestion, presenting a different pattern of digestion when compared to tropical and subtropical-temperate species. This information can contribute to a better comprehension of the digestive physiology of *O. vulgaris* Type II and other subtropical species and are important to formulate specific diets for their cultivation.

P65

The cuttlefish white body: Investigating the transcriptome and proteome

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In the common cuttlefish *Sepia officinalis*, the white body is organized in two lobes surrounding the optic nervous structure and located in the optic sinuses. In some Cephalopods, including the common cuttlefish, it is defined as the hematopoietic organ, where hemocyte production and differentiation take place. Nevertheless, most of the clues concerning this role only rely on microscopic observations, and few molecular data are available. The present work investigates the transcriptome and the proteome of the white body using Illumina sequencing and MALDI-TOF/TOF mass spectrometry analyses. The transcriptomic approach identified signaling precursors involved in immunity (e.g. members of the Toll signaling cascade), but also in hematopoiesis (e.g. members of the JAK/STAT signaling pathway, involved in hematopoiesis in Vertebrates). Moreover, protein precursors of proteins involved in proliferation and differentiation of hemocytes were identified. The proteomic approach identified numerous proteins involved in steps of the cellular metabolism such as transcription, translation, cellular trafficking or energy production, as well as immune and stress response-related effectors. Altogether, these results provide molecular evidence that the white body harbors an intense metabolic activity related to a circulating cell differentiation pathway, and they confirm its hematopoietic role.

P66

The involvement of protein synthesis in the octopus vertical lobe LTP

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The vertical lobe (VL) in the brain of the modern cephalopods is a key structure for learning and memory. The synaptic sites of short- and long-term plasticity in the VL have been physiologically characterized and their cellular manifestations are similar to vertebrates. However, the molecular mechanisms are significantly different. The Octopus VL show a robust NMDA-independent long term potentiatation (LTP) and our latest findings support a novel mechanism based on adaptation of the nitric oxide (NO) system. While the universal mechanism for maintaining the long-term phase of LTP involve de novo protein synthesis, physiological experiments using protein synthesis inhibitors blocked neither induction nor maintenance of the VL LTP. However, behavioral experiments have shown that de novo protein synthesis is important for Octopus long term memory (LTM) and that memory acquisition takes place outside the VL. Thus, we hypothesize that a protein synthesis-independent LTP may coordinate a protein synthesis-dependent LTM acquisition outside the VL and that this acquisition is mediated by neuropeptides strongly expressed in the output neurons of the VL. To test these hypothesis, a "fluorescent non canonical amino acid tagging" (FUNCAT)" for in situ visualization of newly protein synthesis has been adapted for Octopus brain tissue. First, using the FUNCAT method on Octopus brain slices preparations revealed an abundant protein synthesis across the supraoesophageal mass in control while slices exposed to 20μM anysomycin (a protein synthesis inhibitor) displayed a markedly reduced signal. Because previously it was shown that 20μM anysomycin did not block LTP induction and maintenance these results corroborate the conclusion the octopus VL LTP is indeed protein synthesis-independent and that the NO-dependent molecular switch is the mechanism responsible for the very long term maintenance of the octopus VL LTP.

P67

PJ prints: Identifying unique individual markings on *Sepioloidea lineolata*

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Photoidentification is a popular and widely used method of tracking wild animal populations over time. It allows us to keep record of population abundance, natural survivorship, social relationships and migrational patterns in a way that is non-extractive and non-invasive. This method is well established in documenting vertebrates in terrestrial and aquatic habitats by capturing distinctive characteristic such as scars, missing limbs/appendages, individual patterns, dorsal fins, tail flukes etc. It is not as commonly used in ecological studies of invertebrates. Little research has been done on identifying unique body coloration patterns in cephalopods. This study focuses specifically on *Sepioloidea lineolata*, also known as the Pajama squid. It is known for its dumpling shaped body and zebra-like stripes. It is a model organism for research due to their rapid growth, short lifespans, durability and large egg size. Not only is being able to identify and distinguish individuals in the wild important but it can also be extremely useful in lab research. A total of 50 individuals were photographed, measured and sexed from 5 separate populations. It was determined that each *S. lineolata* had their own unique line pattern from the moment they hatch, and remains consistent throughout their lifecycle. The complexity of the strip pattern varies from individual. There was no sexual dimorphism among this species. It is easiest to tell individuals apart when their lines have darkened and are most prominent, paying close attention to the dorsal mantle and facial markings where the most variation occurs. This method also allows us to keep track of intraspecific behaviors such as which individuals are matching, which females are laying and how often, aggression between individuals etc.

P68

Analysis of stem cell markers in the adult *Octopus vulgaris* brain

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Octopus vulgaris, a mollusk, has a highly centralized and lifelong growing brain. It also bears extensive regenerative capabilities and shows complex behaviors such as problem solving and social learning. Deviating from more primitive mollusks like Aplysia with 10000 neurons, cephalopods and in particular *Octopus vulgaris* increased its neuronal number to 500 million. In vertebrates, an increase in number of neurons links to complex behavior and is associated with the appearance of different types of neurogenic stem cells. Furthermore, neurogenesis is continued postnatally in specific regions of the brain. This process allows a continuous supply of neurons that integrate into existing networks, supporting brain plasticity leading to adaptive behavior. It is unknown whether complex invertebrate neural systems such as the *Octopus vulgaris* brain also renew neurons at the adult stage. Here, we attempt to visualize neurogenesis in the adult *Octopus vulgaris* brain using immunohistochemical stainings for markers of cell cycle and proliferation. Using the recently published *Octopus bimaculoides* genome, we identified orthologues of genes involved in vertebrate postnatal neurogenesis. We use in situ hybridization to map the expression of these orthologues on different regions of developing paralarvae and adult *Octopus vulgaris* nervous tissue aiming to identify progenitor cells.

P69

Exploring groundworks of *Octopus vulgaris* memory: gene expression including epigenetics

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The formation of long-term memory (LTM) begins with the activation of many signalling pathways that ultimately affect gene expression and chromatin structure. *Octopus vulgaris* is a "learning animal" and, despite extensive studies on its learning and memory recall, studies on the biological machinery involved and analysis of gene expression associated with learning is very limited. Our working hypothesis is that, following learning, altered expression of genes involved in neural plasticity occurs and this may be associated with the expression of genes coding for the epigenetic machinery. Genes potentially involved in neural plasticity (uch, stm, dat, TH, bdnf, chp1, zif-268, nrxn1a) and genes coding for epigenetic modifiers (kat2b, crebbp, pp1) were identified using a biased approach and tested for qRT-PCR in different areas of *O. vulgaris* brain, after passive avoidance learning. We found increased expression of the immediate-early gene uch and the genes involved in neural plasticity (stm, chp1, bdnf) in response to learned fear, in the posterior regions of SEM, including the vertical lobe (VL), the pivotal centre for learning and memory. Epigenetic modifiers showed a differential expression pattern in different areas of *O. vulgaris* brain. Kat2b and the memory suppressor pp1 showed an opposite trend being, respectively, up- and down-regulated in the median region of the SEM, including VL and superior frontal lobe. These genes are known to play an important role in chromatin remodelling during learning. The data indicate that target genes change their expression in response to learned fear. The results demonstrate a crucial role for the genes selected in neural plasticity and memory formation and highlight the possibility, for the first time in the common octopus, of an implication for epigenetic mechanisms in learning and memory paving the way for the intriguing study of the epigenome contribution to the octopus outstanding behavioural plasticity.

P70

Orcokinin B-like neuropeptides are neurohormones/neuromodulators involved in egg-laying regulation in the cuttlefish *Sepia officinalis*

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The common cuttlefish *(Sepia officinalis*) is a Cephalopod mollusc that lives in the English Channel and breeds in coastal spawning grounds in spring. FLGamide neuropeptides (renamed orcokinin B) are suspected to be involved in the control of egg-laying, oocyte transport in the oviduct, capsular secretion, but also in the release of mature oocytes into the genital coelom by the ovarian stroma. These are indeed the only neuropeptides detected so far by mass spectrometry in the ovary. They are also present in the nerve endings of the accessory glands of the female genital tract. Finally, their presence in the haemolymph of egg-laying females demonstrates that they also have a hormone-like role. Immunocytochemistry analyses confirmed the presence of neuropeptides in the nerve endings as well as in the cells of the accessory glands of the female genital tract and in different regions of the CNS. They are more particularly found in the sub-esophageal mass, a region that innervates the genital tract and the neurohemal area of the vena cava. In vitro tests indicated that neuropeptides FLGa1 and FLGa3 modulated oocyte transport and capsular secretion, and acted synergistically with FMRFamide. Finally, in silico analyses of the databases indicated that FLGamides had extensive structural homology with orcokinins B, which motivated their name change. In addition, the in silico study also led us to su+ggest modifying the annotations of Octopus bimaculoides and Loligo pealei orcokinins B currently annotated "Feeding Circuit Activating Neuropeptides" (FCANs). The two more abundant peptides have been studied by CD and NMR. Whereas they did not display any define structure in water, in presence of SDS micelles both Pept6 and Pept7 displayed strong variation in the CD spectra in good agreement with a helical character. Further analyses by NMR are also consistent with such a 3-D structure for Pept6.

P71

Crustacean cardioactive peptides in the cuttlefish *Sepia officinalis* : Expression, localization, structure, and a possible involvement in regulation of egg-laying

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The cuttlefish (*Sepia officinalis*) is a cephalopod mollusk distributed on the western European coast, in the West African Ocean and in the Mediterranean Sea. On the Normandy coast (France), cuttlefish is a target species of professional fishermen, so its reproduction strategy is of particular interest in the context of stock management. Egg-laying, which is coastal, is controlled by several types of regulators among which neuropeptides. The cuttlefish neuropeptidome was recently identified by Zatylny-Gaudin et al. (2016). Among the 38 neuropeptide families identified, some were significantly overexpressed in egg-laying females as compared to mature males. This study is focused on crustacean cardioactive peptides (CCAPs), a highly expressed neuropeptide family strongly suspected of being involved in the control of egg-laying. We investigated the functional and structural characterization and tissue mapping of CCAPs, as well as the expression patterns of their receptors. CCAPs appeared to be involved in oocyte transport through the oviduct and in mechanical secretion of capsular products. Immunocytochemistry revealed that the neuropeptides were localized throughout the central nervous system (CNS) and in the nerve endings of the glands involved in egg-capsule synthesis and secretion, i.e. the oviduct gland and the main nidamental glands. The CCAP receptor was expressed in these glands and in the subesophageal mass of the CNS. Multiple sequence alignments revealed a high level of conservation of CCAP protein precursors in *Sepia officinalis* and *Loligo pealei*, two cephalopod decapods. Primary sequences of CCAPs from the two species were fully conserved, and cryptic peptides detected in the nerve endings were also partially conserved, suggesting biological activity that remains unknown for the time being.

