Status Review, Disease Risk Analysis and Conservation Action Plan for the

Bellinger River Snapping Turtle (*Myuchelys georgesi*)

December, 2016

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Cover photo: Juvenile Bellinger River Snapping Turtle © 2016 Brett Vercoe

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Executive Summary

The Bellinger River Snapping Turtle (BRST) (*Myuchelys georgesi*) is a freshwater turtle endemic to a 60 km stretch of the Bellinger River, and possibly a portion of the nearby Kalang River in coastal north eastern New South Wales (NSW).

In mid-February, 2015 a significant mortality event was observed in BRSTs. Most affected animals died within a short time of being found and those brought into care were euthanased due to progression of the disease despite nursing care.

Prior to the 2015 mortality event, the BRST was described as locally abundant, with a population estimate of between 1,600 and 4,500 individuals. The current BRST population is estimated to be between 200 and 300 individuals, predominantly juveniles, and is currently listed as Critically Endangered under the NSW *Threatened Species Conservation Act 1995* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

Since the mortality event a disease investigation has identified a virus (Bellinger River Virus or BRV), previously not known to science, as the agent most likely to be responsible for the mortality event. In addition to the disease investigation a captive population has been founded to provide immediate insurance against extinction and to generate turtles for release to aid recovery.

Priority Actions Years 1-5

- **Disease hazard investigation**: transmission, serological test, explore treatment options
- ***Emydura* investigation:** competition with BRST and management options
- **Build captive breeding program**: add founders, juvenile sex determination, develop studbook and plan
- **Engage and mobilise community**: communication plan; public engagement program
- **Prepare for experimental releases**: population survey & monitoring; translocation proposal
- **Plan for long-term hazard mitigation**: explore options for fox control

Before the disease event, potential threats to BRSTs were considered to be their limited distribution and habitat requirements, predation, water quality, and hybridisation and competition with Murray River Turtles (*Emydura macquarii*). Though much is unknown about the role and impact of these factors on BRST viability, it is considered possible that some or all played a role in increasing the susceptibility of the species to the disease, or could prejudice its recovery from it.

In November 2016, the NSW Office of Environment and Heritage brought 16 experts from eight organisations to Taronga Zoo in Sydney, to discuss and recommend next steps in the recovery of BRSTs, based on their agreed interpretation of the information gathered to date. The workshop included assessments of all known risks to BRSTs, with BRV given particular attention.

Immediate priorities for action (1-5 years) are listed in the accompanying box. Longer term priorities (5-20 years) emphasised reducing the impact of fox predation and an integrated program of riparian rehabilitation and in-stream health.

As enabling strategies, high priority was given to community engagement and communication. Community support and involvement in risk mitigation was considered pivotal to successful recovery of BRSTs.

Contents

Acronyms and abbreviations

AAHL	Australian Animal Health Laboratories
APHA	American Public Health Association
ARWH	Australian Registry of Wildlife Health
BRST	Bellinger River Snapping Turtle
BRV	Bellinger River Virus
BSC	Bellingen Shire Council
CBSG	Conservation Breeding Specialist Group
CITES	Convention on International Trade in Endangered Species
DPI	Department of Primary Industries (NSW Government)
DRA	Disease Risk Analysis
EPA	Environment Protection Authority (NSW Government)
IUCN	International Union for the Conservation of Nature
LLS	Local Land Services
NGO	Non-government organisation
NHMRC	National Health and Medical Research Council
NPWS	National Parks and Wildlife Service (NSW Government)
NSW	New South Wales
OEH	Office of Environment and Heritage (NSW Government)
PCR	Polymerase Chain Reaction (a DNA-based diagnostic test)
SCUD	Septicaemic Cutaneous Ulcerative Disease
spp.	Species
SSC	Species Survival Commission
TAP	Threat Abatement Plan
TCSA	Taronga Conservation Society Australia
WSU	Western Sydney University

Introduction and Background

The Bellinger River Snapping Turtle (BRST) (*Myuchelys georgesi*) is currently listed as Critically Endangered under the New South Wales *Threatened Species Conservation Act 1995* and the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999*.

The BRST (family Chelidae) is a moderately large, short-necked, freshwater turtle endemic to a 60 km stretch of the Bellinger River, and possibly a portion of the nearby Kalang River in coastal north eastern New South Wales (NSW).

Prior to 2015, the BRST was described as 'locally abundant' with a population estimated to range between 1,600 and 4,500 individuals in the Bellinger River (Blamires, et al., 2005; R. Spencer, pers. comm., 11 March 2015 in NSW Scientific Committee 2016). Potential threats to the population were considered to be vulnerabilities associated with limited distribution and specific habitat requirements, predation, alteration to water quality, and possible hybridisation and competition with the Murray River Turtle (*Emydura macquarii*) (Spencer, et al., 2007; Blamires & Spencer 2013; Spencer, et al., 2014). More background detail is contained in Appendix I.

Initiating Event

In mid-February, 2015 a significant mortality event was observed in BRST in the Bellinger River. Numerous dead and sick turtles were found, displaying clinical signs such as severe swelling or ulceration of the eyelids, cloudy corneas, lethargy and reluctance to move, and some animals dragged their hind legs behind them. Most sick animals died within a short time of being found, and animals that were brought into rehabilitation care were euthanased within a few days due to progression of the debilitating disease despite nursing care. Periocular ulcers initially suggested exposure to a caustic agent was involved. However, the sensitive oral and cloacal mucosa of affected animals appeared normal. Internal examination of the turtles revealed variable changes in the colour and consistency of the parenchyma of the kidney and spleen, while microscopic examination of the tissues of affected turtles revealed a consistent pattern of acute inflammation and necrosis. This pointed to the presence of an infectious disease process. No pathogens, however, were visible within the lesions when viewed under light and electron microscopy.

Immediate Response

(i) Site examination and removal of affected animals

Due to the grave prognosis for affected animals and concern about the potential presence of a highly infectious pathogen, The Department of Primary Industries (DPI) advised that affected animals be collected and euthanased for animal welfare reasons and to prevent possible spread of the disease. More than 430 turtle deaths were recorded in the period until June 2016. This consisted of dead bodies and affected BRST that were collected and euthanased by a local veterinarian under the

direction of DPI. It is assumed that the actual number of deaths was higher with some bodies thought to have been undetected lying on the riverbed or washed downstream. A flood was also recorded within 72 hours of detection of the mass mortality event and further minor and major flooding events were subsequently recorded in April and May 2015.

(ii) Animal and water quality investigation

Initial disease investigations focused on ruling out the presence of known pathogens of reptiles, aquatic animals, and pathogens known to cause the types of lesions observed. Gross and microscopic post mortem examinations, haematology, serum biochemistry, bacterial and fungal culture, viral culture and DNA-based Polymerase Chain Reaction (PCR) tests for specific pathogens were conducted by the Australian Registry of Wildlife Health (ARWH) at Taronga Zoo, NSW DPI, Australian Animal Health Laboratories (AAHL), the University of Sydney, and Murdoch University. Animal tissues taken during necropsies of bodies collected during the event (sent for analysis April 2015) were analysed for heavy metals, mercury, organo-chlorine pesticides, organo-phosphate pesticides and phenoxy acid herbicides. All results fell within the normal range.

Initially all microbial tests returned negative results, yet the pattern of lesions and pattern of disease spread along the river remained most consistent with the presence of an infectious agent. Given that bacteria, fungi and protozoa should have been visible microscopically within lesions, a viral agent was considered the most likely pathogen type and additional attempts at viral culture were undertaken. Within approximately 6 months of the event, a virus previously unknown to science was isolated in a pattern consistent with it being the likely agent responsible for the mortality event. This virus, which we shall refer to as Bellinger River Virus (BRV), has been identified as the greatest threat to the survival of the BRST.

Concurrent testing of water quality was conducted by both Bellingen Shire Council (BSC) and NSW Environment Protection Authority (EPA). Water samples were collected on 18/3/2015 at 5 geographic locations on the Bellinger River following Australian Drinking Water Guidelines, 2011 NHMRC and methods based on Standard Methods for the Examination of Water and Waste Water, APHA. There were no significant findings.

The current BRST population is estimated to be between 200 and 300, predominantly juvenile, animals (based on recent preliminary surveys). Further surveys with increased coverage of the Bellinger River are planned to provide a more accurate population estimate.

(iii) Establishment of an 'insurance' captive breeding for reintroduction program

The Office of Environment and Heritage (OEH) is coordinating the Conservation Project for the recovery of the BRST. This Conservation Project is focussed on a captive breeding program and a planned reintroduction program. This action was taken based on the findings from preliminary surveys in the Bellinger River which found very few surviving adults and a population of mostly juveniles extant in the river. As a species exhibiting Type III survivorship where mortality rates decrease with age (Spencer & Thompson, 2000; Blamires et al. 2005), the BRST is highly reliant upon the survivorship of adults for ongoing survival. The Taronga Conservation Society (TCS) is managing the Captive Breeding Program in-kind on behalf of OEH.

OEH is seeking advice on appropriate conservation actions and research priorities as an outcome of this Conservation Planning Workshop to further develop the Conservation Project aimed to recover the BRST population.

Subsequent Actions

Mass mortality events in wildlife never occur in isolation but are an expression of the interactions between the affected animals (hosts), the causative agent(s) and the environment (Wobeser, 2006). Therefore, following the initial, emergency response the investigation was broadened to look more holistically at the river system in which this event occurred. The aim was to gain a better understanding of the complex host, agent and environmental interactions that might have precipitated this event and apply any insights to control or prevent further impacts on BRST, other riverine species and, potentially, the adjacent human communities. Figure 1, developed by participants in the conservation planning workshop described below, provides some idea of the complex interactions considered relevant by the invited experts. The workshop aimed to pool the relevant knowledge and expertise available to review and analyse the threats as the basis of a conservation and research action plan.

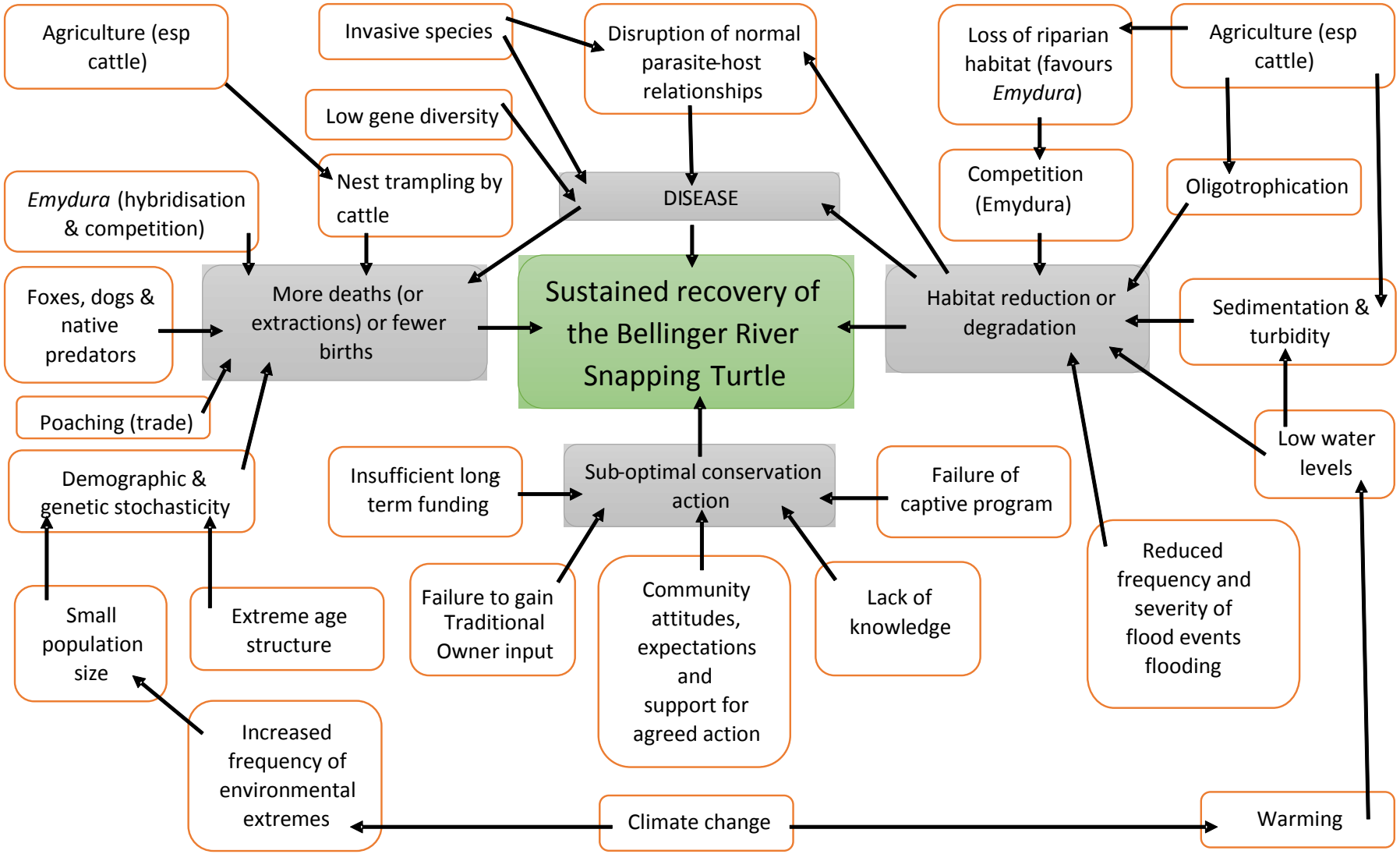


FIGURE 1: CURRENT AND POTENTIAL HAZARDS FOR THE SUSTAINED RECOVERY OF THE BELLINGER RIVER SNAPPING TURTLE *NOTE- THESE ARE NOT ALL PROVEN HAZARDS.

The Conservation Planning Workshop

Sixteen experts from eight organisations gathered in Sydney, NSW, between November 1-2, 2016, to agree on a plan of priority actions for the recovery and conservation of the BRST. The workshop was hosted by Taronga Zoo, organised and funded through the Office of Environment and Heritage (OEH), and facilitated by the IUCN SSC Conservation Breeding Specialist Group.

Before the workshop, participants collaborated to compile two briefing documents: one detailing the general biology and ecology of the species, known threats to its persistence and conservation action currently in progress (Appendix I) and the other considering in detail the potential disease hazards relevant to the species and its current or proposed management, and in particular all information relevant to the recent BRV disease event and the species' response to it (Appendix II). The specific aims of the workshop were to:

- review the information available on the species and its reaction to the recent disease event;
- review the information available on the disease itself;
- review the information available on other existing or potential threats to the species;
- build a consensus interpretation of this information among the experts present;
- use this interpretation as the basis for recommending a plan of action for BRST recovery.

Gerry McGilvray, OEH, welcomed participants on the first day of the workshop and set the context for discussions. The workshop opened with a series of presentations summarising key information and information gaps:

- IUCN SSC CBSG workshop philosophy, process and tools (Richard Jakob-Hoff, CBSG Australasia);
- Status Review – biology/ecology, past & present distribution and status, major threats, conservation activity to date, current investigations (Shane Ruming, OEH);
- Disease – what we know and don't know about the current disease issue, and about other relevant disease hazards (Karrie Rose, TCSA);
- The captive program – primary purpose, current status, major challenges (Michael McFadden, TCSA);
- Use of population models to explore key management questions (Ricky Spencer, WSU).

A visioning exercise followed which resulted in a qualitative description of what successful recovery would look like to those present (Appendix VI). From this, a set of measurable goals was developed after the workshop, with indicators, and these were reviewed by participants as part of the report drafting process.

Participants next confirmed a list of known existing or potential hazards or obstacles to realising the vision and a visual representation of this was created which depicted both the known and the assumed impact on BRSTs, the relationships to other hazards/obstacles, and the root causes (see Figure 1).

Participants then separated into two groups, one to explore the full suite of existing and potential disease-related hazards, the other to explore non-disease-related hazards (recognising that there is overlap between these). Over the next day-and-a-half each group worked separately to agree the current state of knowledge of the hazards considered, to identify critical information gaps and to recommend hazard mitigation activities. Groups reported to each other periodically and sought further input. At the end of the second day, recommended strategies and activities from the two groups were synthesised to create a draft conservation action plan for the next five years and beyond. Outputs from the working groups are detailed below.

Vision

It is 2030. The Bellinger River Snapping Turtle project is a model conservation program for supporting critically endangered native fauna, facilitated by multi-agency collaboration and community engagement.

This program has ultimately led to river health restoration and a sustainable turtle population that is disease free.

Goals:

1. **Bellinger River Virus does not pose a threat to BRST in the wild.** Measured by either absence of virus (not detectable via testing), resolution of issues relating to susceptibility, or immunity or protection provided to the species (by vaccine or otherwise).
2. ***Emydura macquarii* does not pose a threat to BRST in the wild.** Measured by absence of *E. macquarii* threat. Control methods ensure that hybridisation threat is significantly minimised.
3. **BRST are abundant in the Bellinger River.** The adult population is at least 150-385 adults (33-106 females) by 2032 with a total possible population of 700 to 2200 animals.[1].
4. **The community supports the recovery program and is actively engaged in the long-term health of the Bellinger River system.** Government and community organisations including BSC, OEH and Bellinger Landcare are engaged through the Local Stakeholders Group and are implementing/supporting a river health program. Landholder involvement indicated by at least 5 km riparian zone rehabilitated by 2021 and by a citizen science project on river health including a minimum of 15 volunteers.
5. **Multi-agency and academia collaboration is in place and working positively for the program.** OEH, Taronga (including the ARWH), DPI, BSC, University of Canberra and WSU have continued active involvement.

[1] R. Spencer, unpub. modelled data, May 2017

Hazard Analysis

A disease outbreak is widely understood to have precipitated the current critically endangered status of BRST. However, it is also recognised that a range of other factors may have predisposed the species to disease risk, could prejudice its recovery, or pose a future risk to the species should it be successfully recovered. The purpose of the hazard analysis was to confirm, clarify and prioritise the full suite of threats, obstacles and issues currently or potentially impacting on the sustained recovery of the BRST, as a precursor to exploring mitigation.