P72

Changes in the brain connectivity across the lifespan of the mourning cuttlefish *Sepia plangon*

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The mourning cuttlefish *Sepia plangon* inhabits shallow water, is diurnal, but is colour-blind. In spite of this, *S. plangon* possess high-resolution polarisation vision, which can potentially be used as an alternative source of contrast information for body patterning. These dynamic body colourations and colour-blind mimicry are well-known features of cuttlefish, however, the neural mechanisms behind them remain unclear. Through field and behavioural observations, we identified 16 body patterns in the adult *S. plangon*, 10 body patterns in juveniles and five in hatchlings. To analyse the neural mechanism regulating the variety of these patterns, we used high-resolution MRI (16.4 T) with an isotropic resolution of 30 μ. Additionally, we estimated the volumetric growth of 47 brain lobes throughout the different life stages. Finally, whole-brain connectivity was analysed by diffusion magnetic resonance imagery (dMRI). We recognised three structural connection pathways from the colouration control centres, the chromatophore lobes (Ch). Two of these pathways derived from the anterior chromatophore lobes (aCh), were responsible for colouration in the head and arms. The other pathway originated in the posterior chromatophore lobes (pCh), controlled the colouration of the mantle. The relative size of the aCh in relation to the entire brain volume was consistent throughout the life stages, at approximately 0.5%. However, the pCh increased to nearly 2% at the maturity stage, suggesting the enlargement of the pCh could be related to the complexity of courtship displays in adults. The present study represents an innovative approach to recognise the neural pathways transducing visual stimuli into neuronal signals, which may be responsible for controlling the repertoire of body patterns and behaviours across the lifespan of the cuttlefish.

P73

Vampire teeth: Radula morphology of the vampire squid, *Vampyroteuthis infernalis*, indicates a soft-bodied diet

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The feeding habits of *Vampyroteuthis infernalis* have been investigated through gut sample analyses, shipboard observations, in situ observations and stable isotope analyses. These observations have indicated a variety of food items including copepods, shrimps, squids, cnidarians and marine snow. Cephalopod radulae come in two basic forms: homodont with a single cusp on all teeth and heterodont that has more than one cusp on the rhachidian tooth and/or several teeth. Using light and scanning electron microscopy, the morphology of the vampire squid heterodont radula was compared to radulae of other cephalopods, including the Humboldt Squid, *Dosidicus gigas*, California market squid (*Doryteuthis opalescens*) and other midwater species. Scanning electron micrographs of radulae from over 8 species of cephalopods are compared in this study. Vampire squid have a unique homodont radula with a single cusp on all seven teeth. In comparison to the robust cutting heterdont radula and rhachidian teeth of shallow predators, the radula of *V. infernalis* has long gracile teeth that appear to be well suited to tearing apart very soft tissue and perhaps picking apart marine detritus. Despite living in extremely low oxygen concentrations with very low metabolic rates, adult vampire squid can be fairly active swimmers and likely feed on a variety of soft-bodied organisms.

P74

The neural basis of cuttlefish camouflage

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A major goal of neuroscience is to understand the neural basis of visual perception. In response tovisual stimuli, the brain extracts salient features and creates a neural representation of the environment, which permits the generation of appropriate behaviors. The cuttlefish provides a unique system in which to study visual perception. Cuttlefish camouflage to the environment, and thus require a transformation of the neural representation into an accurate recreation of the environment using skin pigmentation and texture. We are using *Sepia bandensis* to uncover the neural circuits underlying visual perception. Our ultimate goal is to record neural activity in a transgenic cuttlefish while monitoring camouflage behavior. To achieve this goal, we are developing molecular methods in cephalopods. These include CRISPR/Cas9 gene editing and cephalopod cell culture that can be used to screen for promoters that drive transgenes in cuttlefish.

P75

Reconsideration on reliability of using internal shell increments for age determination of neritic decapods

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In most of decapods, the length of internal shell (cuttlebone, gladius) is approximately equal, or at least has a good relationship, to the mantle length. This character enhances determination of growth in mantle length through growth in shell length, hence increments of shell lamella. Increments of lamellae in the internal shell of decapods, like in other hard structures, are likely to be a daily basis, but might vary upon context of the life cycle. However, the method for lamella count in internal shell of decapods is with lower expense compared to what in other hard structures. To reconsider the reliability of this method for age determination, two neritic decapods, *Sepioteuthis lessoniana* Ferussac, 1830 and *Sepia pharaonis* Ehrenberg, 1831 were reared in laboratory through their life cycles, obtaining specimens of exactly known age from less fluctuated environment. The information of *Sepiella inermis* Van Hasselt, 1835 is from the literature on laboratory rearing in Thailand. The relationships between ages and numbers of lamellae were linear in *S. pharaonis*, LN = 1.084A, and cubic parabolic in *S. lessoniana and S. inermis,* LN = 7.177+0.383A+0.019A2-9.045x10-5A3 and LN = 4.740+1.064A-1.711x10-3A2-3.744x10-6A3, respectively. Although the total average of daily lamella increment was 0.9-1.3 through their entire life, relationships of the three species shared the transition at the age of around 60 days. The transition and subsequent fluctuation in lamella numbers corresponded to periods of maturity and reproduction. The lamella counts in *S. lessoniana* and *S. inermis* are considered to be reliable for age determination as a daily increment from 10 to 60 days. The count is reliable in *S. pharaonis* from 10 days through its entire life. Lamella numbers are also reliable for age determination at least for the neritic species in tropical zone where environmental conditions are less fluctuated.

P76

Heart-forming gene expression in a heart and gill hearts development in Pygmy squids

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Cephalopod branchial hearts locate on the vein at the base of the gills and pump blood through the gills. Same as the true heart, the branchial hearts show autonomic contraction at own constant rhythm. It suggests that the branchial hearts have own pacemakers and the contraction is driven and sustained by electric signals from them. Although it is generally accepted that the systemic heart evolved once, heart-like additional pulsatile organ have evolved independently in some animals and the pulsatile contract activity is nearly unique to cephalopod. However, cephalopod cardiac developmental mechanism, including even true heart development, is still largely unknown. In heart development, molecular data have shown the similarity in gene regulatory network among animal species, and the evidences support that we can trace back hearts to the common origin arose in early bilaterian lineage. Previous studies have shown that many transcription factors are involved in heat development. Even among those, it is known that homeobox transcription factor Nkx2-5 (also known as tinman in fruit flies) and T-box transcription factors (Tbxs) have a key role in heart development commonly in animals. Here, in an attempt to understand the branchial heart development, we isolated Nkx2-5, Tbxs as well as Hyperpolarization-activated cyclic nucleotide-gated (HCN) channel gene using Japanese pygmy squid (*Idiosepius paradoxus*) and traced the expression in heart formation during the development.

P77

Embryonic development of the upper beak in *Octopus vulgaris* Cuvier, 1797: Implications in ageing accuracy

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Techniques using beaks to estimate age in cephalopods are spreading, and the daily periodicity has been confirmed for growth increments observed in the microstructure of the Rostrum and the Lateral Walls. Nevertheless, the ontogeny of these structures has not been described and the presence of any growth increments at embryonic stages not confirmed. In this study the embryonic development of upper jaws was analysed in *Octopus vulgaris* with following aims: (i) to validate the accuracy of age inferred from the two reading areas (ii) to explore the relationship between shape and function during ontogeny; and (iii) to understand responses to temperature fluctuations. Upper jaws from *O. vulgaris* embryos reared at different temperatures were extracted, photographed with DIC-Nomarski microscope and measured with Image Analysis System. For the latest developmental stages, the microstructure was described by visual observation, the growth rate was calculated and the overall dimensions were compared for incubation temperatures of 16°, 19° and 23°C. Results indicate that the "second inversion", a movement in the egg case that lead the embryo to assume the hatching position, is a turning point in the beak ontogeny. The Rostrum erupts after this movement and the first increment is observable at hatching, whereas increments on Lateral Walls appears earlier in ontogeny. Consequently, only the accuracy of age inferred from the Rostrum surface is confirmed for the early stages. After the second inversion the growth rate registered a drop in the Rostrum region, whereas it slightly increased in the Lateral Walls. This heterogeneity could be due to differences in the function of both structures. Temperatures differing from 19°C caused a rise of the individual variability and beaks of embryos reared at a 23°C were significantly smaller than others: those results confirm that incubation environment could alter hatchlings characteristics, thus affecting the recruitment conditions.

P78

Visual response properties and functional organization of the octopus optic lobe

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Cephalopods are highly dependent on vision, and use it to detect predators and prey, navigate their environment, and find mates. Cephalopods also use visual information to drive uniquely cephalopod behaviors, including rapid body pattern camouflage in response to their environment. However, due to technical challenges there have been very few recordings of activity in individual neurons of the octopus central visual system. Thus, it is essentially unknown how neural circuits in the cephalopod brain process visual information.

In this study, we aimed to identify the visual features extracted by the octopus system, using two-photon calcium imaging of the primary visual processing area of their central brain, the optic lobe. By loading the fluorescent calcium indicator Cal-520 AM-ester into an ex-vivo preparation of the central brain and eyes of *Octopus bimaculoides*, we were able to visualize the activity of hundreds of cells across multiple layers of the optic lobe simultaneously. We presented an array of visual stimuli on a projection screen on the side of the imaging chamber, and recorded the response dynamics of cell populations throughout the lobe. We found that cells have spatially localized receptive fields, and many are selective for distinct features of the stimuli, including direction of motion and luminance polarity (On/Off). These properties varied based on the layer location of the cells. Furthermore, we found a retinotopic organization of response to stimuli across the optic lobe, and patchy organization for On/Off preference. These data are the first measurements of neural coding and functional circuit organization in the central visual system of cephalopods, and provide a foundation to begin studying how the octopus brain performs the computations necessary to guide visual behavior.

P79

Morphologies of eumelanins from the ink of six cephalopods species measured by atomic force microscopy

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The morphologies of eumelanin, isolated from the six cephalopods species *Sepia esculenta*, *Sepia lycidas*, *Sepia pharaonis*, *Sepiella japonica*, *Euprymna berryi*, and *Uroteuthis* (*Photololigo*) *edulis,* were investigated using atomic force microscopy (AFM). The results showed that the hierarchical aggregate structures of irregular spherical particles with different diameters are the common characteristics of these eumelanins. Furthermore, the diameters of these spherical particles present an unevendistribution in a wide range and mainly concentrate in the range of about 20-150 nm. In addition, the eumelanin from different cephalopods species show obvious differences in the morphologies, which is illustrated by different assembly forms of diverse aggregate units and the quantitative features of eumelanin particles derived from the images.