Confirming Hazards

Workshop participants began their analysis by confirming a list of current or potential hazards. The list included both direct biological threats and less direct social and economic impacts. A hazard diagram was constructed to represent the known or inferred relationships between hazards, the route through which they impact on the viability of BRSTs, and where possible their underlying causes (see Figure 1).

Hazard Clarification

As noted above there are gaps in the evidence-base relating to the hazards threatening (or potentially threatening) BRSTs in the wild. Also, experts do not all agree on the best interpretation of the information that is available but agree that urgent action is needed and therefore decisions must be taken against the current background of uncertainty. All decision making involves some assumptions and various constraints. Making these assumptions and limitations explicit is an essential part of wildlife conservation planning as information is often scarce and resources limited. The purpose of the hazard clarification step was to build, among experts present, a consensus interpretation of the information available for each hazard identified. The following descriptions reflect that consensus. In characterising each hazard an attempt was made to be clear about what is **fact**, what is **assumption**, and **which data gaps need to be filled** in order to progress decisions about conservation action.

Fox predation

Description. Introduced foxes are known to prey on nesting female turtles and their eggs, causing direct mortality of both. In *E. macquarii*, fox predation may result in 90% egg mortality (Thompson, 1983).

Cause. Foxes are a permanent presence in the area.

Mitigation options. Rehabilitation of potential nesting habitat that has become overgrown with weeds, to provide more nesting sites, thereby mitigating the impact of predation. Consider carefully the rehabilitation of riparian habitat around nesting areas to increase complexity and as a result interfere with fox olfactory and visual honing skills (due to risk of disturbance this is not considered an appropriate strategy where nesting areas are thought to be working well). Continual removal of

foxes using standard techniques (shooting, baiting, trapping) with monitoring to assess the effectiveness of this for BRSTs; fencing nesting areas; by-pass the threat of foxes by inducing females to oviposit before they nest, collecting eggs and releasing hatchlings (interim measure).

Note. Direct fox control over the next 4-5 years was considered of little benefit as there will be very few adult females in the river.

Key information gaps:

- Where do BRSTs nest? There are significant gaps in our knowledge of BRST nesting ecology which will make it difficult to protect nests or to encourage the restoration of the riparian zone in ways that will support successful BRST nesting.
- BRST nest predation rates (rates presented here are inferred from studies of *E. macquarii*). There are some relevant data in Blamires et al. (2005), however at the time of the study there were relatively few *E. macquarii* in the Bellinger River (R. Spencer, pers. comm.).

Disease (Bellinger River Virus)*

Description. BRV is a newly discovered virus (K. Rose, pers. comm.) not found in any host species other than BRST to date. It appears to be highly specific as a pathogen of this species, appearing to mostly impact adult animals. An understanding of this virus and its epidemiology is critical to successful mitigation and control, including successful management of the captive-breeding-for-release program.

Cause. BRV has been identified as the immediate cause of the recent mortality event. Its ability to cause disease in this species may be influenced by a number of host (age, sex, genetics, diet, body condition, seasonal physiology, population density, immune and health status), agent (virulence, strain variation, method(s) of transmission, mutation rate and ecology) and environmental factors (including water quality, food availability, temperature fluctuation, toxin exposure and seasonal food availability).

Mitigation options. Research to improve understanding of the biology and epidemiology of this virus is critical to the development of effective disease mitigation actions. Potential mitigating measures were evaluated and actions are identified in this report (see action plan, pp 27-41.)

Key information gaps:

- Origin, mode of transmission, current prevalence and distribution of BRV, and susceptibility of species and life-history stages.

*A comprehensive Disease Risk Analysis (DRA) is attached as Appendix IV to this report.

Dog predation

Description. It is assumed that, like foxes, feral and domestic dogs will prey on both BRST eggs and nesting females. Dog predation is separated from fox predation here because its potential impact was assumed to be small compared to that of foxes and because mitigation options (at least for domestic dogs) were assumed to be different.

Cause. Feral dogs are present in the area and domestic dogs can sometimes wander.

Mitigation options. *Domestic dogs:* educate the community about keeping dogs under control during the turtle breeding season (using signage); fencing nest sites. *Wild dogs:* standard removal techniques; fencing nest sites.

Note. As for foxes, for the next 4-5 years the threat of dogs is mostly to *E. macquarii*.

Predation by native species

Description. The following are known to prey on freshwater turtle eggs, hatchlings or juveniles: catfish (Blamires & Spencer, 2013); ravens, water rats and goannas (Thompson, 1983); and bandicoots (G. Kuchling, pers. obs.). Where present, they are assumed to be natural predators of BRSTs.

Effect. It is assumed that, prior to the mortality event, predation by native species did not have a major impact on the viability of BRST. With population size so low however, all predation is now a potential threat to recovery.

Cause. Not applicable – native species are natural predators of BRSTs.

Mitigation options. No specific mitigation was proposed, though some control would result from mitigation of other threats (e.g. nest protection against introduced predators).

Key information gaps:

- What is the likely reaction of native BRST predators to fox and dog control?
- Might native predators expand in number and, as a result, temporarily grow as a threat?
- Are avian predators (e.g. ravens) in unnaturally high numbers in particular areas of the river?

Riparian zone degradation

Description. This includes removal of riparian vegetation (primarily for agriculture) and the resulting colonisation by introduced weeds. Introduced cattle trample nests directly (Blamires, et al., 2005) but also trample soil around narrow entry points to the river, deterring re-growth of native species and causing erosion of the banks and increased siltation of the river bed.

Effect. There is some direct mortality of eggs as a result of cattle trampling nests though the impact is assumed to be limited to lowland sites (R. Spencer, pers. comm.). BRSTs rely on the riparian zone in multiple ways: for food (insects, flowers, fruits); for shaded, protected nesting areas which (it is

assumed) are less favoured by *E. macquarii*; riparian vegetation prevents erosion of river banks, the impact of which is to increase siltation, which in turn covers the cracks between rocks which can be a source of food for BRSTs. Further, an intact riparian zone is assumed to provide a greater variety of micro-habitats to which BRSTs may choose to retreat to regulate temperature, thereby effectively buffering against temperature extremes. For example, overhanging vegetation provides shading. It was assumed that a reduction of these benefits would impact on BRSTs negatively and reduce their ability to compete with the more aggressive and adaptable *E. macquarii*.

Though all agreed that the riparian zone provides benefits to turtles, views on the importance of this to BRST recovery differed as the abundance of BRSTs can be high in areas of higher siltation. It is not clear why this is the case.

Cause. Clearing for agriculture. Inaction on rehabilitation of the riparian zone or failure to maintain weed control after rehabilitation works. Narrow points of access to the river for cattle, concentrating their impact.

Mitigation options. Community-led restoration of the riparian zone.

Key information gaps:

- There is insufficient evidence to conclude what ideal nesting and riparian zone vegetation is for both BRST and *E. macquarii* in the Bellinger River. Initial observations may indicate that that both species benefit from open nesting areas (characteristic of unrestored areas).

Water quality

Description. Water quality variables include turbidity, dissolved oxygen, temperature, pesticides and other toxins, nitrogen, phosphorus and faecal bacteria. Further, a high *E. coli* count has been recorded in some areas. Though the quality of the riparian zone plays a key role in regulation or mitigation of many of these variables there are other contributing factors.

Effect. Not known. It is not known whether BRST switches diet in lower water quality conditions. Cloacal breathing could be a problem for BRST where water quality is low but this is not known and BRST has been seen in areas of high siltation, high nutrients and turbidity (unpublished data from OEH surveys).

Cause. Loss of riffles reduces oxygen levels in the water. Erosion resulting from land clearing and other causes increases siltation and turbidity. Total nitrogen exceeded ANZECC/NSW MER trigger values in the freshwater Bellinger River and lower Kalang estuary once during 2015-16 river health studies of the Bellinger catchment. In contrast, total phosphorus exceeded trigger values at all sites, and with the exception of Never Never Creek and the Rosewood River, these exceedances were persistent through the study period. Bioavailable nitrogen and soluble reactive phosphorus exceeded the trigger values on all sampling occasions at all estuarine sites. Bioavailable nitrogen exceeded the trigger values at all freshwater sites on all but the first sampling occasion and soluble reactive phosphorus exceeded the trigger value once each in the freshwater Bellinger and Kalang

Rivers. High nutrient concentrations did not result in nuisance algal blooms at any site during the study period (Mika, et al., 2016).

Mitigation options. Retaining riffles, reducing the impact of siltation resulting from unsealed roads, improving the riparian zone (see above). These initiatives need to be community-led. There are existing initiatives to support this through: Waterwatch, Bellinger Landcare and North Coast Local Land Services (LLS).

Key information gaps:

- Does BRST switch diet in poor water conditions?
- Is cloacal breathing a problem for BRST in turbid, silty or low oxygen conditions?

Filling these information gaps was considered helpful though not essential.

Hybridisation with *Emydura*

Description. *Emydura macquarii* is an Australian native turtle that appears to have been introduced into the Bellinger Catchment (Georges, et al., 2007; Georges, et al., 2011). *E. macquarii* and BRST are known to hybridise (Georges & Spencer, 2015). The relative survivorship and fertility of hybrids are not known, although they are known to be capable of breeding successfully with each other and with at least one of the parental species. In the past the two species have occupied different areas in the river (Cann, et al., 2015) and hybridisation events are assumed to have been rare (Blamires & Spencer, 2013). There is evidence that *E. macquarii* is now the dominant turtle species in the Bellinger River (Chessman, 2015). It is assumed that the rate of hybridisation could increase under the current situation, for example as a result of maturing BRSTs finding it easier to locate a mate among the larger population of *E. macquarii*.

Effect. It is assumed that an increase in the hybridisation rate will result in the current genotype/phenotype of the BRST becoming rarer. Additionally, *E. macquarii* appears to be resistant to the BRV. If this resistance is conferred on hybrid individuals, it is assumed that this would magnify the effect on the current BRST genotype. The occurrence of hybrids, along with the apparent absence of BRST in the Kalang River has given rise to the hypothesis that BRSTs may once have occurred there but have been out-competed by *E. macquarii*. It was agreed that there is currently too little evidence to make a firm judgement on this. However, based on the evidence and anecdotal information available in regard to both hybridisation and competition with *Emydura* it was considered highly unlikely that the two species could co-exist in the long-term.

Cause. Introduction of *E. macquarii* to the Bellinger River historically and possibly ongoing.

Mitigation options. Investigate options to manage the *E. macquarii* threat that are acceptable to the community.

Any mitigation of this hazard will affect an Australian native species and so will need to be shaped by the values and attitudes of the Bellinger River community and those of Indigenous groups.

Key information gaps:

- Is *E. macquarii* a reservoir for BRV?
- Is BRV in the Kalang River?
- How will the indigenous connection to freshwater turtles be impacted?
- Is hybridisation in one direction (e.g. male *Emydura* and female BRST)?

Competition with *Emydura*

Description. *E. macquarii* are a more aggressive species than BRSTs and are assumed to compete with them in several ways. They may cause general disruption and interference - male *E. macquarii* may chase female BRSTs. Arthur Georges is investigating directionality in his genetic studies. *E. macquarii* are voracious feeders and though their diet is suspected to be broader than that of BRSTs (Allanson & Georges, 1999; Spencer, et al., 2014) there is sufficient overlap for competition over food to be a problem. This may only occur however, when food is limited, which may not be a problem in the short-term due to the reduced number of turtles in the river. There could be competition at the juvenile stage but nothing is known about this. It is assumed that the situation in the Kalang River (where it is thought that *E. macquarii* may have out-competed BRSTs) represents a possible future for the Bellinger River. The consensus view of the group was that the two species cannot co-exist in the Bellinger River long-term.

Effect. Though there is little direct evidence of it in this specific case, sustained competition can be assumed to reduce individual growth rates and body condition, potentially exacerbating disease susceptibility and leading to reduced population growth.

Cause. Introduction of *E. macquarii* to the Bellinger River historically, and possibly ongoing.

Mitigation options. As for hybridisation.

Key information gaps:

- What is the degree of home range and habitat overlap between BRST and *E. macquarii? – This will be answered by radio tracking to begin Oct 2017 under the PhD study (see Appendix VIII).*
- What is the degree of dietary overlap between BRST and *E. macquarii? – This will be answered by stable isotope and stomach flushing studies under the PhD (limited by small numbers of adults extant).*
- What happens to BRST with and without *E. macquarii*? Exclusion trials may be pursued if questions to points above not answered.

Captive program failure

Description. Captive program failure could result from a complete inability to breed BRST in captivity, or from a disease or other catastrophe causing loss of individuals. Note that turtles are

long-lived. Short-term inability to deliver breeding results can often be corrected; however, with only five sexually mature females in captivity, building up numbers is urgent.

Effect. No offspring for release and no insurance against extinction in the wild.

Cause. Long-term failure to establish, for example, long-term dietary requirements or fertility triggers; genetic bottleneck as a result of too few individuals breeding successfully and subsequent losses due to inbreeding depression. Theft.

Mitigation options. Establish a second insurance colony. Catalogue and share all information gleaned from the program.

Key information gaps:

- List of questions to be determined after 1 or 2 seasons.

Poaching

Description. Taking BRST from the river to sell in the pet trade. May also include some extraction by private individuals for well-intentioned but misguided purposes. This is a potential threat. There is no evidence of its occurrence to date, though it is known that turtles are taken from rivers and there is commercial trade of Australian turtles in Asia (R. Spencer, pers. comm., 2016).

Effect. Fewer BRST in the river. Potential disease spread to other areas where poached animals are released.

Cause. Turtles are collectible. Economic incentives increase as the rarity of the BRST increases. Also potential exists for misguided action by those wanting to protect the species.

Mitigation options. Continue with existing strategies. All animals are currently notched and electronically tagged, and locations of animals are not publicised.

Community and stakeholder engagement issues

Description. To be successful the recovery of BRST will need the support of the Bellingen community (i.e. it requires a "social licence" to operate effectively). Thus far the Bellingen community has been supportive, although some misinformation exists. There are conflicts between welfare and conservation objectives and the community does not necessarily speak with a single voice on these issues.

Effect. Lack of community support could derail this project.

Cause. Information and communication to the community may be too slow in some instances. This problem might be exacerbated by a distrust of government in sections of the community. Some sectors of the community may not agree with actions that could compromise animal welfare.

Mitigation options. Invest time nurturing community trust. Communicate early and often. Cultivate influential members of the community (can be adults or children) and get them on board. Give them the tools to spread the word and work for the project. Make use of the existing community/stakeholder reference group. There is a good model in the Western Swamp Tortoise program where key community representatives are included on the Recovery Team.

Indigenous stakeholder input

Description. Acknowledging cultural aspects of the project is important. In any formal recovery plan there is a requirement to demonstrate consultation with indigenous groups but departmental protocols are not always sufficient.

Effect. Lack of support for the project from Indigenous groups will have ramifications for proposed actions.

Cause. In the case of insufficient engagement with local Aboriginal community.

Mitigation options. Go beyond departmental protocols and pursue meaningful engagement.

Stochasticity

Description. Demographic stochasticity (fluctuations in population growth rate driven by chance, variation in birth and death rates and sex-ratio which can de-stabilise and drive decline in populations when they are very small) is a risk for the captive population for the foreseeable future. Catastrophes (rare, unexpected, extreme die-offs) from disease, natural catastrophes etc. pose a greater risk to smaller populations with limited distribution and therefore pose a continuing risk to both wild and captive BRST populations. Year-to-year environmental fluctuations (within the normal range of "good" and "bad" years for the species) cause fluctuations in birth and death rates which, though easily buffered by large populations can de-stabilise and cause declines in small populations and therefore pose a risk to the wild population. Genetic stochasticity can drive a depression in fitness through mechanisms such as inbreeding and chance-driven loss of gene diversity (drift), which can cause declines in populations that either begin with low genetic diversity or remain small through generations, and we assume this to be a risk to both the wild and captive BRST populations.

Effect. Population decline. Stochastic effects can exacerbate each other to create an extinction "vortex" even in the absence of other, deterministic threats.

Cause. Small population size, single populations (captive and wild), limited distribution, low gene diversity.

Mitigation options. Establish a second captive population at a second site to spread the risk. Increase population size in the wild as quickly as is feasible, primarily through breeding for release. Restore the riparian zone to help mitigate the effects of weather extremes on turtle habitat (especially in the face of climate change). Manage the captive population to retain genetic diversity and aim for high levels of gene diversity and low-levels of inbreeding in the release population also.

If after a period of time recovery strategies are failing in the Bellinger River, consider a second wild site.

Climate change

Description. Impact on BRST as a result of changing climate

Effect. Climate change predictions are uncertain. The most likely impacts on the BRST's environment are assumed to be elevated air and water temperatures and more extreme and erratic rainfall, drought and fire events. It is not clear what the net impact of this on BRSTs might be. The increasing frequency and severity of environmental extremes may prove difficult to adapt to, given the BRST's assumed narrow habitat requirements and low gene diversity. Conversely, increased size and frequency of flood events may clear away silt and improve food resources for BRSTs (though this may at the same time scour out other food resources (B. Chessman, pers. comm.)), and a longer period of warm water temperatures may increase turtle growth rates, allowing them to mature earlier and increase reproductive outputs.

It was agreed that the degree of uncertainty makes mitigation planning difficult but likely to be best directed towards maintaining or repairing habitat complexity (to allow individual BRST to move between a range of micro-habitats as needed). It was also agreed that climate changes impacts are likely to act gradually and should not be a major threat in the next 20 years.

Cause. Negative impacts of climate change on BRST are assumed to be exacerbated by a range of human-mediated threatening processes, in particular the simplification and degradation of BRST habitat (riparian zone destruction and reduced water quality).

Mitigation options. Over a 20-year planning period it is assumed that climate change effects will not require specific mitigation beyond actions levelled against other threats. In particular riparian restoration is assumed to increase the resilience of the species, buffering against temperature shifts and increasing the range of available micro-habitats. If the effects of climate change become too severe in the long term, more extreme options such as assisted colonisation may need to be considered and planned for.

Prioritisation

Participants were asked to consider the final list of hazards and prioritise them in terms of importance. Following discussion of what was meant by "importance" it was agreed that prioritisation would consider the question, "Of these hazards, which is it most important for the program to address?" and that this would be more easily answered by considering two time-frames: the first 5 years of the program and the following 15 years. Hazards were therefore prioritised (using colour-coded dots) according to:

- The most important hazards for the program over the first 0 – 5 years (RED dots).
- The most important hazards for the program from years 6 to 20 (BLACK dots).

Participants were each assigned 5 RED and 5 BLACK dots and were invited to distribute dots according to their considered priorities (see Figure 2.). Participants could place all 5 dots on a single hazard or could spread their dots among hazards. The resulting scores and prioritisation ranks are provided in Table 1. These ranks were reviewed by the group at the beginning of day 2 and were agreed to be a reasonable reflection of group priorities, with the exception of the high priority afforded to riparian zone degradation[2]. After discussion it was agreed that this difference of opinion would not be an obstacle to progress. Riparian zone restoration is a current and ongoing community-led activity that operates outside the BRST project, potentially accruing a range of other environmental benefits. It is not considered to compete for resources with other BRST priorities. Therefore, with the short time available, resolution of this difference of opinion was not pursued and its rank remained that assigned during the prioritisation exercise.

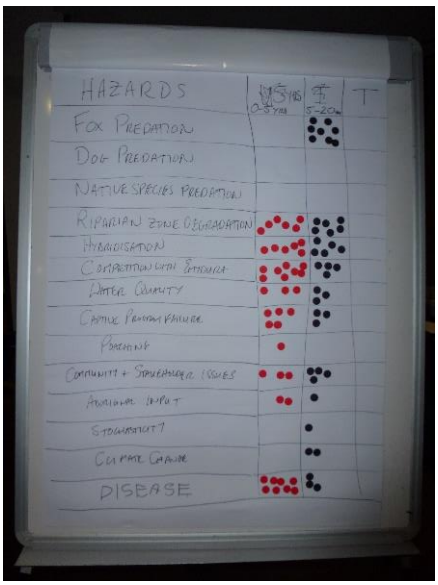


FIGURE 2: PRIORITISATION OF 1-5 YEAR AND 6-20 YEAR HAZARDS IN THE SUSTAINED RECOVERY OF THE BELLINGER RIVER SNAPPING TURTLE

As shown, over the next five years the most important hazards were considered to be: Disease (i.e. BRV), *E. macquarii*, riparian zone degradation and captive program failure. Longer-term (6-20 years), fox predation and community and stakeholder hazards were considered to become more important.

It should be noted here that although particular hazards may have a greater impact on BRST recovery during the 6-20 year time-frame, this does not imply that mitigating action can be delayed until then. For example, it was agreed that sustaining community engagement over the first five years will be key to having in place sufficient support to deliver on 6-20 year high priority hazards such as fox control. Poaching and predation by dogs and native species were assigned low priority as their impact was considered relatively small compared to that of foxes. Stochasticity and climate change were assigned low priority over the time frames addressed by the prioritisation exercise. This assignment may reflect the difficulty of comparing these hazards, whose effects are potentially large but highly uncertain, with better understood, deterministic hazards. Input from Indigenous groups did not rank highly as a potential obstacle to recovery but their engagement was considered important for a variety of other reasons.

[2] This was considered by a majority to be of high importance to both short and long-term viability of BRST but by some to be of low importance on the basis that 1) BRST are observed in relatively reasonable numbers in degraded areas and 2) lack of evidence to support the suggestion that the current state of the riparian zone is impacting BRST viability.

TABLE 1: PROPOSED PRIORITY HAZARDS TO SUSTAINED RECOVERY OF BRST OVER PROJECT YEARS 1-5 AND 6-20.

Note: the main use of this exercise was to guide the allocation of time to discussions of potential mitigating strategies. It does not imply funding priorities.

Hazards	1-5 year priority Score (RANK)	6-20 year priority Score (RANK)	Overall score (RANK)
Competition with *E. macquarii*	8 (1)	4 (4)	12 (1)
Riparian zone degradation	6 (2)	6 (2)	12 (1)
Disease	8 (1)	3 (5)	11 (2)
Hybridisation	6 (2)	5 (3)	11 (2)
Captive program failure	6 (2)	3 (5)	9 (3)
Predation by foxes	0 (6)	8 (1)	8 (4)
Community and stakeholder engagement issues	3 (3)	4 (4)	7 (5)
Water quality	3 (3)	3 (5)	6 (6)
Input from Indigenous people	2 (4)	1 (7)	3 (7)
Climate Change	0 (6)	2 (6)	2 (8)
Stochasticity	0 (6)	1 (7)	1 (9)
Poaching	1 (5)	0 (8)	1 (9)
Predation by dogs	0 (6)	0 (8)	0
Predation by native species	0 (6)	0 (8)	0

Mitigation

An initial discussion of potential strategies for mitigation took place as part of the hazards clarification step. It was recognised that some of the strategy options discussed could contribute to the mitigation of more than one hazard, that is, adequate mitigation of some hazards could be achieved as a by-product of the mitigation of others. For example, it was considered likely that effective restoration of the riparian zone could achieve sufficient mitigation of water quality hazards and adequate buffering (at least for the next 20 years) against climate change. Participants discussed mitigating strategies further to arrive at a subset of broad priorities which in their view, if implemented effectively, would result in the sustained recovery of BRST without duplication or redundancy. The results of these discussions are summarised in Table 2. It was noted that the effective delivery of these strategies will depend on several factors, including choice of approach, long-term resourcing, community support and gaining answers to key questions about BRV and BRST ecology.

Understanding more about BRV is of immediate importance in designing effective mitigation; in particular, understanding whether *E. macquarii* are reservoirs for disease as this will determine the options available for control. It was agreed that even in absence of the current disease, *E. macquarii* control would remain essential to the sustained recovery of BRST, though would not on its own provide sufficient mitigation and must be coupled with other measures.

A “good” riparian zone should impact positively on water quality and provide habitat and nutrient benefits to turtles. The Bellinger River riparian zone is degraded in places and there are some reported issues with water quality (e.g. Mika, et al., 2016). Rehabilitation is a large task and many of the areas requiring rehabilitation are on private land. Community support and engagement is key.

An integrated strategy and action plan for riparian and instream management is proposed, ideally including partnerships with BSC, LLS, Landcare and others, to develop a long-term approach to prioritising areas for management using the evidence available. Further information is needed to understand rehabilitation priorities for turtles. Observations to date indicate that a variety of habitats may be required to support the lifecycle of BRSTs. While areas of degraded land (clear gravel or sand with cattle access to the river) may provide beneficial nesting habitat, other areas may need to be restored through weeding to provide suitable nesting habitat. Other areas may need to be rehabilitated to provide canopy for shade, habitat structure and food. It was noted by participants that though some site prioritisation may be possible, in reality the sites available for rehabilitation will to a large extent be determined by the interest of landowners.

Support for the integrated approach proposed could come through the following avenues, some of which have been in place for some time but may require some refreshment:

- BSC has an Environment Sustainability Advisory Committee and a River and Biodiversity Projects Officer responsible for strategic planning and implementation of river and biodiversity programs and projects dependent on funding. Through the Committee, Projects

Officer and/or Local Stakeholder Group, a proposal could be prepared to seek funding to develop an integrated strategy and action plan to guide riparian and instream management.

- Involvement of NGOs. For example, setting up protocols for groups like OzGreen to follow.
- Use of the Waterwatch model under an OEH Citizen Science program.
- Bellinger Landcare has a river management booklet for members of the public.
- BSC has fact sheets on biodiversity of the Bellinger and Kalang River System, managing erosion and managing stock. These are available on the BSC website or over the counter.
- Encourage indigenous participation in surveys and/or monitoring turtle nests and predation (Citizen Science). A good example is the South Australian Indigenous on Country Program where young indigenous participants carry out wetland restoration.
- Approach Indigenous elders about how they can be involved.

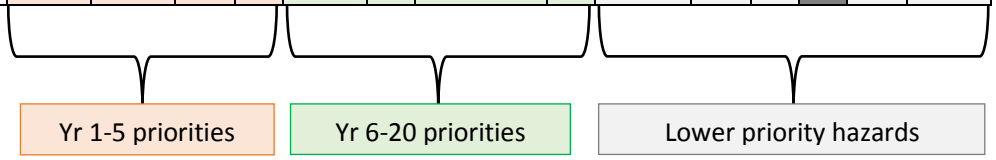
Poaching was considered a relatively minor problem over the long-term, but the financial incentives for poaching increase as turtle populations decline. It is assumed that if a member of the community is poaching BRSTs, the local community can be relied upon to report it. The pros and cons of attempting to list BRSTs on Convention on International Trade in Endangered Species (CITES) appendices were discussed. There are potential negative impacts though the additional attention drawn to BRSTs among international collectors. On balance it was agreed that the current approach of not publicising nest sites and relying on the local community to report breaches would be sufficient.

Mitigation of foxes is not a priority for BRST recovery until years 4-5 as this is when the current BRST cohort in the river is expected to begin breeding. Again, it will be important to have the community on board as baiting will need to occur on private land.

TABLE 2: SUMMARY OF PRIORITY STRATEGIES FOR MITIGATION OF THE IDENTIFIED HAZARDS TO BELLINGER RIVER SNAPPING TURTLE RECOVERY

(details of actions underpinning the strategies listed here are provided in the next section)

Priority mitigation strategies *Feasibility of mitigation not yet known. Key questions need to be answered before this can be determined. **Community-led activities run independently of the BRST recovery project though potentially informed and influenced by it.	Competition with *E. macquarii*	Riparian zone degradation	Disease	Hybridisation	Captive program failure	Predation by foxes	Community and stakeholder engagement issues	Water quality	Input from Indigenous people	Climate Change	Stochasticity	Poaching	Predation by dogs	Predation by native species
***E. macquarii* management:** answer key questions about the threat and evaluate control options using trials. Use results to design and deliver appropriate control.			?											
****Riparian zone restoration:** community-led projects.														
***BRV management:** investigate BRV, answer key questions and use this information to design and deliver appropriate management measures (see Appendices for detailed treatment of this)														
Community engagement: revise and implement communication strategy, manage local stakeholders group, involve community in multi-faceted on-ground action.														
Captive breeding for insurance & release: maintain best practice management of husbandry, disease risk, genetics and demography, spread program across multiple sites, rapidly generate large numbers for release.														
Fox control: targeted fox control and some fencing.														
Engagement of Indigenous groups														
Domestic dog control: install signage to deter uncontrolled dogs during the turtle breeding season.														
Poaching controls: continue tagging and not publicising turtle sites.														



Strategy and Action Plan

Based on the following high priority strategies for recovering the BRST a three-stage recovery process was developed and is illustrated and described in detail below.

High priority strategies:

1. ***E. macquarii* management**
2. **Riparian zone restoration**
3. **BRV management (see Appendix IV for details of how this will be approached)**
4. **Captive breeding for insurance and release**
5. **Community engagement**
6. **Fox control (post 5 years)**

The initial stage involves initiating the captive breeding program and filling key information gaps relating to the disease and to the situation in the river; the second stage involves pursuing recovery whilst continuing to gather information and test and hone strategies; and the third stage involves evaluating program progress and either winding down the program or changing direction. These stages are illustrated in Figure 3.

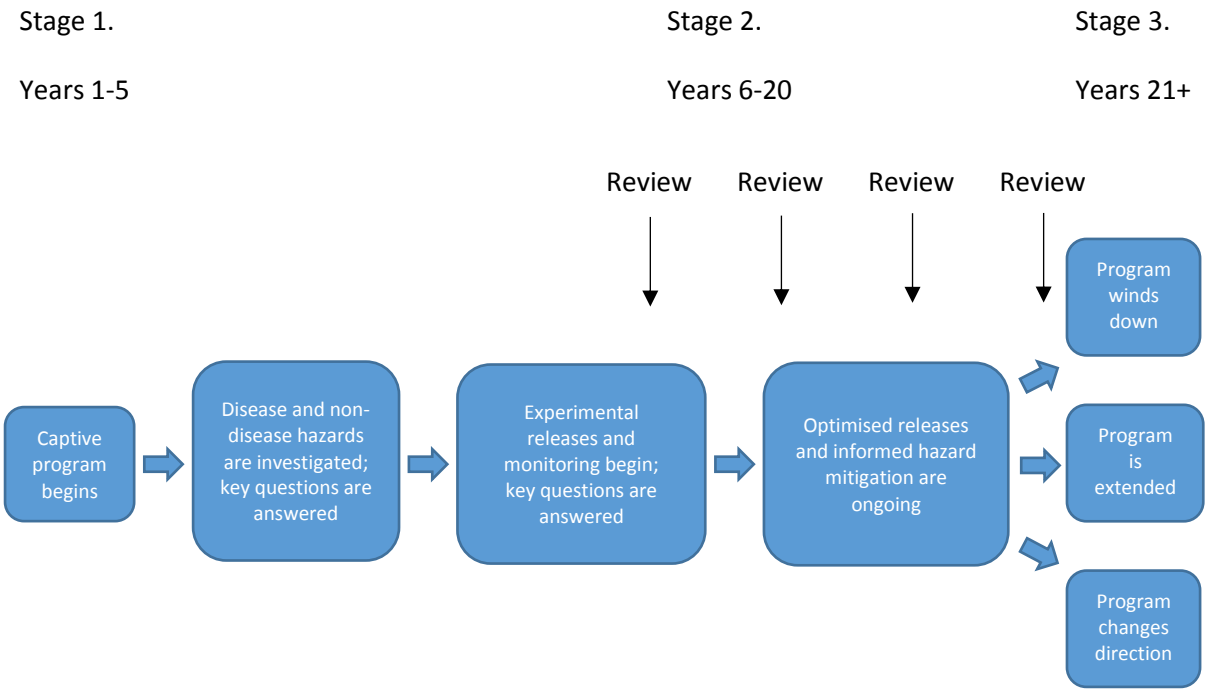


FIGURE 3: PROPOSED 3 STAGES OF THE RECOVERY PROGRAM FOR BELLINGER RIVER SNAPPING TURTLES.

Actions associated with each stage are identified below. Actions for Stage 1 have been developed in detail to provide a clear indication of what is required in the next five years. These detailed actions are summarised in Table 3 (p.41), which also illustrates expected implementation schedules which may extend beyond five years. It is assumed that significant program reviews will take place every five years.

Stage 1: 1-5 years

1. Continue investigation of non-disease hazards and answer key questions

- Study key aspects of *E. macquarii* and BRST biology and ecology
    - Identify nest sites, nesting preferences and location of juveniles of both species (to help design protection for BRST and to assist management of *E. macquarii*). With so few BRST adults, and therefore of nests, use presence of juvenile BRST and *E. macquarii* nesting sites as indicators of potential BRST sites (recognising that nesting habitat preferences may not necessarily be the same for the two species)
    - Study inter-species interactions (to inform management likely to favour BRST)
    - Establish whether hybridisation is one-way (e.g. males of one species, females of the other).

Action 1.1. Identify options for *E. macquarii* management

Detail: Work began on this at the workshop but was stalled by lack of information in some areas. Explore and evaluate options for managing *E. macquarii*. Include in the evaluation feasibility, resource intensity, likelihood of practical success and of community support for implementation. Whether or not *E. macquarii* is identified as a competitor of BRSTs, an increased hybridisation threat or a reservoir for BRV, will have an impact on this evaluation. Community consultation will be an important component of this action.

Lead agency: OEH

Potential collaborators: WSU

Time-line/frequency: Commence Year 2 (June 2017-2018).

Success measure(s): All potential options for managing *E. macquarii* have been evaluated and there is informed agreement on what are and what are not suitable and justifiable option(s) for controlling *E. macquarii* in the Bellinger River.

Related goal(s): 2 (Strategies 1 & 5)

Action 1.2. Evaluate the potential ecological impact of removing *E. macquarii* from the river system

Detail: Investigate pros and cons for the ecology of the River, of controlling *E. macquarii* in the river system.

Lead agency: OEH

Potential collaborators: WSU (Kristen Petrov)

Time-line/frequency: Commence Year 1 (June 2016-2017). Preliminary results in Year 2 (June 2017-2018)

Success measure(s): There is sufficient understanding of the potential ecological impacts of controlling *E. macquarii* to make informed and justifiable management decisions.

Related goal(s): 2 & 3 (Strategies 1 & 5)

Action 1.3. PhD thesis on the recovery of the Bellinger River Snapping turtle is complete

Detail: See detail in Appendix IX

Lead agency: WSU

Potential collaborators: OEH, Taronga (ARWH).

Time-line/frequency: Commence Year 1 (June 2016-2017), January 2017. Complete Year 4 (June 2019-2020), 2020.

Success measure(s): PhD thesis is complete and informs the ongoing recovery of the species.

Related goal(s): 2, 3, 4 & 6 (Strategies 1, 2, 3, 4, 5 & 6)

Action 1.4. Begin *E. macquarii* control assuming community support is secured.

Detail: Method as determined by investigations.

Lead agency: OEH

Potential collaborators: WSU

Time-line/frequency: Commence Year 3 (June 2018-2019). After results of initial dietary studies, end of 2017

Success measure(s): Community support has been secured for *E. macquarii* control and control has begun.

Related goal(s): 2, 4 & 5 (Strategies 1 & 5)

2. Continue to investigate the current disease hazard and answer key questions

Develop proposals and complete trials to establish the following:

- mode of transmission
- current prevalence and distribution of BRV
- species susceptibility.

Action 2.1. Investigate modes of BRV transmission.

Detail: Carry out experimental BRV infection trials to study transmission, incubation, shedding, age/sex susceptibility and pathogenesis. This will involve, initially, development and approval of a grant proposal, work on which should start immediately.

Lead agency: Taronga (ARWH)

Potential collaborators: OEH, DPI, James Cook University

Time-line/frequency: Commence Year 1 (June 2016-2017).

Success measure(s): Heightened understanding of this disease enables the likely effectiveness and feasibility of mitigation strategies to be assessed and informed decisions to be taken on issues such as *E. macquarii* control, vector control etc.

Related goal(s): 1, 3, 4, & 6 (Strategy 3)

Action 2.2. To establish a serological test for BRV with a high sensitivity and specificity

Detail: Establish and deploy the test as part of the epidemiological investigation of BRV disease. Establishing a serological testing method will help identify the virus identified as a primary pathogen. We would expect that during the outbreak, affected animals died so quickly that they did not have time to produce antibodies. Therefore, if antibodies are identified in those animals it is likely that the virus was present prior to the disease event. We are also currently uncertain whether the juvenile animals currently alive in the river are resistant to the virus or have not been exposed to it. The serological test can provide answers to this.