Session: Reproduction

P80

Getting a grip on the squid hectocotylus

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During mating, male cephalopods generally use a modified arm – the hectocotylus – to transfer spermatophores to the female. In decapodiforms, the hectocotylus is used by the male to grasp a bunch of spermatophores from the terminal organ and transfer them to a specific site on the female body. Compared to a regular arm, the hectocotylus commonly has a species-specific external modification, which makes it a useful taxonomic character. However, while the external morphology is generally well known for most decapodiforms, the histology of the hectocotylus still remains poorly studied. To shed some light on the functioning of such an important organ in squid reproduction, we have investigated the histology of the hectocotylus of the loliginid squid *Doryteuthis pleii*. Samples from mature males collected off southeastern Brazil were subjected to electron and light microscopy analyses, including histochemical protocols, such as periodic acid-Schiff (PAS), Alcian blue (AB) and mercury-bromophenol blue (BB). In D. pleii, the distal region of the left ventral arm is modified: while suckers in the ventral row remain unchanged, the dorsal row is devoid of suckers and composed only of narrow stalks. Also, in the region between the dorsal and ventral rows, the epithelium is thicker and thrown into several folds. This folded epithelium is composed of at least two types of secretory cells: cells with large granules strongly reactive to PAS, and cells with small granules strongly reactive to BB, suggesting they secrete neutral sugars and basic proteins, respectively. Given that both cellular types appear restricted to the modified region of the arm, we hypothesize that these secretory cells aid in spermatophore transfer, possibly through an adhesive secretion that helps gripping the spermatophores. Further experimentation is needed to test if these cells cooperate to form an adhesive secretion, or if they act in antagonism through a duo-gland mechanism (i.e., adhesion vs. de-adhesion).

P81

First estimates about reproductive aspects of common octopus (*Octopus vulgaris*) at northeast Yucatan Peninsula, Mexico

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The common octopus (*O. vulgaris*) and the red octopus (*O. maya*), are the most important cephalopod fishery in Mexico. Despite its relevance, the current fishing management in the Yucatan Peninsula are developed using only biological information from red octopus. This study aims to determine for the first time, the reproductive cycle and first maturity size to the common octopus, captured in the Yucatan Peninsula, since May 2015 to February 2016, with a bimonthly periodicity. Mantle length (LM) total weight; sex and maturity gonadic stage were measured for all organisms. The Gonadosomatic Index (IGS) and the Madurity Index (IM) were calculed. It was defined the gonad development through histological methods using Hematoxylin-eosin. A total of 193 octopuses were catches (42% females and 58% males), the males presented a size interval from 70 a 75 mm and females from 60 a 180 mm. The sex ratio was not significantly different from 1H:1M (χ2-test, P 0.05). Four maturity fases were established for both sexes: immature, maturing, mature, and post-spawning (for females) sperm release (for males). The principal mature season in females of *O. vulgaris* occurred in spring and summer (42%) and for males during summer and winter (82%). During fall only immatures organism were found. ithout. The maximum values of the IGS and minimums of IM coincided with the maximum and minimum of maturity to both sexes. The mantle length of first maturity (LM50) was 129 mm in females and 125 mm in males. *O. vulgaris* shows a similar behavior to *O. maya*, in both, the reproduction is continuous with two peaks of maturity, one in summer and one in Winter, although the minimum size of catch (110 mm) is less than the size of first maturity, which means that the current management system is suitable for both species. This work constitutes the first description in an annual period of the reproductive cycle and size of first maturity for this species in the Yucatan Peninsula.

P82

Reproductive behaviour and mating strategy in the pygmy octopus, *Robsonella fontaniana*

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The reproductive behaviour of the octopus *Robsonella fontaniana* is described based on specimens collections carried out in central Chile (33° S, 71° W) during February-December 2015. The interactions were evaluated in laboratory conditions in four types of couples (adult male-female, juvenile male-female, male-male and female-female), and characterized using ethograms divided into pre-copula and copula stages. In all cases, individuals were previously weighed (gr) and measured (mantle length [ML], mm) to evaluate the relationship between size and interaction time. The behaviour of the individuals was recorded through videotapes, establishing a 30-min period as the limit for "no copulatory interaction". Successful copulatory interactions (SCI: mounting and insertion of the hectocotylus) were observed in 22% of the couples, corresponding only to adult male-female encounters (ML> 43 mm). Successful copula was not observed in the other treatments despite evidence of interaction (contact, subjection and aggression). During the pre-copula stage, courtship behaviours were evidenced in 25% of the SCI, including display of coloration patterns, widening of the interbrachial membrane and raising papillae in the mantle and arms. The copulation process began with the male mounting the female, followed by the hectocotylus insertion into the mantle cavity and the subsequent transfer of spermatophores, evidenced by strong funnel pumping movements. There was a positive relationship between the specimens' size and the total copulation time, recording a maximum of 196 min in octopuses around 50 mm ML. The successful copulatory interactions observed in adults and the nearly null interaction between juveniles, suggest the existence of a minimum size for sexual maturity and a low probability of spermatophores storage by juvenile females. (Funding: FONDECYT-CONICYT research grant #11170617 to S.A. Carrasco).

P83

Observations on females of *Todarodes sagittatus* (Lamarck, 1798) (Oegopsida: Ommastrephidae) with spermatangia in bizarre positions

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Males of the majority of flying squid species transfer their sperm packets (spermatophores) to the buccal area of the females. Once the spermatophore reaction is triggered, the spermatophore ejects the spermatangia, which is a sperm reservoir that firmly attaches to the female tissues. Spermatozoa of flying squids abandon the spermatangia and swim over the female squid in a targeted way to the seminal receptacles (SRs) of the buccal membrane of the female, where they remain until spawning. In the European flying squid, *Todarodes sagittatus* (Lamarck, 1798), spermatangia are usually attached to the female buccal mass. Two females of *T. sagittatus* with spermatangia attached in the mantle were sampled from the fishing trawler fleet of two Catalonian ports (NW Mediterranean Sea). Specimen 1 (Barcelona, 29-VII-2014, 398 mm mantle length) had 30 spermatangia in the second third of the ventral surface of the mantle, while specimen 2 (Vilanova i la Geltrú, 10-XII-2014, 267 mm mantle length) had 22 spermatangia in the first third of the dorsal surface of the mantle. No spermatangia was found in the oral region of both females. One of the spermatangia of the specimen 1 was molecularly identified as *T. sagittatus*. No mature male was fished the same day by the trawlers, excluding abnormal copulation inside the fishing net as a stress reaction. Thus, these two bizarre mating events represent a normal component of the mating behavior of *T. sagittatus* in the wild. Until now it is not known the distance that flying squid sperm is able to migrate from the spermatangia to the SRs of the female and if spermatozoa deployed in the mantle are able to migrate this important distance from the mantle to the SRs. Since females are significantly bigger than males and cannibalistic behavior is frequent among squids, I hypothesize that spermatangia position could be the result of quick unspecific mating events performed by males in order to reduce the risk of cannibalism.

P84

Immune condition of post spawning *Octopus maya* (Cephalopoda: Octopodidae) females: Parasite response or reproductive regulation?

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In the majority of the shallow-water cephalopod species, senescence is a short stage in their lifespan, that takes place in the end of sexual maturity that is accompanied of changes and will culminate in the cephalopod's death. Little information is available about immune regulation of female octopus during post spawning period and senescence although the survival of the next generation depends on the maternal care of the single spawn of each female. Females of incirrate octopods usually stop feeding and present maternal care of the eggs until they hatch. The present study was done in attempt to determine immune condition of post spawning *Octopus maya* females and determine if the changes observed present correlation with parasitic status. We characterized a total of 23 *O. maya* females in terms of immunological condition (hemocyte counts, hemagglutination and phenoloxidase activity) and parasitological description (incidence of infection by cestodes and Aggregata sp). Results on immunological variables showed an increased immune process of hemagglutination and phenoloxidase activity in hemocytes. Post-spawned female *O. maya* were parasitized with *Aggregata* sp. (prevalence 86,3%) in the caecum, intestine, and gills and with cestode larvae *Prochristianella* sp. (Cestoda, Trypanorhyncha) in the buccal mass (prevalence 90.9%; mean intensity of infection of 155,4 ± 107,73). Correlation analysis showed that the changes observed in immune parameters are not related to the type of parasites or the intensity of the infection. Observed fluctuations indicate an immunological compensation related to post-spawning days and the maintenance of homeostasis during senescence period at the expense of the consumption of energy reserves. Results indicates that the females are well adapted to this reproductive strategy, which allows them to channel all the energy to the care of spawning and survive in adequate health conditions while the hatching of the early juveniles occurs.

P85

Use of hydroacoustics to map the distribution and extent of the eggs of chokka squid (*Loligo vulgaris reynaudii*) on the south coast of South Africa

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Since 2014, we have been investigating the use of echo-sounders operating at 200 kHz to detect and map the distribution of eggs deposited by chokka squid (*Loligo vulgaris reynaudii*) in their inshore spawning area on the south coast of South Africa during summer months, when spawning is at a peak. The ultimate goal is to develop a possible alternative, cheaper, efficient and effective method of estimating and predicting squid abundance. The objective of obtaining an annual index of the size of the spawning population from an estimate of the extent of the egg beds over a major part of the known spawning grounds is to better manage this economically and socially- important commercial fishery for chokka squid in this area. To date we have succeeded in ground-truthing echoes from known patches of eggs, which has enabled us to map an extensive egg bed convincingly under favourable detection conditions. The challenge now is to increase confidence in the recognition of echoes from squid egg beds in the presence of back-scatter from other targets (including the bottom), such as turbidity or the 'nepheloid layer', and to develop techniques for mapping them over the entire inshore spawning habitat, in which it will be important to utilise information on spawning locations from the commercial fleet. In this poster we will describe the techniques used, present the most recent results obtained, and discuss ideas for meeting the challenges of developing this technique into a cheaper, more reliable survey tool for routine use in management.

P86

Phylogenetic allometry and evolution of male reproductive traits in benthic octopuses

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Octopus males transfer sperm contained into spermatophores through a modified arm called hectocotylus, which consist of two defined segments, calamus and ligula, being the latter in charge of transferring spermatophores to the female's mantle cavity. Previous studies have reported a correlation between octopus body size and spermatophore length. Therefore, if larger octopus species have larger spermatophores, longer hectocotylized arms and ligula should also be expected, because morpho-functional traits may coevolve to efficiently transfer spermatophores to the female. To evaluate this hypothesis, we performed phylogenetic comparative methods using morphometric data of reproductive traits and a molecular phylogeny of 93 species of benthic octopuses. First, phylogenetic multiple regressions between mantle length (ML) and hectocotylized arm length (HAL), ligula length (LL) and spermatophore length (SL) was performed to evaluate phylogenetic allometry. Second, to evaluate evolutionary correlation among reproductive traits, we corrected morphometric traits by body size in order to avoid allometric effects: hectocotylized arm length index (HALI), ligula length index (LLI) and spermatophore length index (SLI). Overall, we observed a positive relationship at inter-specific level between ML and all traits evaluated, but also between HAL vs LL and SL. In all cases, the slopes of phylogenetic regression were

P87

Individual spawning patterns of the common cuttlefish, *Sepia officinalis* L. in the English Channel

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The reproductive biology of the common cuttlefish was studied using samples of female gonads colected during RV "Cefas Endeavour" surveys and from commercial landings. Egg size in this cold-water population is among the largest recorded for this species, varying from 8.5 to 12.2 mm (mean 10.1 mm), egg weight being 221 to 533 mg (mean 337 mg). Egg size increased with female mantle length (ML). Female fecundity varied from 3,200 to 13,000 oocytes. The number of post-ovulatory follicles (POF) in spawning females exceeded the observed batch number by a factor of 3 to 4. Even at the coldest northernmost limit of its range, the cuttlefish still exhibits the intermittent spawning that is normally associated with subtropical populations. Females released ~ 2/3 of their potential fecundity; the rest being subjected to atresia, which begins during the spawning. Size of POF did not change from March to June indicating that individuals laid their eggs in a short time, which is only a fraction of the population's spawning period; otherwise, atresia and a respective decrease in POF size would have been expected to be observed.