Lead agency: Taronga (ARWH)

Potential collaborators: DPI, OEH

Time-line/frequency: Commence Year 1 (June 2016-2017).

Success measure(s): Serological test with high sensitivity and specificity is developed and enables the detection of animals that have been exposed to BRV.

Related goal(s): 1, 3, 4 & 6 (Strategy 3)

Action 2.3. Explore possible antiviral treatment options for reptiles and associated biosecurity methods

Detail: Desktop study of possible treatment options explored.

Lead agency: Taronga (ARWH)

Potential collaborators: DPI, Bellingen Veterinary Hospital

Time-line/frequency: Commence Year 2 (June 2017-2018).

Success measure(s): Treatment options are understood and enable informed management of BRV-affected animals.

Related goal(s): 1, 3, 4 & 6 (Strategy 3)

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3. Establish and build the captive program

- Establish a studbook and captive management plan
- Develop and refine husbandry
    - Establish techniques for sexing young turtles (to allow females to be preferentially retained as needed)
    - Find ways to reduce current restrictions on captive diet (due to disease management) to improve husbandry capability
    - Maintain flow of information between captive and wild studies to enhance operations at both ends
    - Continued involvement of Taronga husbandry staff in BRST fieldwork
    - Test, establish and document husbandry protocols.
- Manage genetic risks
    - Source and capture additional founders to bolster gene diversity
    - Maintain in pairs (and some trios) initially to increase genetically effective size
    - Where options are available, aim to pair individuals with low and similar mean kinship values whilst keeping inbreeding coefficients below detrimental levels (aim for ≤ F=0.125 in the first instance)
    - Emphasise productivity in the first years of the program. The species is long-lived and, providing there is high survivorship, genetic composition can be adjusted later
    - Extend generation time where possible to further slow gene diversity loss.
- Manage demographic risks
    - Establish captive breeding at a second site using additional founders (see above)
    - Retain some of the animals bred initially (emphasising females), to reduce risk of loss of founder genomes and to reduce risks from stochastic events.
- Generate sufficient numbers of turtles appropriate for release

- Maximise output for release while protecting the source population by holding back sufficient individuals to reduce risk of loss (2-3 from each clutch initially).
- Maintain best practice disease risk management.

Note: ongoing discussion with experts and stakeholders will be required to agree the design of the release program, which will need to change over time to respond to new information and insights. Consideration will be given to, for example: minimum numbers for release, characteristics of release animals (e.g. age, gender, weight), age structure of release group, pre and post-release management, disease risk management, post-release monitoring etc.

Note that turtles are long-lived. Provided captive animals can be kept alive there are many breeding seasons ahead and many opportunities to refine captive breeding and release protocols.

Action 3.1. Assess viability of incorporating privately held BRSTs into the captive breeding program

Detail: See Table 15 and Figure 10

Lead agency: OEH

Potential collaborators: Taronga, DPI, WSU

Time-line/frequency: Commence Year 1 (June 2016-2017).

Success measure(s): Rigorous evaluation through health screening and diagnostic testing has been completed and enables a decision to be made.

Related goal(s): 4, 6 (Strategy 4)

Action 3.2. Capture additional founders for a second captive group and establish second quarantine facilities.

Detail: A minimum of 20 animals sought from wild population to form a second captive population (Population 2).

Lead agency: OEH

Potential collaborators: Taronga, WSU

Time-line/frequency: Commence Year 1 (June 2016-2017). Completed November 2016 (19 animals in captivity although population may require supplementation – To be determined early 2017 pending genetic results).

Success measure(s): A second captive group has been established successfully from a sufficient number of founders.

Related goal(s): Linked to achievement of Goals 4 & 6 (Strategy 4)

Action 3.3. Establish sex determination technique for juveniles.

Detail: Further attempts are currently being pursued by Arthur Georges in relation to identification of genetic sex markers which to date have been unsuccessful.

Lead agency: Arthur Georges (Institute for Applied Ecology, University of Canberra)

Potential collaborators:

Time-line/frequency: Commence Year 1 (June 2016-2017).

Success measure(s): Juveniles can be sexed reliably.

Related goal(s): 4 & 6 (Strategy 4)

Action 3.4. Prepare studbook, captive management plan and husbandry manual.

Detail: Prepare a studbook (in standard Species 360 compatible format) to record demographic, pedigree and other relevant data for each captive individual. Prepare a captive management plan describing the goals of the captive program (e.g. gene diversity retention, inbreeding management, harvest for release, disease management etc.) and the genetic, demographic and other management strategies agreed for achieving those goals, including selection of individuals for release. Prepare a husbandry manual outlining recommended practices relating to housing, feeding, lighting, breeding, rearing, disease management and release protocols.

Lead agency: Taronga Zoo

Potential collaborators: OEH, Wildlife Park TBC

Time-line/frequency: Commence Year 1 (June 2016-2017). Brief Husbandry Report March 2017, Husbandry Manual March 2018, captive management plan June 2017, studbook end of 2017

Success measure(s): Studbook, husbandry manual and captive management plan are complete.

Related goal(s): 4 & 6 (Strategy 4)

4. Engage and mobilise community support effectively

- Develop/review and refine a community engagement strategy
- Survey landowners to identify potential collaborators
- Promote community-led riparian zone restoration and identify areas requiring priority attention.

Action 4.1. Develop and implement a communications plan.

Detail: Develop a communication plan for the proposed recovery project. This should include a significant focus on BRV mitigation component but should also cover communication of research and community care for the turtle. Note that the turtles were once a food source and also have cultural significance to the local indigenous community.

Lead agency: OEH (staff to complete as part of the NSW Government Saving our Species (SoS) program)

Potential collaborators: BSC, Bellingen Landcare, National Parks and Wildlife Service (NPWS), DPI

Time-line/frequency: Commence Year 1 (June 2016-2017). New plan drafted February/March 2017.

Success measure(s): Communication is complete. It identifies key stakeholders and focuses particularly on BRV mitigation component but also covers research and community care for the turtle.

Related goal(s): 5 & 6 (Strategy 5)

Action 4.2. Establish a program for engaging the community around hygiene in relation to water contamination.

Detail: The virus was found to be still present in the river as of November 2016, source and means of spread unknown. As a precaution, disease-related signage on the river should be maintained, along with notices to ratepayers and media releases re-emphasising the need for hygiene to be employed on the river, and reporting regularly on the status of the disease in the river**.**

Lead agency: DPI

Potential collaborators: OEH, BSC, ARWH

Time-line/frequency: Current – review Year 1 (June 2016-2017).

Success measure(s): Hygiene practices for people, vessels and vehicles are set in place to minimise transmission by fomites.

Related goal(s): 1 & 5 (Strategies 3 & 5)

Action 4.3. Community education and engagement in ongoing vigilance and immediate reporting of dead and sick animals.

Detail: As part of the communications plan, the promotion of the existing procedure for reporting sick or dead BRST (via standard phone number and email address) is re-emphasised. This would be included in media releases relating to BRST, educational materials, presentations, BSC newsletters. This will remain in place until otherwise advised by DPI.

Lead agency: OEH

Potential collaborators: DPI, BSC, Bellingen Veterinary Hospital

Time-line/frequency: Commence Year 1 (June 2016-2017), early 2017 as per Communications Plan.

Success measure(s): When disease occurs, sick and freshly dead animals are reported by the community immediately, initiating a rapid response to remove those animals.

Related goal(s): 5 & 6 (Strategies 3 & 5)

Action 4.4. Engage community support (including volunteer groups and schools) for riparian restoration and promotion of in-stream habitat

Detail: For in-stream health Bellinger Landcare (2016) states that in-stream debris in the river plays an important role in river health. Debris provides aquatic spawning sites and areas for animals to hide from predators, as well as areas where animals can avoid intense sunlight and high current velocities (Crook & Robertson, 1999, in Ryder, et al., 2011). Debris also provides habitat for biofilm and invertebrates that maintain essential links in the food web for fish (Ryder, 2004, in Ryder, et al., 2011).

River roughness has an important role in river processes, especially during floods. Roughness (from debris, rocks, tree trunks and roots) slows flow and promotes sediment deposition. It helps the river maintain a balance in a dynamic system. Removing roughness speeds up flows and leads to accelerated erosion. Most erosion control projects involve increasing roughness by re-introducing rocks and or debris on the bank and in-stream (Bellinger Landcare, 2016).

Lead agency: BSC/ LLS/ Bellinger Landcare/ OEH

Potential collaborators: Orama Rivercare, OzGreen,

Time-line/frequency: Commence Year 2 (June 2017-2018).

Success measure(s): Community actively supports and participates in riparian restoration and in-stream river health.

Related goal(s): 4 & 5 (Strategies 2 & 5)

Action 4.5. Citizen Science water monitoring program (e.g. Waterwatch)

Detail: Citizen Science program by OEH as a Waterwatch program.

Lead agency OEH (Citizen Science)

Potential collaborators: OEH, Waterwatch, OzGreen, University of New England (UNE)

Time-line/frequency: Commence Year 2 (June 2017-2018). Start 2017.

Success measure(s): Partners are effectively engaged in water quality studies using standardised data.

Related goal(s): 4 & 6 (Strategy 5)

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5. Begin experimental releases and answer key questions

Experimental releases and ongoing disease investigation are expected to take place concurrently. All aspects of the design, execution and monitoring of release events will be discussed and agreed with the reference group. Workshop discussions considered the following areas:

- Testing and refining release strategies (numbers, ages, weights, sites, etc.)
    - Release at least 20-30 hatchlings, of different ages, and monitor survival
    - Consider pre-release phase: soft release (whereby individuals receive some form of pre-release acclimatisation, usually through temporary protection and support on-at the release site) versus hard release (whereby animals are released without support or protection).
- Managing *E. macquarii* (method not yet agreed – see Action 1).

The first phase of releases should be considered experimental and aimed at understanding what strategies are most successful with regard to release animal characteristics (e.g. age, weight, prior conditioning) and site or river conditions. To establish with confidence, the answers to these questions requires release and monitoring of sufficiently large numbers of individuals. Further discussions will be needed to decide the ideal numbers for answering specific questions and to agree the trade-offs between ensuring statistical rigour and getting results quickly. Further work is needed to explore the relative costs and benefits of two potentially competing release strategies:

1) Headstarting, where animals are reared for a period in captivity before release, thereby delivering more adults per clutch (due to lower mortality in captivity than in the wild) but incurring greater care costs and, possibly, providing captive-conditioned individuals less equipped for survival in the river.
2) Releasing animals post-hatch, reducing the cost of care, but delivering fewer adults per clutch (due to elevated mortality in the wild) though possibly providing individuals better equipped for survival in the river.

It is proposed that a combination of the two strategies be developed and the use of soft release be investigated with the reference group to manage risk. Releasing animals post-hatch may not allow for monitoring and so may not be the preferred option unless larger numbers are available for release.

Population Viability Analysis models developed by R. Spencer will be valuable in helping to explore the potential merits and pitfalls of each.

Action 5.1. Population survey and population monitoring.

Detail: Biannual surveys of wild population including disease surveillance, measurements.

Lead agency: OEH

Potential collaborators: WSU (years 1-3)

Time-line: Commence Year 1 (June 2016-2017). Biannual – ongoing pending review

Success measure(s): Wild population surveys and monitoring occur annually and inform an understanding of the epidemiology of BRV disease, population dynamics of BRST and *E. macquarii*, and growth rates.

Data recorded of environmental conditions such as water and air temperatures and samples for PCR testing informs study of epidemiology of the disease.

Data will be subject to standard capture-mark-recapture analyses and current population viability analyses will be continually updated as data is collected. We are currently looking at >60% probability of extinction if nothing was done (R. Spencer, pers. comm.). We want to get this down to <10%. Incorporating data from the captive population and survival and population estimates from the wild will refine that.

Related goal(s): 1, 2, 3, 4, 5 & 6

Action 5.2. Design, implementation and monitoring of a translocation proposal for release of hatchlings/ juveniles

Detail: Completion and approval of a translocation plan will be required before any release of captives back into the wild. This will incorporate an initial experimental reintroduction proposal outlining timing, numbers, location and monitoring program.

Lead agency: OEH

Potential collaborators: Taronga, WSU

Time-line/frequency: Commence Year 1 (June 2016-2017). Potential release of animals Nov 2018 or potentially earlier (to be determined in consultation with BRST reference group). Translocation proposal would need to be completed by June 2018 or earlier.

Success measure(s): A translocation proposal and ethics application are complete and approved.

Related goal(s): 4 & 6 (Strategy 3)

Action 5.3. Agree sites for BRST release (to be informed in part by PhD study).

Detail: Sites identified according to resource availability and ability to control existing threats for optimal release. PhD study will provide data on aquatic plants and invertebrates present at each site.

Lead agency: OEH

Potential collaborators: WSU

Time-line/frequency: Commence Year 2 (June 2017-2018). Preliminary results June 2017, final results June 2018

Success measure(s): Release sites are identified and agreed, informed by science.

Related goal(s): 4 & 6 (Strategy 3)

Action 5.4. Monitored release of BRST hatchlings/ juveniles)

Detail: Assuming the captive program becomes reliably productive and remains free of BRV, and animals are able to be released safely, releases are expected to take place annually. A review of the previous year's release will precede each release event so that lessons learned can be implemented. Releases may be delayed early-on to increase the number of animals and age-classes available for release (to improve the quality of data captured from post-release monitoring).

Lead agency: OEH

Potential collaborators: Taronga Zoo, WSU

Time-line/frequency: Commence Year 2 (June 2017-2018). Annual (unless circumstances prevent it November 2018 or earlier (to be determined in consultation with reference group).

Success measure(s): Hatchlings/ juveniles are monitored. Mortality of animals documented for future improvement.

Related goal(s): 4 & 6

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6. Plan and prepare for future hazard mitigation.

Engage with NSW Fox Threat Abatement Plan (TAP) personnel to consider BRST as a priority species and the Bellinger River a priority site under the current NSW TAP (OEH, 2011)

Action 6.1. Have BRST assessed for consideration as a target species under the NSW Fox TAP 2010 (OEH, 2011)

Detail: Liaise with NSW Fox TAP personnel to have BRST assessed for consideration as a target species, and the Bellinger River a priority site, under the TAP.

Lead agency: OEH

Potential collaborators: LLS? NPWS?

Time-line/frequency: Commence Year 3 (June 2018-2019), June 2018

Success measure(s): BRST and the Bellinger River have been assessed against the criteria identified in the NSW Fox TAP 2010.

Related goal(s): 5 (Strategy 6)

Action 6.2. Engage the community on the issue of fox control

Detail: A community working group will be developed to engage the community on the issue of fox control.

Lead agency: OEH/ LLS

Potential collaborators: NPWS

Time-line/frequency: Commence Year 4 (June 2019-2020).

Success measure(s): The community supports and is actively engaged in fox control.

Related goal(s): 4, 5 (Strategies 5 & 6)

Action 6.3. Establish fox control

Detail: Approach may depend on the outcome of action above relating to NSW Fox TAP, although whatever the outcome, the priority will be the protection of nests and nesting females across tenure during nesting season. Trial/utilise a range of methods from the nest specific (e.g. fencing) to broad area control.

Lead agency: OEH/ LLS

Potential collaborators: NPWS

Time-line/frequency: Commence Year 5 (June 2020-2021). Ongoing.

Success measure(s): Monitoring shows that predation of nests by foxes is reduced

Related goal(s): 3, 4, 5, & 6 (Strategy 6)

Stage 2. 6-20 years

Optimised releases and informed hazard mitigation are ongoing.

- Control foxes incorporating improved strategies (> year 4)
- Build numbers
    - Continue with optimised releases, supported by ongoing hazard mitigation.
- Improve gene diversity
    - Initial years of breeding for release will prioritise increased abundance, to reduce demographic stochastic threats which are expected to be more potent while numbers are small. As numbers increase, greater emphasis will be placed on

increasing gene diversity in the wild population by preferentially breeding and releasing cohorts from genetically less well-represented founders.

- Riparian zone restoration continues
- *E. macquarii* control continues (method not yet agreed).

Other action will be taken as needed, to adapt to the situation on the ground and to the state of knowledge at each decision point.

Stage 3. Years 21+

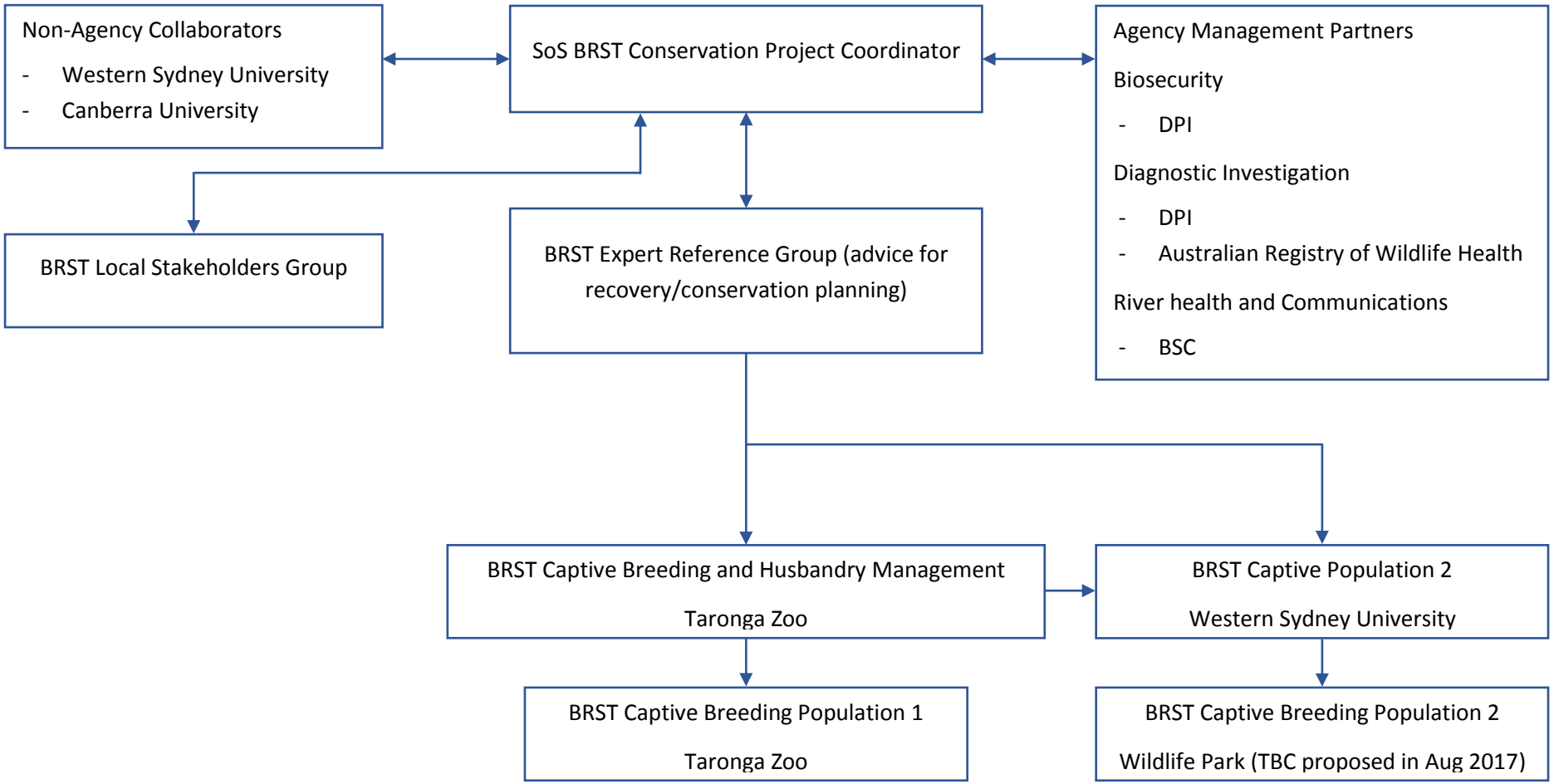
The program described above is complex and in several areas relies on information not yet available and which will take time to acquire. Much hinges on the success of the captive program which, previous experience shows, may take several years to show significant and consistent success. Further, the impact of hazard mitigation *in situ* may not be apparent for some years after it is set in motion. In absence of further catastrophes it is expected to be 15-20 years before program success or failure can be evaluated, at which point decisions will be taken either to wind-down the program, to change direction, or to continue for a specified period. Triggers for these determinations will be developed in advance, and protocols for winding down agreed, to ensure a smooth transition.

TABLE 3: SUMMARY OF STAGE 1 (YEARS 1-5) ACTIONS.

Actions	Related goals	Strategy	Year 1	Year 2	Year 3	Year 4	Year 5	Years 6-20
1. Continue to investigate non-disease hazards								
1.1. Identify options for *E. macquarii* control	2	1,5	→					
1.2. Evaluate ecological impacts of *E. macquarii* control	2,3	1,5	→					
1.3. PhD research	2,3,4,6	1,2,3,5, 6	→	→	→	→		
1.4. Begin *E. macquarii* control	2,4,5	1,5			→			
2. Continue to investigate disease hazard								
2.1. Investigate modes of virus transmission	1,3,4,6	3	→					
2.2. Establish serological test for virus	1,3,4,6	3	→					
2.3. Explore virus treatment options	1,3,4,6	3		→				
3. Establish and build captive program								
3.1. Assess viability of incorporating privately held animals into captive breeding	4,6	4	→					
3.2. Capture founders for second captive group	4,6	4	→					
3.3. Establish sex determination technique for juveniles	4,6	4	→					
3.4. Prepare studbook, captive management plan and husbandry manual	4,6	4	→	→				
4. Engage and mobilise community support effectively								
4.1. Develop and implement communications plan	5,6	5	→	→	→	→	→	→
4.2. Establish engagement program around hygiene and water contamination	1,5	3,5	→	→	→	→	→	
4.3. Education and engagement in ongoing vigilance and reporting of dead and sick animals	5,6	3,5	→	→	→	→	→	
4.4. Engage community support for riparian vegetation and promotion of in-stream habitat	4,5	2,5		→	→	→	→	→
4.5. Citizen Science water monitoring program	4,6	5		→	→	→	→	→
5. Begin experimental releases and answer key questions								
5.1. Population survey and monitoring	1,2,3,4, 5,6		→	→	→	→	→	→
5.2. Translocation proposal	4,6		→	→				
5.3. Identify agreed sites for release	4,6			→				
5.4. Monitored release of hatchlings	4,6			→	→	→	→	→
6. Plan and prepare for future hazard mitigation								
6.1. Have species assessed for consideration as Fox TAP species	5	6			→			
6.2. Engage community on issue of fox control	4,5	5,6				→		
6.3. Establish fox control	3,4,5,6	6					→	→

Implementation Framework

The proposed action plan for recovery of the Bellinger River Snapping Turtle will operate through the following organisational framework.



Appendix I: Conservation Planning Briefing Paper 1: Biology and Conservation

Information is current as of October 2016

Species summary

Taxonomy

Conventionally accepted as *Myuchelys georgesi* (Cann, 1997).

Common name is Bellinger River Snapping Turtle (BRST). This is being used to be consistent with previous planning, media and documentation associated with the mortality event and incident management of February-May 2015. Other published common names include Georges' Turtle, Georges' Snapping Turtle, Georges' Helmeted Turtle, Georges' Short-neck Turtle; Bellinger River Sawshelled Turtle.

Description

The BRST is a medium-sized freshwater turtle with a shell length up to 185 mm in males and 250 mm in females. Most easily distinguished from *E. macquarii* by blotchy plastron and darkened scute margins present (as opposed to *E. macquarii,* which has a clear plastron with no (or indistinct) darkened scute margins). Also, the iris of the Bellinger River snapping turtle is silver as opposed to variable in *E. macquarii* (including often yellow) but not silver.

Distribution

The BRST is known only from the Bellinger catchment on the north coast of NSW (Georges, et al., 2007). Within the catchment it is restricted to the Bellinger and, possibly, Kalang Rivers (Figure 4).

In the Bellinger River, the species occurs along a 60km stretch of the river from Bellingen township upstream to Brinerville (Spencer, et al., 2007). All BRST within the river should be considered as a single population. Waterholes in the river do not contain discrete populations and dispersal both up and down stream occurs during flooding (Spencer, 2006). Even during normal river conditions, there is no reason to suspect that the species has difficulty moving between waterholes (Blamires & Spencer, 2013).

The status of BRST in the Kalang River is uncertain. Cann (1993) states that the species was present at a few scattered locations in the Kalang, although several surveys since 2000 have failed to locate the species (these surveys have confirmed the presence of *E. macquarii* as well as hybrids between the two species).

Although the Bellinger and Kalang Rivers occur in the same catchment, they do not meet until both reach the sea at Urunga (Figure 4). Also, short-necked turtles such as BRST rarely migrate terrestrially (Cann 1998, in Spencer, 2006). Therefore, any naturally occurring migration between the two rivers would be virtually non-existent for the species.

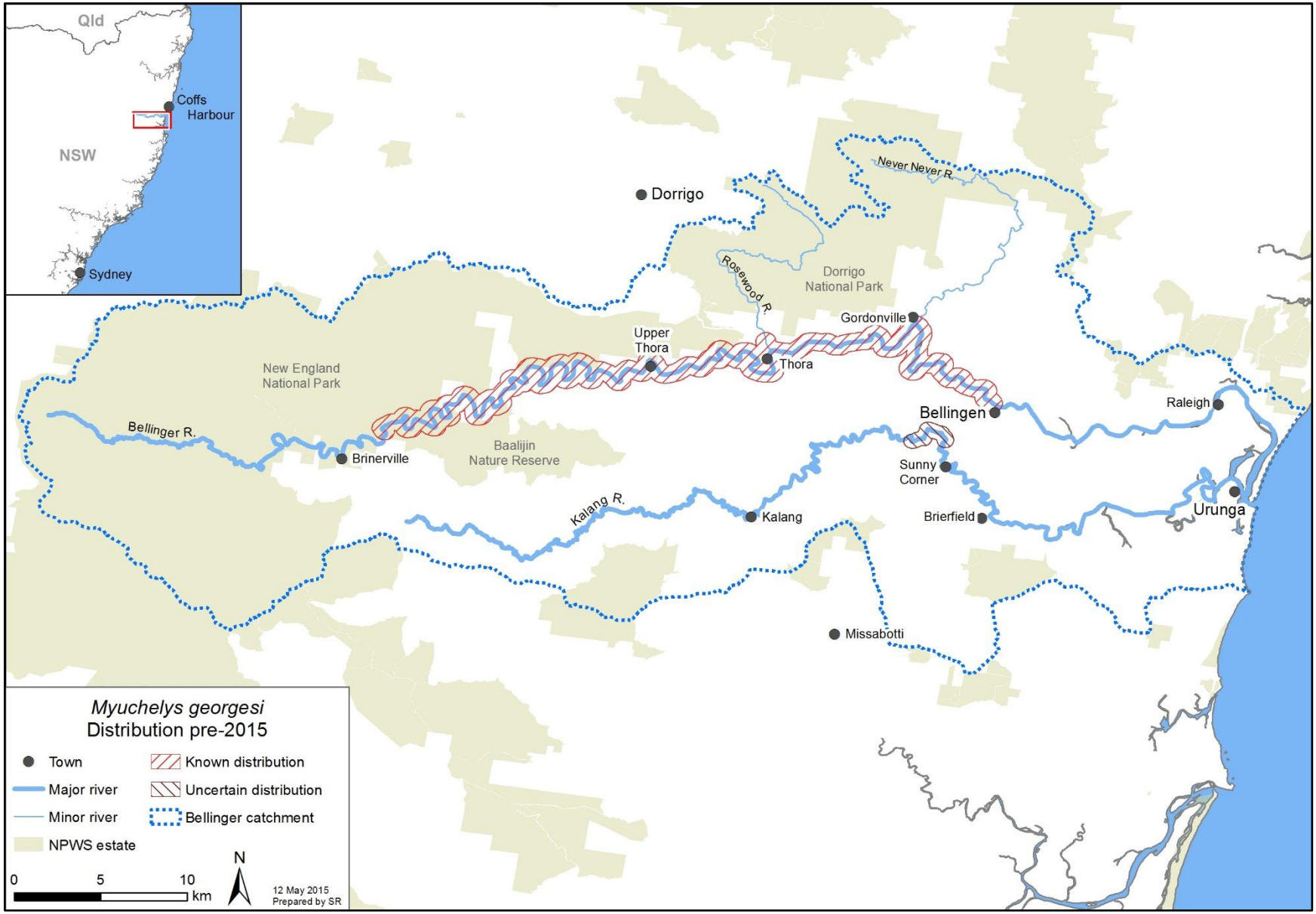


FIGURE 4: DISTRIBUTION OF BRST PRIOR TO MORTALITY EVENT OF 2015

Abundance

Prior to the mortality event of 2015, BRST was described as common (Spencer, et al., 2007) and locally abundant (Georges, et al., 2007) in the Bellinger River. In 2005 the total population was estimated to be approximately 4,500 ± 1,400 (arithmetic mean of sample population estimates ± standard error) (Blamires, et al., 2005), although this high standard error indicates that the value may be substantially more or less than this. In 2015, this figure was revised to a pre-mortality event estimate of between 1600 and 3200 individuals (R. Spencer, pers. comm., 11 March 2015).

Extant Population Size

Since the mortality event there have been three major surveys of the extant population in the Bellinger River (November 2015, March 2016 and November 2016). Although current population size is unknown, these surveys indicate that the number of animals remaining in the Bellinger is low, with an approximate estimate of 200-300.

Importantly, the vast majority of BRST remaining in the Bellinger River are juveniles. Very few adults remain in the River, indicating that adults appear to be the age class most affected by the mortality event.

Chessman (2015) reports that *E. macquarii* now appears to be the dominant turtle species in the Bellinger River, having increased from 2% of captures in 1988-2004 (Blamires, et al., 2005) to 17% in 2007 (Spencer, et al., 2007) and 63% in 2015. The moderate proportion of juveniles in the *E. macquarii* catch of November 2015 suggests that the increase in the percentage of *E. macquarii* since 2007 is due more to collapse of the BRST population than to mass recruitment of *E. macquarii*.

While a precise estimate of overall mortality is unavailable, an approximation can be made by comparing the relative proportions of juveniles in the BRST population (B. Chessman, pers. comm., in Threatened Species Scientific Committee, 2016). In 2007 the proportion of juveniles was approximately 5% while in 2016 it was approximately 84% percent. Assuming no substantial change in detectability of adults or juveniles across surveys, this corresponds to a mortality of adults of close to 99 percent. However, as there was some juvenile mortality during the disease outbreak, but much less than adults, 90% population decline is a better approximation (B. Chessman, pers. comm., in Threatened Species Scientific Committee, 2016).

Life cycle

Many Australian freshwater turtles exhibit type III survivorship where mortality rates decrease with age (Spencer & Thompson, 2000) and BRST follows this type of survivorship (Blamires, et al., 2005). Therefore, the stability of the BRST population is sensitive to changes in adult survivorship and the species' ability to recover from a catastrophic loss such as the mortality event is limited.

Female BRST are gravid between September and November and nest between October and December (Cann, 1997, Blamires, et al., 2005). Clutch size varies between 10 and 25 eggs, averaging between 15 and 20 (R. Spencer, pers. comm., 13 March 2015). Hatchlings appear after 72 days (Cann, 1997).

Modelling and life table analysis by Blamires et al. (2005) calculated that female BRST should have a maximum life expectancy of 28.9 (±4.5) years. In the same study, generation time was defined as the minimum female reproductive age, which was calculated to be 7.9 ±1.2 years (mean ± standard error) (Blamires, et al., 2005). The generation length (as defined by IUCN, 2014) is approximately 20 years.

Existing Conservation Actions

Captive breeding program and maintenance of insurance population

Sixteen (9 male and 7 female) BRST are maintained at Taronga Zoo in a captive breeding program.

Animals are housed outdoors in a netted area in 5,000 L tanks with areas to retreat, ramps for basking and access to sand boxes for nesting (Figure 5). Animals are maintained in the same configuration for mating as was determined at WSU.

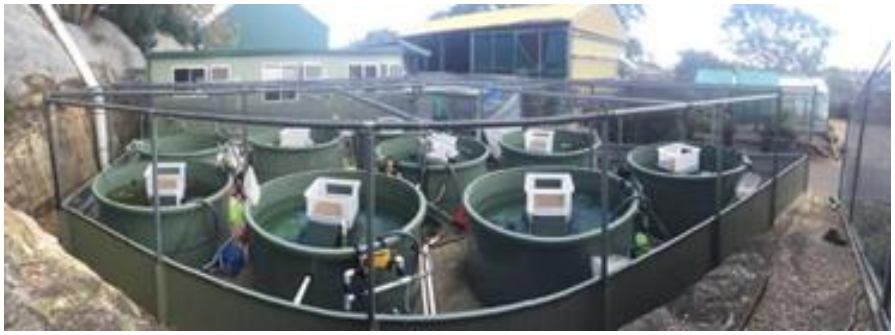


FIGURE 5: TARONGA CAPTIVE BREEDING FACILITY (PHOTO - MICHAEL MCFADDEN).

The captive individuals show genetic variability representative of that found in the native Bellinger population with what appears to be exceptionally low genetic variability in BRST (Georges & Spencer, 2015). The breeding of these animals is to be directed by a studbook program managed by Taronga Zoo.

Taronga has committed to providing Version 1.0 Husbandry and Captive Breeding Requirements by March 2018 with a brief husbandry report to be provided March 2017.

A second insurance population of extant juvenile animals is being pursued in the week beginning November 21, 2016. After a period of quarantine at WSU these animals are proposed to be transferred to new wildlife park facilities for captive breeding.

Surveys and monitoring

Biannual major surveys are proposed to continue in November and March each year. Capture-mark-recapture techniques are used for surveys with capture by snorkelling. Both BRST and *E. macquarii* are targeted in the surveys.

Further monitoring is proposed under research projects by project partners including use of telemetry. Questions relating to threats and the proposed mitigation measures may be answered by the survey and monitoring program.

Genetics studies

Arthur Georges has analysed genetic material collected from both BRST and *E. macquarii* to investigate:

- Hybridisation and introgression of the two species
- Relatedness of BRST individuals
- Identification of genetic loci that have undergone frequency shift following the mortality event
- Whether there is bias in directionality of the hybridisation (e.g. only male BRST mating with female *E. macquarii*).

Attempts to determine a genetic sex marker have been unsuccessful to date.

Application of population modelling

To examine strategies for captive breeding and reintroduction, Ricky Spencer has conducted population viability analyses.

Community engagement program

The Bellingen community is engaged with the plight of BRST. There has also been considerable media interest both locally and nationally.

A local stakeholders group has been established to provide a means of exchanging information between OEH and relevant stakeholders (see Appendix VII). OEH representatives have produced educational materials for schools and the general community and continue to deliver presentations to a variety of interested parties with program partners. Taronga Zoo is also undertaking presentations to local schools.

Threats (for discussion)

Prior to the conservation planning workshop at Taronga, preliminary threats were identified. Information on these threats was used as a starting point for discussions by experts at the workshop.

The greatest immediate threat to BRST is the disease outbreak associated with the Bellinger River Snapping Turtle Mortality Event that was first observed in February 2015. Prior to the disease outbreak and the associated mortality event, potential threats to BRST were limited distribution/specific habitat requirements, predation, alteration to water quality, possible hybridisation and possible competition (Spencer, et al., 2007; Blamires & Spencer, 2013; Spencer, et al., 2014). The effects of the mortality event on species abundance may potentially amplify the impacts of these other threats. Information on these and other threats is provided below.

1. Disease and the Bellinger River Snapping Turtle Mortality Event

On 18 February 2015, a number of BRST were found dead and dying in the Bellinger River east of Thora. The EPA inspected the Bellinger River at 4 locations when the situation was first reported and water quality sampling did not identify any contamination impacting the river. Due to this preliminary exclusion of pollutants by the EPA and the observation that only this species of turtle appeared to be affected, this mortality event was subsequently treated as an emergency animal

disease event and an incident management team was in operation until the end of March to work on the event.