P88

Roles of the pores on the surface of accessory nidamental glands in *Sepia pharaonic* (Cephalopoda: Sepiidae)

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Accessory nidamental glands (ANGs) in decapodiform cephalopods harbor numerous bacteria in their tubules and are considered a reason why decapodiform eggs exhibit antibacterial activity during embryonic development without parental care. In the present study, we investigated the morphological characteristics of the development of ANGs in female pharaoh cuttlefish, *Sepia pharaonis*, by using histology and electron microscopy. In the larva of S. pharaonis, portions of a thin primordial ANG epithelial layer, which are located on the ventrolateral surface of ink sac, invaginated inward to form tubules. Few to numerous tubules were observed in ANGs depending on different developmental stages of the sub-adult while the phenomenon of epithelial invagination on the surface of ANGs consistently occurred. As female *S. pharaonis* became sexually matured, the size of ANGs enlarged and numerous dense tubules that harbor symbiotic bacteria are enclosed in the outer wall of connective tissue. And we found the residual pores only distribute in the downward regions of the mature ANGs near the protruded openings of NGs. The cuboidal epithelial cells of ANG tubules became squamous when symbiotic bacteria proliferated, causing the tubules to become enlarged and elongated. Mitochondria, the Golgi apparatus, RER, and electron-dense granules were observed in the cytoplasm of the tubular epithelial cells. Moreover, the epithelial cells comprising the tubules that harbor the symbiotic bacteria in ANGs formed perforations through the connective tissue between ANGs and NGs, thus allowing the contents of the ANG tubules to be transported into NGs. Our results implied the epithelial invagination on the surface of the ANGs during development may be related to obtaining bacteria from the environment into the ANGs of *S. pharaonis*. Also, we revealed a previously undescribed structural connection between ANGs and NGs, which are two glands with different functions that participate in embryo protection.

P89

Reproductive behavior in chambered nautilus and oval squid with implications for conservation

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My goal is to identify key behavioral and ecological influences on cephalopod reproduction that may then inform conservation measures. Little is known about reproductive behavior in the solitary, heavily olfactory *Nautilus pompilius*. As a mechanism for identifying and locating potential mates, I hypothesized that Nautilus respond differently to female, male, and individual olfactory cues. I presented individuals with the scent of two conspecifics in a y-maze. Nautiluses showed scent preferences that differed between sexes as well as individuals. Similarly, little is known about oval squid (*Sepioteuthis lessoniana*) preferred spawning and mating habitat, though they have been observed spawning in seagrass beds. Oval squid were housed in a tank divided into varied seagrass and coral habitat types. I hypothesized that certain habitats are necessary for natural mating behaviors. Squid showed some differences in mating frequency by habitat type in two experiments. Together, these projects will identify conspecific and environmental factors that influence cephalopod reproductive behavior, and can contribute to targeted conservation measures.

P90

Fatty acids evidence for Argentinean shortfin squid *Illex argentinus* finance reproduction using concurrent energy intake

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Life-histories of short-life organisms are frequently shaped by trade-offs between somatic growth and reproduction, specifically for the cephalopod species, whereby the reproduction is the most energy-intense periods. Although previous knowledge has been gathered regarding the income breeding in Argentinean shortfin squid *Illex argentinus*, little direct information is available about its reproduction financed by concurrent food intake. Because of little modification in predators and the rapid turnover rate in digestive gland of cephalopod species, fatty acids have been proved to be the useful dietary tracers and the potential for reproductive investment investigation. Here, the fatty acid profiles in the tissues, including mantle, digestive gland and ovary, were investigated for this species during sexual maturation. With an exception of mantle tissue, the total content of fatty acids in digestive gland and ovary increased significantly with maturation. DHA, EPA, C16:0, C18:0, C18:1n9c and C20:1 were found to be the specific fatty acids in tissues of mantle, digestive gland, and ovary. The content of these fatty acids in ovary was positively correlated with that in digestive gland. Furthermore, the similarity percentage of fatty acids between ovary and digestive gland was consistently larger than that between ovary and mantle tissue after reaching physiologically maturing stage (mean: 83.21±2.53% vs. 75.87±4.82%). In combination, these lines of evidence confirmed that the energy sourcing for reproduction in *I. argentinus* was financed by that acquired and mobilized rapidly from recent diet.

P91

Transcriptomic analysis of optic glands of males *Octopus maya* revealed the importance of external stimulus as temperature at reproductive level

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The optic glands (OG) are endocrine organs known to be implicated in the control of sexual maturation in cephalopods. The OG regulate the proliferation of the reproductive cells, the maturation of gonads and the yolk production. In males octopus, the removal of the OG that are already producing sperm is followed by a decreased in the weight of the testis and the eventual cessation of sperm and spermatophore production, and this effect is most marked in the largest males. The OG plays an important role in the spermatogenesis of males octopus, because of this, the present study pretends to identify the genes that regulates the spermatogenesis in males *Octopus maya* under mating conditions and chronic thermal stress. *O. maya* males were exposed to different temperatures (24°C - preferred temperature and 30°C - stress temperature) for 30 d. After thermal exposure, males were mated with females maintained at preferred temperature. A transcriptomic analysis (RNA-Seq) of OG (24°C and 30°C before mating - PRE and after mating - POST) were realized. RNA-seq analysis generated 23,565,629 clean paired-end (PE) reads, which were assembled into 76,588 transcripts (Contig N50 = 1,008 bp and average contig = 671.15 bp). Using Blastx, 16,691 contigs have blast hits in the Swissprot database with an E-value cutoff of 1e-5. The differential expression analysis using EdgeR revealed that a total of 831 genes showed ≥2 fold up-regulation among different treatments. Biological processes of spermatogenesis, response to stress and steroid metabolic enzymes, among others, were identified in the different treatments. The results demonstrated that a external stimulus as temperature plays a key role in the reproduction and spermatogenesis processes in males *Octopus maya*.

P92

Reproductive performance in *Octopus maya* males is affected by thermal stress: A physiological and transcriptomic approach

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Octopus maya endemic to the Yucatan peninsula, Mexico, is an ectotherm organism particularly temperature-sensitive. In females *O. maya* studies reveal that temperature affects the spawning, number of eggs, fecundity and the viability of embryos. The aim of this work was directed to evaluate the physiological condition, the reproductive success, and transcriptomics of the testis of males *O. maya* exposed to chronic thermal stress. *O. maya* males were exposed to different temperatures (24°C and 30°C) for 30 d (PRE-before mating). After thermal exposure, six males of each experimental temperature were mated with females acclimated at 24°C (POST-after mating). In octopuses maintained at 30°C the growth rate and health was negatively affected. An increment in the haemocytes count and a reduction in the oxygen consumption were registered. In octopuses exposed to 30°C, the testis histology showed dilation of the seminiferous tubules, shrinkage of the proliferative stratum and high quantities of acidophilic bodies. The sperm quality showed that high temperatures increments the spermatophores production. The reproductive success assessed through paternity analysis confirmed multiple paternity in this species. In temperatures from 24°C -28°C, paternal contributions were assigned to experimental sires. Meanwhile at 30°C there was no parental contribution of the experimental sires. At transcriptomic level, we identified a total of 85,249 contigs in the testis, where 1,370 contigs were differentially expressed (>= 2-fold) at 30°C. We identified putative proteins that regulates processes as spermatogenesis, gamete generation, response to stress, inflammatory response and apoptosis among others, where, in some cases, the up-regulation and down-regulation of these genes can be linked to sterility in males, and could explain the lack of parental contribution. These results indicate that high temperatures directly affect reproductive performance and success of *Octopus maya* males.

P93

Cloning, characterization, and expression profile of estrogen receptor in common Chinese Cuttlefish, *Sepiella japonica*

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Sex steroid hormones are widely detected in molluscs and play important roles in sex determination, gonadal tissue maturation, and gametogenesis. Nevertheless, the signaling pathways of sex steroids in cephalopod have not yet been clearly elucidated. In the present study, a full-length sequence encoding the estrogen receptor (ER) was isolated from common Chinese cuttlefish, *Sepiella japonica*. The sjER cDNA clone was found to contain 1,788 nucleotides including a 1,470 bp open reading frame encoding 489 amino acid (aa) residues. The deduced ER protein consisted of six nuclear receptor characteristic domains. Based on a phylogenetic analysis, the ER DNA-binding domain and ligand-binding domain are highly conserved compared to other mollusc ERs. Highest aa identities were found for sjER with common octopus (*Octopus vulgaris*) ER (89%) and pacific oyster (*Crassostrea gigas*) ER (61%). Tissue expression analysis confirmed that sjER was widely distributed among tissues and predominantly expressed in the brain, liver, gonad (testis and ovary), and other accessory sexual gland (nidamental gland). The ER expression was temporally upregulated in the brain, liver, and ovary during the early sexual maturation period in *S. japonica*, which is coincident with the fluctuation of ovary estradiol content. These suggest that sjER may be involved in regulating the reproductive cycle of *S. japonica*. A fusion protein transient transfections assay showed that sjER was mainly located in the nucleus, suggesting a possible orthodox working mechanism of *S. japonica* ER in the nucleus through a ligand-dependent activation of specific gene transcription.

P94

Spawning grounds of *Dosidicus gigas* in the northeastern tropical Pacific Ocean

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Newly hatched paralarvae (PL) are an accurate indicator of a recent spawning event in cephalopods. This study aims to detect and characterize the spawning areas of the *Sthenoteuthis oualaniensis-Dosidicus gigas* Complex (SDC) using less than 2 mm mantle length PL and environmental factors. The zooplankton samples (247) were collected with two types of tows (oblique and surface), during two oceanographic cruises covering the west coast of the Baja California peninsula (WC) and the Gulf of California (GC) during the summer of 2014 aboard the R/V BIPO-INAPESCA. There were significant differences in PL abundance by type of tow and between regions. Surface tows were better indicators of spawning grounds of SDC than oblique tows. SDC PL were more abundant on the GC (390 PL/1000m3) than on the WC (170 PL/1000m3). Mantle length (ML) of PL ranged from 0.8 to 3.5 mm with 64% and 75% of the PL measuring ≤2.0 mm ML for the WC and GC, respectively. The quotient curves (temperature and salinity) allowed to determine the SDC hatching habitat, while a canonical analysis using 1 mm ML size groups and environmental factors, showed two associations: small PL (1.0-2.0 mm ML) related to high temperatures, and large PL (2.1-4.0 mm ML PL) related to high salinities. During summer 2014, the warmer Tropical Surface Water distributed in the southern portion of the study area, and can be considered the most important spawning and hatching habitat for the SDC. Less abundant but larger PL in our samples, seem to use the more productive GC environment as nursery ground distributing in the boundary of the Tropical Surface Water and Gulf of California Water. Given the seasonal variability present in the northeastern Tropical Pacific, spawning areas would expect to modify depending on spatial differences in environmental conditions and mesoscale physical processes.