433 individual BRST are confirmed to have died (426 of these in a 59 day period) (Threatened Species Scientific Committee 2016). This directly observed mortality is equivalent to at least a 14-27% reduction in the population, the vast majority of these being adults (See Figure 6). The actual number of dead is unknown and believed to be much higher as the majority of affected animals were found on shore close to the river. Additionally, a flood event occurred on 21 February 2015, only a few days after the outbreak was noticed, preventing many of the carcasses being found. Flood events also occurred in early April and May 2015 which were also suspected to have washed carcasses away. Observations of dead animals at the upper distributional limit of the species in June 2015 indicated that the disease was present across the entire known range of the species in the Bellinger River.

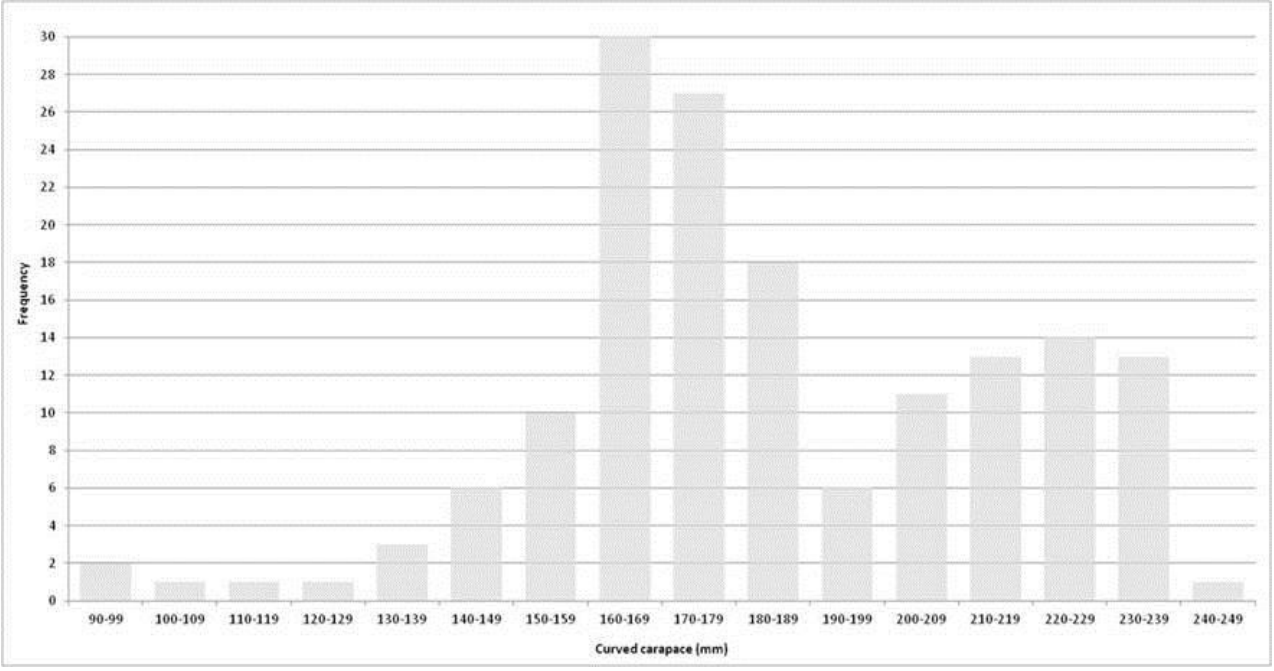


FIGURE 6: SIZE DISTRIBUTION OF SICK/DEAD BRST COLLECTED FEBRUARY TO MARCH 2015 (OEH DATA).

The event has been interpreted as a disease outbreak (Moloney, et al., 2015; New South Wales Scientific Committee, 2016). A novel virus, associated with the lesions in the turtles, has been identified (Figure 7) (B. Kay, in New South Wales Scientific Committee, 2016) although the extent of its role is yet to be clarified and the disease may be a multi-factorial syndrome (Moloney, et al., 2015). The BRST is the only species known to be affected. Sympatric *E. macquarii* have shown no ill effects despite some animals testing positive for the virus by PCR tests. No further confirmed reports of disease-affected turtles in the Bellinger River have been made since May 2015.

The BRST has a lifecycle where mortality rates decrease with age and population stability relies largely on very high adult survivorship (Spencer & Thompson, 2000; Blamires, et al., 2005). Therefore, the death of a large number of adults from disease will affect any potential recovery of the species. Current recovery efforts aimed at increasing numbers via captive breeding or increased

nesting success will have little effect on any population recovery in the immediate future. It will be many years before the species is able to recover, even with management to increase juvenile recruitment through nest protection and captive breeding. Therefore, not only does the mortality event of 2015 have an immediate effect on the abundance of the species, but by removing a large proportion of adults from the population, it will also influence the long term recovery of the species.

In epidemiological terms, it is not known how the aetiological agent is transferable/ transmissible/ infectious, until transmission trials are run.

FIGURE 7: EXTERNAL SYMPTOMS OF DISEASE ASSOCIATED WITH THE BRST MORTALITY EVENT 2015.

2. Stochastic impacts on population with limited distribution and specific habitat requirements

The restricted distribution and specific habitat requirements of BRST means the species is potentially at risk from human-induced or natural perturbations (Spencer, et al., 2007). Like many other species with a small population size, limited distribution and specific habitat requirements, the BRST is susceptible to any demographic or environmental stochastic event that has the potential to affect the entire population, as has been demonstrated by the disease associated with the mortality event of 2015.

All 17 animals taken from the Bellinger River into ex situ quarantine were sourced from a single waterhole representing the upstream limit of the species. Despite this, the captive colony appears to capture what is exceptionally low genetic variability in BRST (Georges & Spencer, 2015).

3. Predation

Predators are a major potential source of mortality in freshwater turtle populations (Georges, et al., 1993; Spencer & Thompson, 2005). Goannas (*Varanus varius*) and European red foxes (*Vulpes vulpes*) have been identified as the major predator of turtles (both BRST and *E. macquarii*) along the Bellinger River (Spencer & Thompson, 2000; Blamires, et al., 2005; Spencer, et al., 2007). Blamires et al. (2005) reports a turtle nest predation rate of 72% (for a mix of natural and artificial nests) in the Bellinger River, although elsewhere in Australia it is known to exceed 90% from foxes alone (Thompson, 1983).

While goannas specifically target nests, foxes prey on both nests and nesting females (Spencer & Thompson, 2000; Blamires, et al., 2005; Spencer, et al., 2007). It is thought that short-necked turtle species are particularly susceptible to predation by foxes because of their inability to fully retract

head and limbs (Spencer & Thompson, 2005). Predation pressure from foxes may limit many populations of freshwater turtles, but a decline in numbers will be slow to become evident because of their longevity (Thompson, 1993). Predation by the European red fox is a Key Threatening Process in NSW.

It has also been suggested that large catfish are the most probable water-based predators of hatchling and juvenile BRST (Blamires & Spencer, 2013). Analysis of stomach contents of catfish (*Arius graeffei* and *Tandanus tandanus*) museum specimens from the Clarence River found that turtle species were the most abundant item in the stomachs of individuals greater than 400 mm in length. Adult and juvenile BRST seem to be able to use waterholes where catfish are absent, although catfish predation is potentially detrimental to the species (Blamires & Spencer, 2013).

4. Hybridisation and introgression

Strong evidence of hybridisation between the endemic BRST and the introduced (i.e. Australian native but not native to the Bellinger) *E. macquarii* necessitates reconsideration of the management of the turtle populations in the Bellinger River, with particular consideration given to options for managing the impact of hybridisation and introgression on the integrity of BRST as a species (Georges & Spencer, 2015).

Hybrid animals may feature morphological features of both BRST and *E. macquarii.* There are however cases of hybrid and backcross animals which are cryptic (Georges & Spencer, 2015).

Similar habitat and dietary preferences between the two species is conducive for further potential hybridisation and introgression (Spencer, et al., 2014). The presence of introgression, and the contamination of the genotype of BRST by horizontal transfer of genes from *E. macquarii* is of management concern (Georges & Spencer, 2015).

The hybridisation and introgression detected in the Bellinger River may occur only rarely, or with low survivorship of the F1 hybrids. When fertile F1 hybrids do arise, they are able to breed with each other and back to at least one of the parental species (Georges & Spencer, 2015).

The removal of a large proportion of BRST adults from the Bellinger River following the mortality event in 2015 may have implications for a further increase in the number of *E. macquarii* which, in turn, may have flow-on effects to the possible incidence of hybridisation.

5. Possible interspecific competition

Competition between *Emydura* species and *Myuchelys* species is likely to occur when in sympatry (Spencer, et al., 2014). The two genera commonly coexist in many catchments, although one genus is usually locally abundant, with competition potentially limiting population numbers of the other (Cann, 1998). In the Bellinger River, interspecific competition may occur between BRST and *E. macquarii* due to similar habitat preferences, diets and life histories (Spencer, et al., 2014).

The likelihood that *E. macquarii* is a recent introduction to the Bellinger River identifies it as a potential invasive species (Georges, et al., 2007; Spencer, et al., 2014) and there is a strong possibility that it is increasing in abundance (Spencer, et al., 2014). The removal of a large proportion of BRST adults from the river following the mortality event in 2015 may have implications for a

further increase in the number of *E. macquarii* which, in turn, may influence the degree of competition between the species (Threatened Species Scientific Committee, 2016).

6. Alteration to habitat and water quality

The Bellinger River is an unregulated river with continuous-flowing clear water in the middle and upper reaches (Allanson & Georges, 1999). The in-stream macroinvertebrate fauna is diverse and appears to have been little impacted upon by human activity (Allanson & Georges, 1999). A large amount of the food of BRST is from the macroinvertebrate fauna closely associated with the river bed and any increase in sedimentation could potentially alter the sedentary benthic macroinvertebrate fauna, impacting upon the species (Allanson & Georges, 1999). Any water quality based changes such as this may exacerbate the impact on BRST even further because of the species' restriction to a single small drainage system (Allanson & Georges, 1999).

Changes in water quality can further impact BRST because habitat preferences for the species are linked to water quality (Spencer, et al., 2007). As an example, sand and silt run-off from unsealed roads upstream of Thora can affect turbidity and as silt enters the river it is deposited on patches of aquatic vegetation and rock substrates. Both of these factors are key habitat features that limit the distribution of the species in the river (Spencer, et al., 2007).

Downstream of Thora, the lower Bellinger River is particularly degraded, with the channel width greatly enlarged and indistinct pools (Cohen, et al., 1998). In 2007 densities of BRST in the upper reaches of the Bellinger River were three times higher than populations in the lower reaches of the river (Spencer, et al., 2007, Fig. 6). Unpublished OEH survey data from 2015 and 2016 indicates that this may no longer be the case.

Loss of riparian canopy and increased accumulation of fine sediments has occurred as a result of agriculture in the Bellinger catchment. These changes to the habitat are considered conducive to the establishment of populations of *E. macquarii* which may compete with BRST for resources (Spencer, et al., 2014).

While BRST probably has a high degree of resilience to flood events in the Bellinger River, major flood events have been known to severely affect the species. In 2001 flooding destroyed much of the upper river aquatic vegetation. In some waterholes, 100% of ribbonweed beds were removed and had not returned after one year. During this time five BRST were found to have no stomach contents and there were signs that reproduction had not occurred. (Spencer, 2006; Spencer, et al., 2007). Floods are also known to destroy turtle nests (Blamires, et al., 2005).

7. Climate Change (background included for discussion)

Climate change may cause environmental stress at multiple scales ranging from direct effects on a species' physiology to complex effects caused by changes in biotic interactions. By affecting species-specific environmental tolerances, it may result in changes in local species abundances and ultimately changes in distribution patterns (Geyer, et al., 2011).

The impact of climate is expected to be especially important for terrestrial and freshwater ectotherms, such as amphibians and reptiles, whose body temperatures are tightly linked to their

external environment. The ability to cope with local shifts in temperature and precipitation is expected to vary between taxa (Waterson, et al., 2016).

The impacts of dry conditions differ among species of freshwater turtles according to their use of various aquatic and terrestrial habitats for feeding, nesting and dormancy (Gibbons, et al., 1983). Therefore the ecology of each species needs to be understood in order to identify those that are most vulnerable (Chessman, 2011).

The NSW Climate Impact Profile (DECCW, 2010) assesses the biophysical risks of climate change to NSW at the regional scale. Expected climatic changes for the North Coast region that may affect BRST include:

- Average daily maximum temperatures are virtually certain to increase in all seasons
- Average daily minimum temperatures are projected to increase in all seasons
- Rainfall is likely to increase slightly in summer and autumn. Spring rainfall is not expected to change, while winter rainfall is expected to decrease slightly. (Note - changes in weather patterns that cannot be resolved by the climate models mean that rainfall in coastal regions is difficult to simulate)
- Evaporation is likely to increase moderately during spring, summer and autumn. A slight to moderate increase in evaporation is likely in winter
- The impact of the El Niño–Southern Oscillation is likely to become more extreme. Current literature indicates that the pattern of climate variability associated with ENSO will continue. This assumes that the ENSO phenomenon will continue to drive climatic variability across NSW. It is noted, however, that ENSO is a weaker influence on annual average rainfall in coastal areas than in inland areas. It is assumed that ENSO years will continue to be drier than average but also become hotter, leading to more extreme impacts. La Niña years are likely to continue to be wetter than average but will also become warmer. In El Niño events, water stress is likely to be more intense because of higher temperatures.

Physical responses of the environment to climate change in the North Coast Region relevant to BRST are expected to include (DECCW, 2010):

- Increased evaporation is likely to lead to drier conditions for most of the year. Despite projected increases in rainfall in summer and autumn, soil conditions are likely to be drier for most of the year, particularly in spring and winter, as a result of increased temperatures and evaporation
- Average annual run-off will likely increase slightly as a result of substantial increases in summer run-off. Substantial increases in run-off depths and the magnitude of high flows are very likely in summer. A moderate decrease in run-off depths is likely in spring
- Short-term hydrological droughts are likely to become more severe
- Flooding behaviour is likely to change. The combination of rising sea levels and catchment-driven flooding is likely to increase flood frequency, height and extent in the lower portions of coastal floodplains. Increases in the intensity of flood-producing rainfall events are likely to change flood behaviour everywhere, but catchment conditions at the time of each rainfall

event (soil moisture conditions and levels in major water storages) will affect the degree of change.

Higher summer rainfall and rainfall intensity in the region are also likely to increase sheet and rill erosion on the steeper slopes of the hinterland. Sediment inundation of coastal and hinterland floodplains is likely where major erosion occurs. Significant channel alteration on coastal rivers is more likely than not (DECCW, 2010).

Higher temperatures, altered fire regimes and altered hydrology (with wetter summers and drier winters) are likely to bring about changes to many ecosystems including changes to structure, species composition and species abundances. Ecosystems most at risk include high-altitude and fire-sensitive species, wetlands and those ecosystems which have a reduced resilience to disturbance due to fragmentation or isolation.

Appendix II: Review of Disease Hazards Recorded in Australian Freshwater Turtles (Chelidae)

Prior to the Sydney workshop, published literature describing diseases affecting Australian chelids was reviewed and used to create a preliminary summary of disease hazards that may be significant for captive, translocated or wild BRST (Table 4).

Native chelid species that are referred to in this disease risk analysis include (OEH, 2015):

- Bell's Turtle, Western Saw-shelled Turtle, *Myuchelys bellii*
- Bellinger River Snapping Turtle *Myuchelys georgesi*
- Broad-shelled Turtle *Chelodina expansa*
- Eastern Long-necked Turtle *Chelodina longicollis*
- Manning River Turtle *Myuchelys purvisi*
- Murray River Turtle *Emydura macquarii*
- Saw-shelled Turtle *Myuchelys latisternum*

Non-native species of turtle that are referred to are:

- Red-eared Slider turtle *Trachemys scripta elegans* (Family Emydidae)

In-contact species

One aim of this disease risk analysis is to assess the likelihood of contact between the identified hazards and the species of concern, and the consequences to them if contact occurs. In-contact species are identified below ([TS] indicates a species identified as threatened on the NSW *Threatened Species Conservation Act 1995*).

Reptiles

- Black-bellied Swamp Snake *Hemiaspis signata*
- Carpet Python *Morelia spilota*
- Eastern long-necked Turtle *Chelodina longicollis*
- Eastern Water Dragon *Intellagama lesueurii*
- Golden-crowned Snake *Cacophis squamulosus*
- Lace Monitor *Varanus varius*
- Murray River Turtle *Emydura macquarii*
- Red bellied Black Snake *Pseudechis porphyriacus*
- Southern Dwarf Crowned Snake *Cacophis krefftii*
- Stephens' Banded Snake *Hoplocephalus stephensii*[TS]

Amphibians (Common names as per NSW Bionet)

- Bibron's Toadlet *Pseudophryne bibronii*
- Broad-palmed Frog *Litoria latopalmata*
- Brown-striped Frog *Limnodynastes peronii*
- Common Eastern Froglet *Crinia signifera*
- Dainty Green Tree Frog *Litoria gracilenta*

- Dusky Toadlet *Uperoleia fusca*
- Fletcher's Frog *Lechriodus fletcheri*
- Giant Barred Frog *Mixophyes iteratus*[TS]
- Great Barred Frog *Mixophyes fasciolatus*
- Leaf-green Tree Frog *Litoria phyllochroa*
- Ornate Burrowing Frog *Platyplectrum ornatus*
- Peron's Tree Frog *Litoria peronii*
- Red-backed Toadlet *Pseudophryne coriacea*
- Revealed Frog *Litoria revelata*
- Rocket Frog *Litoria nasuta*
- Smooth Toadlet *Uperoleia laevigata*
- Spotted Grass Frog *Limnodynastes tasmaniensis*
- Stoney Creek Frog *Litoria wilcoxii*
- Stuttering Frog *Mixophyes balbus*[TS]
- Tusked Frog *Adelotus brevis*
- Verreaux's Tree Frog *Litoria verreauxii*

Fish (Gilligan, 2010)

- Australian Bass *Macquaria novemaculeata*
- Australian Smelt *Retropinna semoni*
- Bellinger Climbing Galaxias *Galaxias* sp. B
- Blue Catfish *Neoarius graeffei*
- Bullrout *Notesthes robusta*
- Common Jollytail *Galaxias maculatus*
- Cox's Gudgeon *Gobiomorphus coxii*
- Duboulay's Rainbowfish *Melanotaenia duboulayi*
- Dwarf Flat-headed Gudgeon *Philypnodon macrostomus*
- Empire Gudgeon *Hypseleotris compressa*
- Firetailed Gudgeon *Hypseleotris galii*
- Flat-headed Gudgeon *Philypnodon grandiceps*
- Freshwater Herring *Potamalosa richmondia*
- Freshwater Mullet *Trachystoma petardi*
- Long-finned Eel *Anguilla reinhardtii*
- Mountain Galaxias *Galaxias olidus*
- Sea Mullet *Mugil cephalus*
- Short-finned Eel *Anguilla australis*
- Snub-nosed Garfish *Arrhamphus sclerolepis*
- Softspined Rainbowfish *Rhadinocentrus ornatus*
- Southern Blue-eye *Pseudomugil signifier*
- Striped Gudgeon *Gobiomorphus australis*
- Western Carp-Gudgeon *Hypseleotris klunzingeri*
- Willung (Bellinger Freshwater Catfish) *Tandanus* sp. 2

Other

Macroinvertebrates of the following Orders:

- Coleoptera
- Decapoda
- Diptera
- Ephemeroptera
- Lepidoptera
- Odonata
- Trichoptera

TABLE 4: SUMMARY OF POTENTIAL INFECTIOUS DISEASE HAZARDS IDENTIFIED FOR BELLINGER RIVER SNAPPING TURTLES

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
VIRAL						
Novel virus Bellinger River Virus	A novel virus has been isolated following the mortality event in all affected *M. georgesi* animals	Host range unknown	Unknown	Severe	Severe	
Ranavirus and other iridoviruses	DNA-based viruses of the genus Ranavirus, in the family Iridoviridae. (U.S. Geological Survey, 2016)	Fish, amphibians, reptiles. Ranavirus infection was an important differential diagnosis for BRV and it is considered an emerging infectious disease of chelonians. Clinical signs and pathological findings in chelonians infected with these viruses were nearly indistinguishable from BRV. Iridovirus in chelonians can be a highly fatal disease with upper respiratory, oral, and skin lesions. There is some evidence of a cross-taxon infection of ranavirus from amphibians to reptiles	Globally, ranavirus diseases in amphibians have been diagnosed in North and South America, Europe, Asia, and Australia (U.S. Geological Survey, 2016). Iridoviruses are reported in European tortoises, American turtles and tortoises, and Chinese soft-shelled turtles	Severe	Potentially severe	See summary later in document (Gibbons & Steffes, 2013)
Hepatic necrosis, Lung-eye-trachea disease, Gray patch disease, Herpesvirus, Fibropapillomatosis	Herpesviruses: Gray patch disease in marine turtles (chelonian herpesvirus 1) (The Merck Vet Manual, 2015) Fibropapillomas in marine turtles Viraemia and death in a range or turtles and tortoises	Herpesviruses have been isolated from freshwater turtles, tortoises, and marine turtles. Infection ranges from asymptomatic to lethal. Herpesvirus infection may be accompanied by lethargy, anorexia, subcutaneous oedema of the neck, nasal discharge, necrotising to diphtheritic stomatitis and neurological dysfunction (The Merck Vet Manual, 2015; Marschang, 2011a; Divers, S.J. and Mader, D.R. eds., 2005).	Global. Identified in Australian marine turtles by Anita Gordon	Potentially severe	Potentially severe	See summary later in document
Adenovirus	Adenovirus	Snakes (gaboon vipers, ball pythons, boa constrictors, rosy boas, and rat snakes),	Globally, adenoviruses have been diagnosed in a	Potentially severe	Potentially severe	(Australian Wildlife Health

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		lizards (Jackson chameleons, savannah monitors, and bearded dragons), crocodilians, tortoises, box turtles. Increasingly reported in terrestrial chelonians as a cause of individual and mass mortality, with signs of dermatitis, diphtheritic stomatitis and enteritis, myocarditis, fibrinous splenitis, renal tubular necrosis, and multifocal bone marrow necrosis. There may or may not be evidence of intranuclear basophilic inclusion bodies in epithelial and endothelial cells	variety of reptiles. In Australia adenovirus detection in reptiles is increasing in step with increased diagnostic capacity. Adenovirus infection is reported in captive bearded dragons in Australia			Network, 2009) See summary later in document
Paramyxovirus (including ferlavirus)	Ferlavirus is an enveloped RNA virus 146 to 321 nm in diameter	Paramyxovirus infections are most common in snakes, but reports are emerging of infection in chelonians associated with dermatitis and pneumonia	The disease has been described from snakes in Europe, the Americas and the Canary Islands, but is likely distributed worldwide	Severe	Potentially severe	(Wildlife Health Australia, 2009) See summary later in document
Papillomas	Papillomavirus	Broad range of reptiles. Fewer reports in chelonians, but documented to cause raised oral lesions in marine and terrestrial turtles		Mild	Mild	See summary later in document
Asymptomatic infection	Togaviruses, Flavi-viruses, Bunyaviruses	Broad range of reptiles including many chelonians. Reptiles likely form an important role in viral persistence. No clinical illness detected in nature and experimental infection, but uncertain impacts for unusual viral incursions	Global	Likely none	Likely none	See summary later in document
BACTERIAL						
Septicemic Cutaneous Ulcerative Disease (SCUD)	*Citrobacter freundi, Beneckea chitinovora* and sometimes other bacteria	Aquatic turtles may suffer anorexia, lethargy, pitted shells or skin lesions associated with	Global	Mild to severe	Usually individual animal in captivity	(The Merck Vet Manual, 2015)

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		bacterial infections that can progress to spread systemically in the blood stream to affect various internal organs. Often associated with poor husbandry and/or nutrition				
Aeromonas septicaemia	*Aeromonas hydrophila* *A. shigelloides*	Can cause individual and mass mortality events. Several mass mortality events reported in terrestrial and freshwater turtles emerging from estivation	Global	Mild to severe	Mild to moderate	(Jacobson, E.R. ed., 2007)
Upper respiratory tract disease, rhinitis	*Mycoplasma*	A broad range of Mycoplasmosis is a disease of high concern for terrestrial chelonian conservation, particularly wild and free-ranging gopher and desert tortoises. Infection may be inapparent or associated with upper respiratory tract infection, particularly in co-infection with other reptile pathogens	Global	Mild/ Moderate	Mild to severe	(The Merck Vet Manual, 2015)
Enteric bacterial disease	*Salmonella spp., E. coli, Clostridium spp.*	Broad host range, including all reptiles and mammals. Can be normal flora. Cause localised disease or septicaemia. "*Salmonella* lives anywhere and can do anything" (Prof. John Iverson – epidemiologist - speaking on the diverse pathogenicity of *Salmonella*)	Worldwide, common	Potentially Severe	Mild to severe	(The Merck Vet Manual, 2015)
Chlamydiosis	*Chlamydophila pneumonia* and newly described "Chlamydia-like" microorganisms *Parachlamydia acanthamoebae* and *Simkania negevensis*	These organisms are emerging as potential pathogens in reptiles, predominantly snakes, but a smaller number of others, including chelonians. The organisms have been identified in granulomatous inflammation, necrotising to	Worldwide	Mild/ Moderate	Mild to severe	(Bodetti, et al., 2002; Hotzel, et al., 2005; Soldati, et al., 2004; Jacobson, E.R. ed., 2007; Cope, et al., 2014;

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		proliferative enteritis, myocarditis, and proliferative pneumonia. Mass mortalities have occurred in farmed green turtles				The Merck Vet Manual, 2015)
Mycobacteriosis	*Mycobacterium ulcerans*, *M. chelonae*, *M. haemophilum*, and *M. marinum*	All reptiles are susceptible to focal to multisystemic granulomatous inflammation associated with a broad range of *Mycobacterium* species. Many of these organisms are common in the environment.	Worldwide	Severe – No reports of successful treatment	Usually individual animal	(The Merck Vet Manual, 2015)
Necrotic stomatitis	*Pseudomonas*, *Aeromonas* and a range other bacterial species	Necrotic stomatitis has been documented in a wide range of reptiles, including terrapins. Husbandry and hygiene deficiencies can contribute to the development of disease	Worldwide, common	Moderate - Severe	Usually individual animal	(The Merck Vet Manual, 2015)
Pneumonia	*Aeromonas, Pseudomonas, Mycobacterium, Chlamydophila* and a wide range of other bacteria	All reptiles are susceptible to pneumonia. Vitamin A deficiency, hygiene and husbandry can play in important role in the onset of disease	Worldwide, common	Moderate - severe	Usually individual animal	(The Merck Vet Manual, 2015)
Otitis	*Proteus spp, Pseudomonas spp, Citrobacter spp, Morganella morganii, Enterobacter spp,* and other bacteria have been isolated	Otitis has been documented in a range of reptiles. Disease is commonly associated with a broad range of different bacteria, and vitamin A deficiency associated hyperkeratosis can be a contributing factor	Worldwide, common	Moderate - severe	Usually individual animal	(The Merck Vet Manual, 2015)
Cloacitis	Can occur secondary to trauma, renal calculi, urate retention	All reptile species are susceptible. Can result in cloacal prolapsed or ascending infections into the urinary tract, GIT, or reproductive tract	Worldwide, common	Moderate - severe	Usually individual animal	
Abscesses/ granulomas	*Mycobacterium, Chlamydophila, Peptostreptococcus, Pseudomonas,*	Granulomas are not uncommon in a range of reptile hosts and can be caused by	Worldwide, common	Moderate - severe	Usually individual animal	(Divers, S.J. and Mader,

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
	Aeromonas, Serratia, Salmonella, Micrococcus, Erysipelothrix, Citrobacter freundii, Morganella morganii, Proteus, Staphylococcus, Streptococcus, Escherichia coli, Klebsiella, Arizona, and *Dermatophilus,* have been recovered from reptilian abscesses	a wide variety of bacteria, particularly intracellular organisms. Migrating helminth parasites, skin infections, enteritis, and poor hygiene may contribute to the pathogenesis.				D.R. eds., 2005)
FUNGAL						
Epidermal, pulmonary or systemic mycosis	Paecilomyces lilacinus	Broad range of reptiles including aquatic and terrestrial chelonians. Common in lesions in skin and internal organs. Most often considered an opportunistic pathogen	Worldwide, common	Mild to severe	Mild, usually individual animal disease	(Divers, S.J. and Mader, D.R. eds., 2005)
Dermatophilosis	*Dermatophilus congolensis*	Broad range of reptiles. Predominantly lizards. Raised skin lesions with severe hyperkeratosis. Organisms may be difficult to see histologically and difficult to grow in culture. Potential zoonosis.	Worldwide, common	Mild	Mild, usually individual animal disease	(Divers, S.J. and Mader, D.R. eds., 2005)
Scute lesions. Internal mycosis	*Fusarium incarnatum, Trichosporon* sp.	Causing necrotising scute disease in various chelonians in Europe. *Fusarium* sp are common isolates in fungal wounds in chelonians in Australia.	Worldwide, common	Mild	Mild, usually individual animal disease	(Divers, S.J. and Mader, D.R. eds., 2005; Jacobson, E.R. ed., 2007)
Mucormycosis	*Mucor spp.*	Identified in very young, captive Florida soft-shelled turtles and wood turtles. Common isoalate in fungal wounds in chelonians in Australia. Multiple, sometimes coalescing, raised, grey or yellow/tan papules and plaques on the carapace,	Worldwide, common	Mild	Mild, usually individual animal disease	(Divers, S.J. and Mader, D.R. eds., 2005)

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		plastron, head and limbs				
PARASITIC						
Nematodiasis	Spirurida, Serpinema (freshwater turtles) Spirurida, Spiroxys (freshwater turtles) Strongyloida, Chapiniella (tortoise) Oxyurids, various (cheloniae)	Intestinal nematodiasis is common and generally inconsequential to the host. Preserving parasite-host relationships is an important part of conservation medicine	Worldwide, common	Mild	Mild	(Divers, S.J. and Mader, D.R. eds., 2005)
Ectoparasites	Cloacaridae - *mites* Hirudinea - *leeches* Neopolystoma spp - *mongenean trematodes* Ticks – variety	Mites described from the cloaca of turtles. Inconsequential to the host Leeches are reported to cause ulcers in aquatic turtles. May transmit blood parasites and theoretically, may introduce *Aeromonas* or other secondary bacterial infections Six species are reported to cause parasitic conjunctivitis from the conjunctival sac of terrestrial and freshwater turtles Freshwater and terrestrial turtles can be affected by a range of ticks	Widespread, including Australia	Mild	None	(Divers, S.J. and Mader, D.R. eds., 2005; Jacobson, E.R. ed., 2007)
Amoebiasis	*Entamoeba* species, *Entamoeba invadens* is the most pathogenic	Have been indicated as the cause of individual and mass mortalities in captive terrestrial and freshwater turtles. Infection largely involves the liver and intestinal tract. One report of mortality of 200/500 red-footed tortoises over a 2 month period	Global. Predominantly captive reptile populations	Mild to severe	Mild to severe	(Divers, S.J. and Mader, D.R. eds., 2005; Jacobson, E.R. ed., 2007)
Hexamitiasis	*Hexamita parva*	Reported in a range of freshwater and terrestrial chelonians causing general ill health and weight loss, renal pallor, chronic nephritis and	North America and Europe. Predominantly captive reptiles	Mild to severe	Usually individual and captive animals	(Zwart & Truyens, 1975; Jacobson, E.R. ed., 2007)

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		proliferative glomerular lesions				
Intranuclear coccidiosis	*Coccidia*	Rare but important cause of proliferative pneumonia in tortoises. Infection is systemic and involved alimentary, urogenital, respiratory, lymphoid, endocrine, and integumentary systems. Reported as a cause of chronic rhinitis in 5 Sulawesi tortoises Rare but considered an emergent disease in chelonians, and is capable of causing multisystemic signs. As such this was a differential diagnosis in the BRV outbreak	Primarily individual animals in North American collections, but many were confiscated and of Asian origins	Mild to severe	Mild. Rare and predominantly an individual animal disease	(Garner, et al., 2006; Innis, et al., 2007; Gibbons & Steffes, 2013)
Cryptosporidiosis	*Cryptosprodium sp*	Emerging in numerous species of chelonians as an important cause of chronic diarrhoea, anorexia, weight loss and lethargy. Ranges from an incidental finding, to a cause of gastric hyperplasia and enteritis	Global. Increasing detections	Mild	Mild	(Jacobson, E.R. ed., 2007; Gibbons & Steffes, 2013)
Haemogragarine	Haemogregarine parasites	Common incidental finding in freshwater and terrestrial turtles	Global. Common	None to mild	None to mild	(Jacobson, E.R. ed., 2007)
Pentastomiasis	*Diesingia spp*	Rare reports of pentastome (thorny headed worms) infection in lung and liver	South America, southeast Asia	Mild	None to mild	(Jacobson, E.R. ed., 2007)
NON-INFECTIOUS						
Lead toxicity	Lead	Anorexia and CNS signs reported in common snapping turtles and Greek tortoise Treated with chelation therapy with sodium calcium edentate		Mild to severe	Mild to severe	(Divers, S.J. and Mader, D.R. eds., 2005)
Ivermectin toxicity	Ivermectin	Should not be used with chelonians				(Divers, S.J. and Mader, D.R. eds., 2005)
Chlorhexadine toxicity	Chlorhexidine	Chelonians are extremely sensitive to the toxic effects of				(Divers, S.J. and Mader,

DISEASE	CAUSATIVE AGENT	HOST RANGE	GEOGRAPHIC DISTRIBUTION	CONSEQUENCE OF INFECTION FOR *M. georgesi*		REFERENCE
				Individual	Population	
		chlorhexadine and may cause acute CNS disease and death				D.R. eds., 2005)
Hypovitaminosis A	Low dietary vitamin A concentrations	May cause oedema affecting eyelids, heart and kidneys. May affect upper alimentary and respiratory tract. May cause excessive sloughing. May cause hyperkeratosis and parakeratosis Primarily a disease of chelonians				(Divers, S.J. and Mader, D.R. eds., 2005)
Hypothiaminosis (leukoencephalopathy)	Low dietary concentrations of thiamine, predominantly due to prolonged frozen storage	Found in reptiles fed thawed frozen fish, clams and some vegetation Non-specific clinical symptoms including muscle twitching, incoordination, blindness, seizure, torticollis, abnormal posture, spiral locomotion, jaw gaping, dysphagia and death. In chelonians commonly causes sunken eyes				(Divers, S.J. and Mader, D.R. eds., 2005)

Appendix III: Disease Synopses

Freshwater turtle morbidity and mortality investigations - QLD

Several investigations have been undertaken in freshwater Krefft's turtles (*Emydura macquarii krefftii*) from central Queensland where ulcerative skin disease was documented at a 39% (n=869) prevalence in 2002, and where two localised mass mortality events occurred along the Burnett river catchment in January and November 2009 (Flint, 2009; Flint, et al., 2011; Tucker, et al., 2002). Unfortunately animals from each of the two mortality events were too decomposed for pathological investigation. A primary pathogen or disease process was not clearly identified in these events.

Two follow-up health surveillance investigations were conducted in Krefft's turtles along the Burnett catchment near the sites of the two mortality events:

- In May 2009, near Ned Churchward Weir, where there was the perception that a high proportion of the population was in poor body condition. Of the animals examined (n=56), 38% had shell ulcers, 13% had unilateral or bilateral opacity of the lens or anterior chamber of the eye(s), and 70% had erythema of the ventral soft tissues (Flint, et al., 2011)
- In January 2010, in two pools in the Burnett river catchment near Goomeri, 45 turtles were examined and 31.1% were found to be in poor condition. A high proportion of these animals had haematology and serum biochemical results outside normal ranges and these were interpreted to represent suppressed immune function (Flint, et al., 2010).

Infectious Diseases

Viral Diseases

Bellinger River Virus

Clinical Signs and Pathology

The most consistent findings of clinical disease associated with BRV have been generalised weakness, reluctance to move, swelling to ulceration of the eyelids and corneal oedema. Many affected turtles had a slight clear nasal discharge. A small proportion of animals exhibited hind limb paresis. Some animals had tan patches of skin along the ventral hind-limbs, but it is uncertain whether these were associated with the infection. At necropsy, animals were thin, had bilateral eyelid oedema, anterior uveitis, and multifocal to diffuse pallor of splenic and renal tissues (Moloney, et al., 2015).