P95

Some evidence for female monogamy in the firefly squid, *Watasenia scintillans*

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Paternity analysis using molecular markers has revealed polyandry or promiscuity is common in cephalopod mating system. Here we show several lines of evidence for female monogamy in the firefly squid, *Watasenia scintillans*. Seasonal dynamics of sex ratio indicate that males disappear (die) by April, whereas females continue spawning until May, suggesting that females store spermatozoa for at least one month. The spermatangia are attached in the seminal receptacle under collar on bilateral sides of nuchal cartilage and kept until end of her life. Notably, nearly equal number, with approximately 7, of the spermatangia was found stored on each side of the seminal receptacle. This well-ordered left-right symmetry would discount the possibility of copulations by multiple males and consequently, all the spermatangia attached could be delivered from a single male. Accordingly, males produce limited number of the spermatophores with maximum of 64 (the mean of 29), which estimates the number of lifetime mating opportunities for males being between 2 and 4. From these anatomical and ecological analyses, we hypothesized that females are monogamous, which is the first reported case, to our best knowledge, in cephalopods. We are currently undertaking the DNA paternity analysis if genetic monogamy is the rule in this species.

P96

Sexual dimorphism in *Brachioteuthis beanii* (Cephalopoda: Brachioteuthidae) in the northwest Atlantic

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In the northwest Atlantic, two morphotypes of *Brachioteuthis* sp. were collected during exploratory cruises to the Gully Marine Protected Area conducted by Canada's Division of Fishes and Oceans in 2007. CO1 barcoding of fresh tissue indicated that both morphotypes were Brachioteuthis cf. beanii. Here we demonstrate that there are consistent external morphological characters that can distinguish between males and females, and trace the development of these features back to their earliest expression.

Over 200 specimens of fixed and vouchered *Brachioteuthis* specimens 24 – 111 mm ML were measured, dissected, and sexed. Males and females were first identified based on the presence of the spermatophoric complex or nidamental glands. The smallest specimen that could be identified as male was 37 mm ML, and the smallest female was 52 mm ML. A mix of juveniles, males and females were found between 40-49 mm ML, but all specimens ≥ 52 mm ML were either male or female.

External features correlate with the development of the internal sex organs. Adult males are fusiform in shape, have small, sparse photophores on the mantle, and are lighter in color than the females. Females have a more flaccid mantle with larger and denser chromatorphores on the mantle and head. In addition to color differences, males have tubercules on the mantle that develop during maturation. Males, females and juveniles all have an arm formula of II>III>V>I, but male arms are slightly longer than female arms, especially arm pair III. Suckers are numerous and more dense on juvenile arms than in the adults. Club length is 25% of the tentacle length in juveniles, but < 20% in adult males and females.

P97

Bacterial community is changed in accessory nidamental gland during ovarian development in oval squid, *Sepioteuthis lessoniana*

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Accessory nidamental glands (ANG) is the accessory reproductive gland of female in squid. In ANG, symbiotic bacteria growth is associated with the ovarian development. A colorful ANG (filled with bacteria) is observed in mature female. To understand the developmental stage (vitellogenesis) by the superfamily of large lipid-transfer proteins (LLTPs) genes, two vitellogenins (VTG1 and VTG2), two apolipophorins (APOLP2A and APOLP2B), and a cytosolic large subunit of microsomal triglyceride-transfer protein (MTTP) found in the oval squid. Our data showed that only VTGs (VTG1 and VTG2) expressions were correlated to the female reproductive cycle. Thus, VTGs is used to identify the ovarian stage. According to the histological data, the bacteria clonies were observed in the ANG while the follicle cells penetrated into the oocytes (yolkless oocytes). The colonies of symbionts were observed the yellow and red pigments in the ANGs while oocytes entered vitellogenic stage. Combined to histological data of ANG, we found that ANG development is followed with ovarain development. Furthermore, according to amplicon analysis data, the population of bacteria in ANG is chnaged during ANG development. Thus, these results demonstrate that a stable symbiosis between the ANG and bacteria is regulated by the ovarian tissue.

P98

Comparison of nutritional composition of yolks of fertilized eggs of two cuttlefish (*Sepia lycidas* and *Sepia pharaonis*)

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Sepia lycidas and *Sepia pharaonis* are two main cuttlefish species widely distributed in the South China Sea, with their high economic value and characteristics of large size and fast growth rate. The aim of this study was to compare the nutritional composition of yolks of fertilized eggs of *S. lycidas* and *S. pharaonis,* then to provide reference data for the development of formula feed of cuttlefish larva. 500 yolks of fertilized eggs of *S. lycidas* and *S. pharaonis* were selected randomly respectively, and the contents of nutritional composition were determined. The results (dry weight basis) show that: 1) the crude protein content of *S. lycidas* and *S. pharaonis* were 55.14% and 57.23%, respectively; essential amino acid (EAA) and total amino acid (TAA) content ratio of EAA /TAA were 42.69% and 44.50%, respectively; Leu content was the highest in both *S. lycidas* and *S. pharaonis* (5.03% and 5.52%, respectively). 2) In yolks of fertilized eggs of *S. lycidas* and *S. pharaonis*, the contents of crude lipid were 6.65% and 9.54%, respectively; DHA contents were up to 26.75% and 26.58%, and DHA /EPA were 2.58 and 1.99, respectively. 3) Ca, Na, Mg, K, Fe, Zn, Mn and Cu were both rich in the yolks of fertilized eggs of *S. lycidas* and *S. pharaonis*. The main nutritional composition *of S. lycidas* and *S. pharaonis* are very similar. Therefore, in the process of developing formula feed of large cuttlefish in the South China Sea, it is suggested that the major nutritional requirements reference values are 57.23% for crude protein, 3.94% for Lys, 1.33% for Met, 9.54% for crude lipid, 2.53% for DHA, 1.27% for EPA, 44.4 mg /kg for Zn, 38.4mg /kg for Cu, 25.9mg /kg for Mn.

Session: Systematics and Biogeography

P99

Graneledone - how many species?

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We gathered as many tissue samples from Graneledone specimens as possible over the years and sequenced these for a number of genes. We supplemented the dataset with sequences from Genbank and the Barcode of Life project (COI only) where these were available. We conducted three analyses: (1) We built a maximum likelihood trees based on six genes (with at least four out of six genes available for most species); (2) We generated statistical parsimony networks in TCS from COI; (3) We conducted species delimitation analyses using bGMYC based on the COI sequence data. We establish that the rule of thumb normally applied to statistical parsimony networks does not adequately delimit species within Graneledone. In our phylogenetic analysis representatives of other genera were found within the Graneledone clade.

P100

Progress in cephalopod systematics

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In the proceedings of the 2012 CIAC meeting, we summarised the impact molecular data had had on cephalopod systematics. Here, we review progress in the field over the last six years. The deeper level phylogeny has been tackled through a multigene NGS study, transcriptome analyses and through analyses of mitogenomes, suggesting a close relationship between *Idiosepius* and sepiolids (although there is no complete consensus between methods), and a close relationship between *Spirula* and oegopsids. Additional studies have examined relationships within lower taxon groups (e.g., loliginids and ommastrephids). Meanwhile sequencing of the octopus genome has shown that the large size is not due to widely hypothesised genome duplications, but that expansions of gene families associated with neuronal development are far greater than expected. mRNA editing appears to be rife across coleoid cephalopods and may be a trade off with genome evolution - with coleoid genomes consequently evolving more slowly than expected, which raises further interesting challenges in resolving evolutionary relationships among coleoid taxa.

P101

Spatio-temporal variation of *Octopus maya* distribution and its relationship with environmental factors

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Octopus maya supports the most important octopus' fishery in the Yucatan peninsula and the largest in America. Knowledge about its spatiotemporal dynamics is fundamental for its conservation and sustainable management but it is yet limited. The aim of this study was to determine the spatiotemporal variations in the age structure of *O. maya* and the possible relationships with environmental factors that determine its distribution. Two-year georeferenced data (2012-2014) were obtained from the catch per unit effort, as well as the size structure of *O. maya* catches by the small-scale coastal fleet of the Yucatan peninsula, Mexico. Through a modal progression analysis and the multimodel inference method it was constructed a length-age key in terms of probability, the CPUE was calculated by age groups. Boosted Regression Trees model was used to describe the association of presence-absence with environmental factors and a predictive model of the probability of presence was made for each age group. Generally, *O. maya* presents spatiotemporal variations according to age. It has affinity for temperatures greater than 24 °C and less than 30 °C in areas with low turbidity, and also for shallow waters (<15 m). An important recruitment zone was perceived in front of Celestun and Sisal ports between March and June. The most abundant organisms in the fishing season were those of 5-9 months old, mainly in the Campeche zone. These preferences suggest that *O. maya* may have migratory movements in search of suitable conditions for their age. The spatiotemporalvariations in the population structure affect the catches of commercial fleets, so this study provides relevant knowledge for planning and management of regulatory strategies, in order to reach sustainable management of this resource.

P102

Phylogenetic diversity of cephalopoda along the Saudi Arabian Red Sea Coastline

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Although the Red Sea presents a unique environment with high temperature and salinity, it remains an area that is understudied. This lack of information is reflected in many areas, one which is biodiversity. Despite increasing work on biodiversity throughout the Red Sea and an increase in Cephalopoda studies worldwide, Cephalopoda in the Red Sea remain underrepresented, including molecular analyses. Class Cephalopoda are considered to be major contributors to coral reef ecosystems, serving an integral part of the food chain and exhibiting population increases due to targeted teleost fisheries and global climate change. To assess the biodiversity of Cephalopoda in the Saudi Arabian Red Sea, 87 specimens were collected from 25 reef locations between 17°N and 28°N latitude, as well as from the largest fish market in Saudi Arabia. Taxonomic identification of specimens was determined using morphological comparisons with previously reported species within the Red Sea and molecularly using the barcoding region Cytochrome Oxidase I. 84 Red Sea sequences were compared with sequences from GenBank and analyzed using a complement of Neighbor- Joining, Maximum-Likelihood, and Bayesian inference trees. From 17 cuttlefish, our study yielded three species, two of which matched previously reported species in GenBank. In addition, two distinct clades of Sepia pharaonis were identified. Of 35 squid collected, four species were identified, one of which did not match any other accepted species in literature, while Sepioteuthis lessoniana in the Red Sea formed a distinct clade. From 30 different specimens a total of five genera of Octopoda were present, forming six distinct species. Five Octopoda species collected did not match previously reported species, although specimens were paralarvae or juveniles, so morphological comparisons could not be used. Further investigation into the biodiversity, and role which cephalopods play in supporting biodiversity in the Red Sea is essential.

P103

Cephalopod paralarvae community structure in Mesoamerican Barrier Reef System

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Paralarvae are the first growth stage after hatching in all squids and many octopods. We describe the community structure (species richness, abundance, and distribution) of cephalopod PL at the Mesoamerican Barrier Reef System (MBRS), the second largest coral reef system in the world considered a prime biodiversity hotspot. The oceanic zone from Isla Contoy to Chinchorro Bank in the east coast of Quintana Roo, Mexico, was surveyed by an oceanographic research cruise during March and April 2006. Zooplankton samples were collected using a MOCNESS at four depth strata: a) 100-75 m, b) 75-50 m, c) 50-25 m, d) 25-0 m. The PL abundance registered was grouped by latitudinal transects, day-night collections and distance to the coast to test for significant differences.

A total of 601 PL were collected belonging to 42 taxa. Most abundant families were Enoploteuthidae (51%), Onychoteuthidae (18%) and Ommastrephidae (13%). In general, largest abundances were found south of the study area, near Chinchorro Bank. The northernmost transect was the most different regarding PL abundance and specific composition. The species richness in the diurnal and nocturnal seasons was similar, however, the abundance was higher during the diurnal tows. More coastal stations (0-75 km) reported greater abundance and richness of PL. As a sharp contrast with the Mexican Pacific were paralarval communities are influenced by the environmental and oceanographic change at different spatial and temporal scales, the oceanographic homogeneity in the MRBS water column precluded to show differences in the composition of PL. Current velocities better explained the distribution of PL in the MRBS.

P104

Shallow water octopuses from off Mexico

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The missing field guides to identify octopuses at local level have repercussions on the species determination and thus their management as fishery resource. The morphologic characterization of octopods at the Mexican seacoast enables to report morphometric and meristic information. The aim of this work was to analyze the specimens of the family Octopodidae of the Mexican coasts. The biological material deposited in the Malacological Collection of Escuela Nacional de Ciencias Biológicas was reviewed with the original descriptions and taxonomic keys. Once the morphological information was obtained, scientific illustrations of the species and important taxonomic characters were made. With the collection data we elaborate a general map of species distribution made with the software QGIS 2.18.12. The octopod collection counts with 134 specimens which previously, contemplate 16 species of *Octopus* genera, after the characterization updates and corrections were made resulting ten species in four genera: Amphioctopus, Callistoctopus, Octopus and Paroctopus and four more species of the artificial group Octopus sensu lato. Re-descriptions of the species were made where stands out morphologic and meristic information unknown to date of five species: *Amphioctopus burryi, Callistoctopus sp., Octopus maya, "Octopus" briareus* and *"Octopus" fitchi* and the range of distribution for several species extends. In conclusion, we recognized 14 species of the family Octopodidae of the 23 reported at the taxonomic lists of the Mexican seaboard.

P105

Phylogenetics, mophometrics, and life history of two unclassified species of small near-shore octopuses in Hawai'i, USA

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Within the tropical – subtropical Pacific there have been multiple studies describing the phylogenetic relationships among the numerous near-shore octopus species, in particular within the southern and western regions, however octopus diversity in the central Pacific has not been well studied and life history information for the majority of Pacific species is scarce. In this study the phylogenetic relationships of two unclassified species of octopuses (*Abdopus* sp. 1 "Crescent octopus" and *Octopus cf. vitiensis*) which inhabit the near-shore coral reef ecosystem of Hawai'i, USA were determined by sequencing the cox1, cox3 and cytb mtDNA loci from multiple individuals of each species caught around Hawai'i Island, Hawai'i and also obtaining 16s rRNA sequences from the same species from Genbank. The sequences were then analyzed using maximum likelihood and bayesian methods both individually and concatenated (cox1, cox3, cob, cox1+16s, cox1+cox3) against sequences from other octopus species available on Genbank that inhabit the topical/subtropical Pacific Ocean. Mophometrics for each species were recorded including egg size, paralarval mantle length, arm length, sucker count, and chromophore pattern and for juvenile and adult animals arm length, mantle length and mass were measured. Finally, to better understand the life history of these two octopuses their eggs were incubated at 5 temperatures (20, 22, 24, 26, 28, 30°C) and development and hatching times were recorded. The growth and lifespan of wild caught individuals maintained in captivity was also monitored. Our results will help start filling in the knowledge gap of the many unclassified octopus species in the central Pacific and will help facilitate further work on these species.

P106

First record and description of *Planctoteuthis* (Cephalopoda: Chiroteuthidae) paralarvae in the Gulf of California, Mexico

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We report for the first time the presence of doratopsis stages of *Planctoteuthis* sp. 1 (Cephalopoda: Chiroteuthidae) in the Gulf of California, Mexico, including a description of the morphological characters obtained from three of the five best-preserved specimens. The specimens were obtained from zooplankton samples collected in oblique Bongo net tows during June 2014 in the southern Gulf of California, México. Chromatophore patterns on the head, chambered brachial pillar, and buccal mass, plus the presence of a structure, possibly a photophore, at the base of the eyes covered by thick, golden reflective tissue are different from those of the doratopsis stages of *Planctoteuthis danae* and *Planctoteuthis lippula* known from the Pacific Ocean. These differences suggest *Planctoteuthis* sp. 1 belongs to *Planctoteuthis oligobessa*, the only other species known from the Pacific Ocean or an unknown species. Systematic sampling covering a poorly sampled entrance zone of the Gulf of California was important in the collection of the specimens.

P107

Beak identification of four dominant octopus species in East China Sea based on traditional measurements and geometric morphometrics

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Octopus is the most abundant genus in Octopodidae family and account for more than half of the total cephalopod landing in neritic fishery. Taxonomic problem is still exist due to the synonymous scientific name and limited genetic information. Cephalopod beak is a stable structure that give us an effective solution for the species and stock identification in previous studies. Researchers concern more about the beak shape variation than the beak measurements in recent years. We used the beak as a material in this study, combined with geometric morphometrics (GM) and machine learning methods, to compare the discrimination result between traditional and GM method of four Chinese neritic octopus species. According to our analyses, *Octopus vulgaris* has the largest beak morphology (both upper (UB) and lower beak (LB)) than other species. The result of ANOVA showed that all of the beak measurements had significant difference among four species except for LRWs. Significant differences of both UB and LB shapes among four species were identified in the MANOVA analysis based on the GM method. The result of GM based discriminant analysis is better than that of traditional measurements, and machine learning methods also performed a higher classification rate than the linear discriminant analysis. GM is a useful method to reconstruct the shape cephalopod beak and also effectively distinguish different species. We should also concerns about the effective improvement of classification accuracy with machine learning methods, making this a promising method for determining species structure moving into the future.

P108

Unconventional characters in cephalopod systematics are able to solve the controversial taxonomic status of *Todaropsis eblanae* (Ball, 1841)

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Traditionally, three subfamilies for the family Ommastrephidae Steenstrup, 1857 have been recognized: Illicinae Posselt, 1891, Todarodinae Adam, 1960 and Ommastrephinae Posselt, 1891. Controversy exists regarding the actual number of subfamilies. Particularly difficult is the taxonomic position of *Todaropsis eblanae* (Ball, 1841), whose placement varies according with the taxonomists´s criteria, being allocated as Illicinae or Todarodinae, and even a monotypic subfamily -Todaropsinae Nigmatullin, 2000- has been proposed to allocate this species. Different molecular systematics works showed a closer relation between *T. eblanae* and Illicinae instead that between *T. eblanae* with members of the Subfamily Todarodinae, as traditional taxonomy considered. Since traditional taxonomic morphological characters seem to be unable to solve the taxonomic position of this particular species, some unconventional characters in cephalopod systematics were studied. *T. eblanae* shares with Illicinae the biflagellate spermatozoa. Both taxa also shared an earlier development of chromatophores during the embryonic phase in comparison with other ommastrephids. These synapomorphies provide further morphological support to the clade formed by both groups in molecular systematic works. Three hatchling morphological autoapomorphies isolates *T. eblanae* from the remaining ommastrephids: its larger hatching size and the higher degree of development of the arm crown and the respiratory system. Although the absence of seminal receptacles in Illicinae isolates this group from *T. eblanae* and the remaining ommastrephids, the morphology of the seminal receptacles of *T.eblanae* is unique among the family. The new described synapomorphies of the clade (Todaropsis(Illicinae)) preclude the inclusion of *T. eblanae* within Todarodinae. However, both Illicinae and *T. eblanae* have enough private apomorphies to consider them as separated subfamilies.

P109

The common octopus of the Veracruz Reef System (southwestern Gulf of Mexico) is *Octopus insularis*: morphological and genetic evidence

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Historically, commercial landings of the artisanal fishery operating in the Veracruz Reef System (VRS) (southwestern Gulf of Mexico) have been assigned to *Octopus vulgaris*. Despite the importance of proper identification of commercially valuable species, to date, no studies have been performed in the VRS to verify the identity of this species. Moreover, a recent comparison of the VRS common octopus with the most recent morphological description of *O. vulgaris* showed marked differences between both taxa. Hence, to assess this matter, the aim of this study was to determine the taxonomic identity of the species using both morphometric and genetic approaches. In all, we sampled 52 octopuses that were haphazardly selected from the common octopus catches of the artisanal fishermen of the VRS. Morphological and genetic data confirmed that the VRS common octopus is not *O. vulgaris* and determined that it is the recently described *O. insularis*. In relation to *O. vulgaris*, the VRS common octopus has a smaller size, less suckers in the hectocotylized arm (162-192 vs 103-146), smaller calamus, bigger ligula, smaller enlarged suckers and smaller spermatophores. On the other hand, morphometric measurements and counts (e.g. 103-146 suckers in the hectocotylized arm) as well as other characteristics such as specific color patterns (e.g. a red/white reticulate in inner arms), almost exactly matched what had been reported for *O. insularis* in Brazil. Sequences from mitochondrial and nuclear markers revealed that the octopus from VRS are in the same high supported clade than *O. insularis* from Brazil and Mexico, showing the lowest genetic distances to this species (0-0.8%). Our findings add to the recent reports of octopus's species misidentification involving the members of the "*O. vulgaris* species complex" and highlight the need for more morphological and genetic studies with similar taxa in other geographical areas.

P110

Studying tropical cephalopods: The Southern Caribbean case

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Tropical cephalopods are very abundant; however, they remain one of the most understudied groups. While the tropics and the deep sea are considered hotspots for biodiversity, research funding hotspots are unfortunately not in these areas. The Caribbean Sea is considered the most biodiverse marine area in the Atlantic Ocean; it is however still poorly known. There is no reliable species list for most of the Caribbean, and many of the existing published records are misidentifications. The Southern Caribbean is a very rich area; however, due to little research only a few species have been identified and most of them product of fortuity or bycatch captures. To understand an area, a long-term observation and research must be done. In 5 years of studying the Taganga Bay, 14 different octopods species were collected, making this the most extensively researched bay in Latin America and probably all of the tropics. Some species were captured after 3 years of sampling, some are seasonal, and some represent small populations (rare) for the area. There is yet another species that was observed but never captured. One of the most common squids is a new species, previously misidentified as *Loliguncula brevis*; this is not an exception. In the southern Caribbean, with only one small area well studied, more than 50 species are known, most of them poorly understood, and many waiting to be described to science. At the moment one Spirula, five Sepiolids (two new species), 20 Squids (four new species), one Vampiroteuthis, 25 Octopods (10 new species) can be distinguished. More research is needed, with targeted focused collections, and more personal trained in natural history to be sent into the field.

P111

Octopods of Taganga Bay

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The Taganga Bay in the Tayrona sector of the southern Caribbean is the best studied bay in the area in terms of octopod fauna. There are 14 different species belonging to 4 genera who inhabit these little bay most of the year. One large species complex, the *Octopus tayrona* complex, can be found there. With 6 members, only one is described: *Octopus tayrona*, the most common in the area. All others species are easily recognizable.

The first molecular approach to the octopod fauna of the southern Caribbean was made to help clarify relationships between the species. Despite minor inconsistencies between the mitochondrial (COI) and nuclear (Rho) maximum likelihood phylogenies, monophyly of species such as *Octopus hummelincki, O. briareus* and *O. taganga* could be confirmed. The phylogenetic framework also showed evidence for the distinction of a new Callistoctopus species under description. However, the *Octopus tayrona* complex, that belongs to the *Octopus vulgaris americanus* complex, could not be resolved with the sequences used.

P112

The cirrate octopod *Opisthoteuthis bruuni* (Cephalopoda: Opisthoteuthidae) from SE Pacific and notes about cirrates phylogeny

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The cirrate octopods are typical cephalopods inhabiting at deep sea, there are collected in few numbers, and together with their gelatinous and delicate consistence, situates this group in constant taxonomic revision. In this study, we compare morphological and mitochondrial gene 16S rRNA data of cirrate specimens belonging to the genus Opisthoteuthis collected from SE Pacific, off Chile. Morphometry of fixed cirrate octopods were performed for specimens recently collected and also for specimens from the Museo Nacional de Historia Natural, Santiago, Chile. Additionally, we sequenced *Opisthoteuthis chathamensis* and *O. mero* from SW Pacific as well as Cirroctopus glacialis from Antarctic waters. Morphological examination suggests specimens collected from SE Pacific belong to *O. bruuni*. Molecular phylogenetic, analysis using Parsimony, Maximum Likelihood and Bayesian inference, confirms the status of *Opisthoteuthis bruuni* from SE Pacific as member of the family Opisthoteuthidade and phylogenetic comparisons with species from GenBank support the hypothesis for the existence of four families of cirrate octopods: Cirroctopodidae, Cirroteuthidae, Grimpoteuthidae and Opisthoteuthidae.

P113

Completed global revision of the Octopoteuthidae Berry, 1912 (Cephalopoda: Oegopsida)

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Octopoteuthids have been collected from every ocean except the Arctic and are an ecologically important group, being both key prey for apex marine predators and likely active predators themselves. However, owing to the family's taxonomic disarray and the difficulty of identifying them to species, they have remained poorly understood. A summary of results from the first global familial taxonomic review are prsented. Sixteen species were recognised within the family, with eleven accommodated in Octopoteuthis and five in Taningia; species of Octopoteuthis grouped into four morphologic species groups. Additional suprageneric taxa were proposed to better describe the finer-scale intrafamilial topology now recognised. A concurrent genetic investigation undertaken on 130 individuals comprising thirteen of the sixteen species yielded support for all species and species groups for which material was available. Discrete geographic distributions were identified for almost all octopoteuthid species, with most tending to be limited to a single ocean basin or a part thereof. Species-specific beak-to-body size regressions were calculated for four species and one species group (pooling data for two very similar but rare species), and were found to differ from the previously published "genus-wide" equations in specific ways. Opportunistic observations were made on several aspects of octopoteuthid reproductive biology, including sexual dimorphism and potential spawning strategy.

P114

Cephalopods of the continental slope of northeastern Brazil, with description of a new incirrate octopod

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The deep water cephalopod fauna along the continental slope of Northeastern Brazil is poorly known as no deep water commercial fishing occur in this tropical environment and, no previous bottom trawl surveys were performed in this region. To comply with the Brazilian environmental agencies requirements on nektonic diversity for licensing deep water oil drilling, two bottom trawl surveys were performed in 2009 and 2011 between latitudes 36° 6' 6" S and 36° 55' 10"S and longitudes 4° 19' 54" W and 4° 48' 26" W at depth and 150 and 1950 m. In 33 hauls, over 40,000 fish and 56 cephalopods were collected. Combined morphological and molecular (COI and 16S) analyses discriminated 14 species and 8 families. A single specimen of Spirula spirula occurred along the 1000 m isobaths. Four teuthids were caught: the small mesopelagic *Abrlia vernayi*, *Ornithoteuthis antillarum* and the large benthopelagic amoniacal squid *Pholidoteuthis adami*, all within their previously reported geographical range, the ommastrephid *Illex coindetii* confirmed the tropical Southwestern Atlantic within its distribution range. Two sepiolids of the genera Semirossia from the upper slope and Austrorossia from the mid slope require identification. Incirrate octopods were recorded only in the upper slope and included *Scaergus unicirrhus*, subadults of presumably *Mussoctopus cf januarii*, *Octopus vulgaris* and subadults of an undescribed species of *Scaergus*. Two cirrate octopus *Opistoteuthis cf agassizii* and *Opistoteuthis sp*. occurred in the hauls along the 1000 m. One specimen of Vampiroteuthis infernalis was recorded along the 2000 m isobaths. Despite the used gear was not efficient for cephalopods, the number of species and the catch rate compared with fish suggest an overall poor deep water cephalopod fauna along the slope of the studied area.

P115

Biological status of the common cuttlefish, *Sepia officinalis* (Linnaeus, 1758) in the Gulf of Cadiz in the autumn-spring 2017-2018

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Common cuttlefish, *Sepia officinalis* (Linnaeus, 1758) is one of the most valuable cephalopod species inhabiting the Gulf of Cadiz. Despite that to the moment there is no specific management options applied to cuttlefish fisheries located in this area. To support the development of fisheries management, the monitoring on various biological traits of cuttlefish was performed. The cuttlefishes were sampled in two bottom trawl surveys, ARSA 1117 and ARSA 0318, performed in November 2017 and February-March 2018, respectively. In total 170 specimens were sampled. Sampling included measurement of the dorsal mantle length (DML), sex and maturity determination (using Hatanaka, 1979 scale for males and Gabr, 1998 scale for females) and extraction of the beaks for age estimation. Later, rostral sagittal sections (RSS) of the upper beaks were processed following standard methods, developed for age estimation of the *Octopus vulgaris*. During both surveys females prevailed in catches, their percentage ranged from 53 to 63%. The basis of cuttlefish catches in autumn was composed by immature individuals (34,3% females and 18,5% males) and pre-spawning males (24,1%), in spring percentage of pre-spawning and spawning females significantly increased (to 9,7 and 17,7%, respectively) on the background of decreasing percentage of immature and maturing individuals. Length of studied cuttlefish ranged from 55 mm (immature female) to 342 mm (pre-spawning male). Average length of maturing, mature and pre-spawning specimens slightly decreased from autumn to spring. Especially evident this change was in mature males (174,2 mm in autumn and 164,3 mm in spring) and pre-spawning and spawning females (from 206,3 to 156,5 mm and from 209 to 172,5 mm, respectively). Age of studied specimens ranged from 132 to 470 days, and was bimodal, representing peaks of hatching in the beginning and in the middle of the year. Combined with the change in size structure of autumn and spring catches this can indicate the presence of two seasonal spawning cohorts.

P116

Oceanic cephalopods around Atlantic seamounts

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Several old and recent collections of oceanic cephalopods sampled during research surveys in Atlantic waters, were combined to improve our knowledge on the distribution of cephalopods and the pelagic biodiversity in the Atlanctic Ocean. Sampling area covers the Irving, Tyro and Atlantis seamounts south of the Azores Archipelago and the Gorringe Bank, Seine, Ampere and Josephine seamounts in the Madeira-Tore region, the oceanic waters between the Portuguese southern continental shelf and the seamounts and waters surrounding the Cape Verde Islands. Samples from 1994 were taken with the German RV Poseidon within the framework of the research project Eurosquid II. Samples from 2015 and 2016 were collected by the Portuguese RV Noruega within the framework of the research project Biometore. Additional samples were taken with German research vessels in oceanic waters surrounding the Azores and the Cape Verde Archipelago. Cephalopods were collected with a Pelagic Trawl (PT) and an Isaacs-Kidd Midwater Trawl (IKMT) to sample mesopelagic and epipelagic adults and juveniles, and Bongo net and MultiNet to sample paralarvae in the upper 200 m. The early life stages of many cephalopod species are virtually unknown, which makes the taxonomic identification of cephalopods captured by plankton nets very difficult. Furthermore, the identification of early stages of many well known species is problematic due to strong morphologic similarities between related taxa within each family. A few new identification keys and descriptions, available since the comprehensive compilation by Sweeney et al. (1992), were used to identify the early life stages in our collections. Illustrations and some original new descriptions of oceanic paralarvae of different ontogenic stages are provided to contribute to a new identification guide for paralarval and juvenile cephalopods arising from CIAC2018.

P117

Historical biogeography of the genus Octopus in America

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Benthic octopuses of the family Octopodidae comprises over 170 species inhabiting tropical and temperate ecosystems worldwide. Specifically, the Octopus genus comprises over 68 species, 30 of them inhabiting the Americas at both side (Atlantic and Pacific). Geomorphological events associated to the formation of the Isthmus of Panama trigged evolutionary processes such as dispersal and vicariance, which caused diversification of the Octopus genus in America. To test this hypothesis, three mitochondrial genes (COI, COIII and 16S) were sequenced in 33 specimens belonging to 14 species (Octopus, Robsonella, Enteroctopus, Muusoctopus) from North, Central and South America. Additionally, sequences from 83 species of the superfamily Octopodoidea were obtained from GenBank. The phylogenetic relationships, origin and divergence time as well as the ancestral distribution were estimated, including a total of 97 species. As in previous studies, phylogenetic results showed that the genus Octopus is polyphyletic; however, one of the Octopus clades corresponding only to the American species revealed to be monophyletic, contrasting with other Octopus species from different continents. Biogeographic, phylogenetic and divergence time evidenced a concordance with the geological time of the formation of the Isthmus of Panama, initiating this divergence during the Oligocene in the Caribbean Sea, which through dispersion and vicariance processes originated the current species that inhabit the Atlantic and Pacific coast of America.

P118

Octopods of the Deep Reefs Off Curacao

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Coral-reef habitat around the world is in steady decline due to many factors. Because mesophotic and rariphotic reefs (40 - >300m) are protected from destruction by storms and typically are characterized by cooler temperatures than shallow reefs, researchers hypothesize that deep reefs could form refugia for shallow reef coral, fish, and invertebrate species. However, before studying the changes in faunal populations over time, we must first assess the current biodiversity of these reefs. While recent exploratory research on deep reefs in the Caribbean Sea has yielded information for various deep-reef fishes and invertebrates, researchers have yet to study Caribbean deep-reef cephalopods. Using images, videos, and specimens collected by a manned submersible at Curaçao, southern Caribbean, as well as existing DNA sequences derived from the specimens, I attempted to survey the cephalopod diversity of the mesophotic and deeper-reef communities off Curaçao. Among the fifteen available specimens, I found three *Octopus hummelincki*, one *Pteroctopus cf tetracirrhus*, two *Scaeurgus unicirrhus*, and one *Paroctopus mercatoris*. In addition, I found two groups of octopods that did not correspond to any known species, two specimens in one group, and six in another. These most likely represent undescribed species.

P119

Genetic variability of *Octopus mimus* (Gould, 1852) along two marine biogeographic provinces

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Octopus mimus inhabits the shallow waters of Peruvian province but recently, it has been suggested, based on mitochondrial markers that it could be found in the Panamanian Province. We hypothesize that the biogeographic boundary between Panamanian and Peruvian province act as a genetic break which has led to the recognition of two *O. mimus* clades but the oceanographic processes have allowed enough gene flow along the Pacific coast yielding lower levels of genetic structure. We use cytochrome oxidase subunit I to investigate our hypothesis. Sequence divergence ranged from 0 to 1.5%. We identify 19 haplotypes and they were not distributed evenly among the sampled localities. *O. mimus* samples cluster in two groups: one exclusively Peruvian and other with individuals from Panamic and Peruvian provinces.

P120

Paralarvae abundance and distribution in the Slope Sea off the northeast United States

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The Slope Sea is a highly dynamic oceanographic region bounded by the Gulf Stream to the south and the northeast United States continental shelf to the north and west. Plankton sampling in this region has historically been very limited, with paralarvae examined from only a few cruises. Recently opportunistic plankton sampling was initiated on summertime marine mammal surveys in the Slope Sea. Paralarvae were sorted from over 200 plankton samples, and individuals are in the process of being identified and measured. Preliminary analyses indicate that at least 21 unique taxa are present in these samples with Enoploteuthidae and Pyroteuthidae being the two dominant families. Future work will focus on completing the identification of these samples, and contrasting the cephalopod community in this region with the community sampled over the adjacent northeast United States continental shelf.

P121

In situ images document the benthic cephalopod fauna in the mid-Atlantic canyons of the eastern US continental margin

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Much of what is known about deep-sea cephalopods is based on midwater and benthic trawling activities. In situ observations of deep-sea cephalopods are rare, yet provide essential information about species diversity and distributions without negatively impacting the organisms or their habitat. Here we document the presence of over 100 unique benthic and benthopelagic cephalopods in the deepwater canyons of the mid-Atlantic by examining 52,295 photographs taken during 36 dive transects conducted as part of NOAA's Northeast Deep-Sea Coral Initiative. Images were collected using a towed-camera system (the Woods Hole "TowCam") aboard NOAA ship Henry Bigelow in 2012 and 2014. TowCam was flown approximately 5 m above the seafloor and took an image every 10 seconds. Washington (n=7), Accomac (n=1), Leonard (n=2), Wilmington (n=6), Spencer (n=6), Lindenkohl (n=4), Carteret (n=4), Tom's (n=5), and Hendrickson canyons (n=1) were all explored. Images were bulk color-corrected and then examined for the presence of cephalopods and other benthic megafauna including fish, crustaceans, and echinoderms. All images were first annotated with keywords describing the taxa present, habitat characteristics, and other highlights. Navigational and environmental data were linked to each image so individual cephalopods could be georeferenced and mapped. Each dive transect documented at least 2 cephalopods, with a maximum of 17 individuals found in one dive transect in Wilmington Canyon. The benthic incirrate octopod *Graneledone verrucosa*, and the sepiolid *Rossia* sp. were the most commonly encountered benthic taxa. *Stauroteuthis syrtensis*, *Mastigoteuthis* sp., and *Taonius pavo* were all documented in the water column. Incidental observations include a *Graneledone verrucosa* female with eggs, a *Rossia* sp. female with eggs, juvenile octopods, octopod tracks, and predation events.

P122

The distribution of *Benthoctopus* and *Bathypolypus* in the North Atlantic

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Despite the review of *Benthoctopus* and *Bathypolypus* carried out by Muus (2002) these species require more work to identify and separate them. One of the ways this can be done is through DNA barcoding which enables identification through molecular methods. Eight research cruises were conducted between the years 2005 and 2012 on the RV Scotia by Marine Scotland. The cruises were focussed on sampling areas around the seamounts located in the Atlantic Ocean around the north of Ireland and Scotland. Universal primers were used to amplify the Folmer region of cytochrome oxidase subunit I (COI) as a DNA barcode for 102 of the specimens collected. These sequences were then compared to sequences already available on Genbank through the building of haplotype networks. The networks were constructed using statistical parsimony in TCS 1.21 (Clement et al., 2000). The haplotype networks confirmed six species were present. Four formed single discrete networks with material previously identified and sequenced by LA; *Graneledone verrucosa, Benthoctopus normani, Benthoctopus johnsonianus* and *Bathypolypus sponsalis*. With further morphological analysis we confirmed the other two species are *Bathypolypus ergasticus* and *Bathypolypus arcticus*. Building up a barcode inventory of cephalopod specimens can assist in future identifications and also help improve our knowledge of cephalopod distribution.

P123

Updated taxonomy of the genus *Ommastrephes* d'Orbigny, 1834 (Oegopsida: Ommastrephidae)

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Cryptic speciation, where distinct species share morphological features, has been increasingly reported among marine invertebrates. In general, the recognition of these distinct species leads to a scenario in which the distribution of each newly discovered species represents a small fraction of the original distribution range. Because conservation and natural resource management politics rely on recognition at the species level, resolving the taxonomic status of species-complexes is a crucial task. Until the present study, *Ommastrephes bartramii* (Lesueur, 1821) was considered a monotypic species of flying squid with a cosmopolitan and discontinuous distribution range. However, some morphological and metabolic difference between individuals from different regions raised some uncertainties about its taxonomic status. In the present study, molecular data of cytochrome c oxidase subunit 1 (COI) and 16S rRNA revealed that *O. bartramii* actually represents four species. Three previously synonymized names from the literature were resurrected for the additional species recognized in this genus: *Ommastrephes brevimanus* (Gould, 1852) comb. nov., *Ommastrephes caroli* (Furtado, 1887), and *Ommastrephes cylindraceus* D'Orbigny, 1835 In 1834-1847. The distribution range of each species was tentatively drawn based on the available molecular data and putative oceanographic features that are acting as reproductive barriers. Only one of the four newly recognized species is currently exploited by commercial fisheries; therefore, understanding the true distribution range of this species important for fisheries management because it is much more restricted than previously believed.

P124

Effects of fixation and preservation on deep-sea octopodiform specimens

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Following their capture, deep sea cephalopod specimens are usually directly fixed and then preserved. Only in select cases, photographs and measurements of the specimens have been taken prior to fixation. Thus, species descriptions are generally based on preserved specimens, although preservation was shown to lead to a change in body shape and overall outer appearance. In order to document the effect of fixation on octopodiform samples, specimens collected in 2016 in the North Pacific during SO-249 BERING were photographed and measured directly after capture, after twelve months of fixation in 4% formaldehyde, and after four weeks storage in 70% ethanol. The outer appearance of the cirrate specimen changed significantly after twelve months storage in formaldehyde. After transfer of the specimen to 70% ethanol and storage for four weeks, further shrinkage of the specimen was observed. In contrast to this cirrate specimen, the incirrates were hardly altered after the same fixation and preservation steps. After ten months in 4% formaldehyde, the overall appearance remained unchanged. After four weeks storage in 70% ethanol, measurements and outer appearance did not change as well. Thus, the effect of preservation is significantly smaller on incirrates than on cirrate specimens. The present study provides photographs and measurements of selected octopodiform taxa treated in the same way.

P125

Hatching into the Future: cuttlefish development and early behaviour under high CO2 conditions

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As atmospheric CO2 levels rise, continuous uptake by the oceans is changing the seawater chemistry, resulting in ocean acidification (OA). The expected levels of CO2 can compromise key biological traits of marine organisms and potentially reverberate through the entire ecosystem. Increasing research has provided evidence of detrimental effects of OA on the behaviour of a wide range of marine organisms, including cephalopods. Nonetheless, previous studies have focused solely on squids and impacts over cuttlefish behaviour remain unaddressed. Here we investigate the effects of OA in the development and behaviour of recently-hatched cuttlefish (Sepia officinalis) exposed to either present-day (~ 400 μatm) or end-of-the-century levels of CO2 (~ 900 μatm | Δ 0.3 pH units) since their early embryogenesis. We conducted a comprehensive assessment of the embryogenesis duration, percentage of premature hatchings, growth rates and early survival rate. Furthermore, different aspects of the cuttlefish behavioural ecology, including their shelter-seeking and predatory behaviour, as well as their response to alarm cues, were analysed in order to achieve a holistic overview of the OA impacts in cuttlefish hatchlings. In contrast with previous studies on other cephalopod species, our preliminary results suggest a certain behavioural resilience of the cuttlefish hatchlings towards these acidified conditions. The benthic lifestyle and further adaptations to the fluctuation-prone coastal environment may favour the odds of the common cuttlefish recruits in an acidified ocean. Nonetheless, these same environments are also particularly susceptible to anthropogenic pressure and other climate change-related variables. Thus, the cumulative effects of multiple stressors should be further addressed in order to accurately predict what the future reserves to this ecologically important and economically valuable species.

P126

CephPAD: An Open Access Database of Pathogenic Agents from Cephalopods

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Here we present "Cephalopods' Pathogenic Agents Database" (CephPAD), a free online resource collating data on parasites and other pathogenic agents of cephalopods. This is presented in an interactive database format and based on the information from 'Diseases of Marine Animals' (Kinne, 1990—chapters concerning cephalopods; Hanlon and Forsythe, 1990a, 1990b; Hochberg, 1990). The current data set includes viruses, bacteria, fungi and fungus-like organisms, Chormista and Protozoa. Metazoan parasites, i.e. Diciemyda, Monogenea, Trematoda, Cestoda, Acanthocephala, Nematoda, Annelida and Crustacea, are also included. The database also provides indications of possible effects of these agents in their host, such as affected tissue, anatomy-pathological findings, clinical presentation and prevalence of mortality. Additionally, information on structural abnormalities and neoplasia are also incorporated. If available, information about the cephalopod origin (i.e., wild or cultured) and place of capture is also included. Several species of cephalopods are included (about 130), and the these are presented based on the original taxonomic description and the updated one according to World Register Marine Species (WoRMS). This database is intended for researchers, students, technicians, care-takers and veterinarian to help ensure cephalopod welfare in captivity. We plan to regularly update this database with new published information as it becomes available. This work is the result of the activities of the COST Action FA1301.