Microscopic lesions in affected turtles included inflammation extensively throughout the eyelids, peri-orbital tissues, and sinuses, sometimes extending along the olfactory/optic nerve into the meninges. There was also histological evidence of splenitis, nephritis and multisystemic fibrinoid vasculopathy. All turtles had acute lesions, which seemed insufficient in duration to account for the animals' thin body condition. It was suggested that the nutritional plane of the animals may have been poor in advance of the outbreak, perhaps predisposing the animals to the severe consequences of infection by a novel pathogen (Moloney, et al., 2015).

Transmission

Unknown.

A multi-agency, collaborative research grant application has been lodged with the NSW Environmental Trust to conduct experimental trails to elucidate transmission pathways of BRV. An initial Expression of Interest was assessed favourably in early 2016, leading to an invitation to submit a Full Application in June. The outcome of this application will be known sometime in November 2016.

The phylogeny of the organisms offers no hints as to the likely means of transmission.

Diagnosis

As we are still working to understand the impacts of BRV it is important to collect a detailed clinical history, establish the exact location where animals were located, and undertake either a thorough clinical exam or a thorough gross and microscopic post mortem examination. This data will help define the syndrome description and spectrum of lesions associated with infection.

Establishing a diagnosis of infection with BRV is currently achieved via a specific PCR assay developed by Dr. Peter Kirkland's lab at NSW DPI.

Optimum sample from live animals: sterile applicator swab applied to the conjunctiva and cut off into NSW DPI viral transport medium, which is then shipped immediately or frozen.

Optimum samples from dead animals: aseptically collected eyelids (including lacrimal gland) spleen and kidney samples into individual sterile cryovials or NSW DPI viral transport medium and either shipped immediately or frozen. Change to clean instruments between handling each tissue.

Exclusion of other pathogens known to cause similar disease in reptiles and aquatic animals is also important, so collect a range of samples, including whole blood from live animals, and a range of frozen and formalin fixed tissues from dead animals.

Treatment

No successful treatment is known. Nursing care of affected animals has been unrewarding.

Control

No studies of disinfectant efficacy have been reported to date. Washing equipment, boats and trailers, and clothing with soapy water and then application of a broad-spectrum disinfectant known to have virucidal activity is recommended.

Be aware of the use of chlorhexidine around turtles, as toxicity has been reported.

Prevention

- Adherence to strict biosecurity measures may limit the spread of the virus to other species or other waterways (Keep a 'clean' routine: Bellinger River) (DPI, 2015)
- Quarantine and repeated specific PCR tests on animals entering quarantine are important to prevent infection entering breeding programs
- Viral PCR-positive individuals should be kept physically separated from negative individuals to prevent direct transmission
- Care must also be taken to prevent indirect transmission through fomites
- The role of vectors in the transmission of viruses is unknown

- Minimize exposure of individual turtles to environmental and nutritional stressors.
- Management considerations prior to release should include parasite management, pre-release husbandry and nutrition, and behavioural adaptation to re-wilding
- Choice of habitat at release site:
    - Low density of competing species *E. macquarii*
    - Food supply for release animals (ribbon weed, invertebrate density).
- Season for release – non-torpor period September to March
    - Water temperature/rainfall and its impact on the animals themselves.

Epidemiological Factors

The full host range, geographic distribution, and transmission pathways of BRV have not been established.

Surveillance activities in the Bellinger and other river systems have been initiated to try to address knowledge gaps inherent in the identification of a novel pathogen.

The outbreak investigation has focused on elucidating the primary pathogen and identification of factors that could have predisposed the animals to disease. Experimental infection trials are planned, subject to funding, to establish transmission pathways, pathogenesis, to develop additional diagnostic tools and to establish the role of BRV as a primary pathogen.

Iridoviridae (including Ranavirus)

Clinical Signs and Pathology

The Iridoviridae family is comprised of 5 genera and 11 species of large, double stranded DNA viruses that undergo temperature specific amplification, and are thus specific to ectothermic animals such as reptiles, amphibians, fish and invertebrates. Many ranavirus-associated mass mortality events have been recorded in amphibians and fish, and are increasingly reported in reptiles. Ranavirus infections of reptiles appear to target multiple organs including the stomach, oesophagus, lungs, spleen, liver and kidney, although some isolates may have a propensity for infecting the respiratory tract (Ariel, 2011). These viruses are recognised as a significant emerging infectious disease of chelonians, and they are capable of causing both multisystemic illness and mass mortality in free-ranging populations (Ariel, 2011; Gibbons & Steffes, 2013).

Ranaviruses have been identified in many chelonian species worldwide since the late 1990's (Marschang, 2011b). Chelonian infection with ranavirus has been associated with lethargy, anorexia, upper respiratory signs, conjunctivitis, subcutaneous oedema, ulcerative stomatitis and skin ulceration. Microscopic lesions in infected animals include hepatitis, enteritis, splenitis, pneumonia, oral and skin ulceration and fibrinoid vasculopathy. Basophilic and eosinophilic intracytoplasmic inclusions are described at the margins of hepatic and gastrointestinal lesions and have been observed in some infected animals; and sometimes in circulating leukocytes, but inclusions can be difficult to identify in degenerating cells and they are not a consistent finding (Gibbons & Steffes, 2013).

Ranavirus infection is an important differential diagnosis for BRV. Clinical signs and pathological findings of reptiles infected with these viruses can be nearly indistinguishable, particularly in a

description of box turtle and tortoise mortalities reported by Johnson (Johnson, et al., 2008). Johnson and colleagues (2008) conducted a retrospective investigation to identify five ranavirus infection related mortality events in captive and free-ranging box turtles and tortoises across several American states, where the description is very similar to that described above for BRV. Ranavirus infection was confirmed in Burmese star tortoises (*Geochelone platynota*), gopher tortoise (*Gopherus polyphemus*), eastern box turtles (*Terrapene carolina carolina*), and a Florida box turtle (*Terrepene carolinabauri*). Sequences of a portion of the major capsid protein from each event were identical to Frog virus 3 (FV3), the type-specific genus of Ranavirus. Amphibians infected with ranavirus were identified at the sites of two of the chelonian mortality events.

Ranavirus infection has been identified within a large group of captive Hermann's tortoises (*Testudo hermanni*) that died suddenly. The diagnosis was based on electron microscopy of basophilic intracytoplasmic inclusion bodies in multifocal, necrotising hepatic and respiratory lesions. Similar reports of ranavirus infection are documented in gopher tortoises (*Gopherus polyphemus*), and box turtles (*Terrapene* species) (Ariel, 2011; Marschang, 2011b).

Transmission

Some ranaviruses have been shown to infect both fish and frogs, suggesting the possibility of cross-taxon transmission.

The mechanisms of iridovirus transmission in chelonians has not been clearly documented. Arthropods and the presence of asymptomatic chelonian carriers of these viruses may be important in disease transmission (Gibbons & Steffes, 2013).

Cross-species susceptibility to ranavirus in chelonians is described in an experimental infection trial where western ornate box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*) were injected with virus isolated from a Burmese star tortoise. Inoculated animals developed clinical signs, as described above, and some animals died (Johnson, et al., 2007). Bohle iridovirus, isolated in amphibians, has experimentally been demonstrated to be highly virulent when inoculated into the coelomic cavity of hatchling Australian turtles (*Elseya latisternum* and *Emydura krefftii*) (Ariel & Owens, 2011). Further cross-species transmission of ranaviruses are documented in clinical outbreaks involving mixed-species groups of tortoises (Blahak & Uhlenbrok, 2010).

Diagnosis

A diagnosis of ranavirus is generally based on a combination of clinical signs, and post mortem findings, supported by either, electron microscopy, viral culture or PCR.

Ranaviruses have the capacity to form either eosinophilic or basophilic cytoplasmic inclusions in H&E stained histological sections and in circulating leukocytes in cytological preparations, but can be obscured by necrosis and they are not evident in all cases, even in experimental infection trials. Inclusions may be most commonly found in hepatocytes, trachea, lung, tongue, oesophagus, spleen, endothelial cells and leucocytes (Gibbons & Steffes, 2013).

Prof. Richard Whittington, University of Sydney, manages Australia's Iridovirus reference laboratory under the auspices of the World Organisation for Animal Health (OIE). CSIRO's Australian Animal Health Laboratory also offers viral culture and PCR testing to rule out the presence of iridoviruses.

Treatment

Treatment for ranavirus infection is poorly described, and where described offers limited success. Treatment may include topical or systemic antibiotic therapy to reduce the risk of secondary bacterial infection, fluid therapy, nutritional support, analgesics, and more rarely, antiviral agents (Gibbons & Steffes, 2013). Antiviral, fluid therapy and nutritional support are recommended on a case-by-case basis (Gibbons & Steffes, 2013).

Control

Control of ranavirus infections has been investigated at the continental scale by García-Díaz et al. (2016). This control is focused on the development of sophisticated biosecurity systems to detect potential incursions of exotic ranaviruses introduced via the importation of amphibians.

Prevention

Prevention of ranavirus in captive chelonians can be achieved through the maintenance of a closed population, as much as possible. Quarantine of new animals for 6-12 months should reduce the risk of bringing an infected animal into a group. If there is concern regarding the potential presence of ranavirus, animals may be tested by PCR upon entering quarantine (oral, conjunctival and cloacal swabs). Maintaining reptiles separate from amphibians may reduce the risk of ranavirus exposure.

Organic debris may protect ranaviruses from disinfectants that do not contain detergents. Cleaning surfaces and equipment with soapy water is recommended prior to disinfection with an agent that has broad virucidal activity.

Epidemiological Factors

The epidemiology of ranavirus infection in chelonians is not described beyond the discussion above.

Herpesvirus

Clinical Signs and Pathology

Herpesviruses are large, enveloped, pleomorphic, 120 to 200 nm diameter, double stranded DNA viruses that are well adapted to a particular species, and are often ubiquitous in a population. These viruses may cause lifelong latent infections that are characterised by the periodic recurrence of viral shedding with or without detectable clinical signs. Infection can range from inapparent to life-threatening. More severe forms of disease often occur when hosts are infected with a herpesvirus that is not adapted to that species.

Herpesviruses have been described in a broad range of captive and free-ranging chelonians (Frye, et al., 1977; Cox, et al., 1980; Jacobson, et al., 1982a; Jacobson, et al., 1991; Marschang, et al., 1997; Quackenbush, et al., 1998; Lackovich, et al., 1999; Quackenbush, et al., 2001; Origgi, et al., 2004; Greenblatt, et al., 2005; Johnson, et al., 2005; Hunt, 2006; Marschang, et al., 2006; Origgi, 2006; Stacy, et al., 2008; Marschang, et al., 2009a; Bicknese, et al., 2010), including a captive Australian Krefft's river turtle (*Emydura macquarii krefftii*) (Cowan, Raidal & Peters, 2015).

Herpesvirus infection in terrestrial and freshwater chelonians may be asymptomatic or may be accompanied by lethargy, anorexia, subcutaneous oedema of the neck, nasal discharge, necrotising

to diphtheritic stomatitis and neurological dysfunction. Post mortem examination findings may include stomatitis, rhinitis, conjunctivitis, hepatomegaly, hepatic pallor, acute hepatic necrosis, splenic neoplasia, enteritis, pulmonary oedema, pneumonia, and encephalomyelitis (Divers, S.J. and Mader, D.R. eds., 2005; Ariel, 2011; Marschang, 2011a;). Infection can be persistent and the expression or disease has been associated with a range of factors, including water temperature and host immune function (Haines & Kleese, 1977; Curry, et al., 2000) Eosinophilic to amphophilic intranuclear inclusion bodies are most commonly evident within hepatocytes at the margins of foci of necrosis, and may be also be evident in spleen, kidney, lung, upper respiratory tract, pancreas and gastrointestinal tract (Frye, et al., 1977; Cox, et al., 1980; Jacobson, et al., 1982a; McArthur, et al., 2002; Stacy, et al., 2008; Heckers, et al., 2013).

Several herpesviruses have been documented in marine turtles, associated with a range of lesions in epithelial tissues. A herpesvirus was identified in green sea turtles (*Chelonia mydas*) associated with a syndrome called gray patch disease in hand reared 2-3 month old animals (Rebel, et al., 1975), in green turtles with buoyancy and respiratory disturbance, and caseous conjunctivitis (Jacobson, et al., 1986). Herpesviruses have been associated with fibropapillomas on green and loggerhead turtles (*Caretta caretta*) (Lackovich, et al., 1999; Yu, et al., 2000; Quackenbush, et al., 2001). Two herpesvirus associated syndromes have been identified in loggerhead turtles: Loggerhead genital-respiratory herpesvirus, which was associated with tracheal and urogenital ulcers, and loggerhead orocutaneous herpesvirus, which was associated with oral and cutaneous ulcers and plaques and pneumonia (Stacy, et al., 2008).

Herpesviruses associated fibropapillomatosis are described in a variety of marine turtles around the world and are reviewed by (Marschang, 2011b). A novel circovirus, sea turtle tornovirus 1, has been identified through metagenomics in fibropapillomatous tissues, but not other tissues collected from two green turtles. The virus was not thought to have been the primary cause of fibropapillomas, but may have been either an opportunistic invader, or possibly altered the immune function of the turtles to predispose them to disease (Ng, et al., 2009).

Transmission

Transmission pathways for herpesviruses are poorly understood. Horizontal transmission is considered to be the most common pathway, but vertical transmission may be possible in some species. Latent infections, with periodic viral shedding, have been documented following stressful events. Viral shedding may or may not be associated with concurrent signs of disease.

Diagnosis

A diagnosis of herpesvirus infection can be achieved through a variety of modalities. A suspicion of herpesvirus infection is often raised based on the collective findings from an investigation including epidemiology, clinical history, clinical examination, and microscopic post mortem examination.

Microscopic post mortem findings may range from in-apparent to sever tissue necrosis/ulceration where cells at the margin of viable and non-viable tissues contain large eosinophilic intranuclear inclusion bodies. These foci of necrosis and inclusion body formation are most common in degenerating epithelial cells of the tongue, palantine mucosa, oesophagus, intestines, stomach, cloaca, liver, trachea, bronchi, alveoli, in endothelial cells of capillaries, including glomeruli, and in

spinal cord and brain. Lymphoproliferative lesions may accompany herpesvirus infection, and these lesions are most often documented in liver and spleen.

Diagnostic techniques for herpesviruses can include antibody assays, electron microscopy, viral isolation, and molecular characterisation.

ELISA testing is available overseas, where paired serum samples, collected 8 – 12 weeks apart, are tested to identify specific antibody production associated with active herpesvirus infection.

Rapid diagnosis of herpesvirus infection may be achieved through direct electron microscopy of fresh fluids collected from vesicular lesions, washes of epithelium of oral or tracheal lesions, intestinal contents, or fresh biopsy samples from acute lesions. Electron microscopy can also be conducted on glutaraldehyde fixed lesions, or on formalin fixed, paraffin embedded lesion margins.

Virus has been isolated from reptile pharyngeal swabs, tongue, liver, spleen, oesophagus, intestines, lung, brain and trachea.

Reptilian pan-herpesvirus PCR tests can be useful to rule-out or detect herpesvirus infection, and these tests are available on a research basis through Murdoch University and the University of Sydney.

Treatment

There is no specific anti-viral therapy available for chelonian herpesviruses. Treatment with Acyclovir may shorten the duration of viral shedding. The application of 5% acyclovir topical ointment or 80mg/kg oral acyclovir every 72 hours may be considered. Acyclovir may cause kidney damage in some species and should be used with caution after careful investigation and consultation.

Supportive care may improve clinical outcomes of reptile herpesvirus infections. This care may include fluid maintenance, nutrition, increased ambient temperature, and the use of suitable antibiotics to treat secondary bacterial infections.

Quarantine of animals suspected or confirmed to be infected with herpesvirus should be undertaken. The effective quarantine period should be life-long to prevent the spread of infection as a result of viral shedding through recrudescence in latently infected animals.

Control

Infected animals should not be released into the wild.

Infected animals should be isolated to reduce the risk of spread to other animals.

Maintain strict quarantine and use suitable diagnostics tests for any animals exhibiting suspicious lesions.

Prevention

Maintenance of a closed population, as much as possible, can minimise the risk or herpesvirus infection in managed reptile groups. Thorough and prolonged quarantine periods may reduce the risk of bringing an infected animal into a group. Due to latency in herpesvirus infections, even

lengthy quarantine (> 12 months) may not prevent the introduction of an infected animal into a group.

Hygiene is critical in maintaining a high plane of health, which may reduce the risk of viral recrudescence in latently infected animals, and reduce the risk of secondary bacterial or fungal infections in animals with herpetic lesions.

Most herpesviruses are sensitive to common disinfectants, high heat (56^{o}C for 5-10 minutes, or 37^{o}C for 22 hours), and acidic conditions (pH less than 5).

Organic debris may protect herpesviruses from disinfectants that do not contain detergents. Cleaning surfaces and equipment with soapy water is recommended prior to disinfection.

Epidemiological Factors

- Herpesviruses have been recorded in freshwater, marine and terrestrial chelonians
- Herpesvirus-associated disease has been reported in multiple tortoise species
- Isolated from tortoises in Europe, USA, and Africa, wild and long-term captive animals.

Adenovirus

Adenoviruses are double stranded, linear, non-enveloped DNA viruses with an icosahedral capsid. Reptilian adenoviruses are most commonly observed in lizards, predominantly bearded dragons (*Pogona* species), but more recently have been described as an emerging infectious disease of captive chelonians (Gibbons & Steffes, 2013). Clinical disease in infected chelonians most often presents as multisystemic illness, including hepatitis, enteritis, oesophagitis, splenitis and encephalopathy, but death can occur in the absence of observed clinical signs (Gibbons & Steffes, 2013).

The following adenovirus infections in chelonians have been described:

- A leopard tortoise (*Stigmochelys pardalis*) with biliverdinuria, wasting and haemorrhage was found to be infected with both herpes and adenovirus (Wilkinson, 2004)
- An adenovirus has been detected in a box turtle (*Terrapene ornata ornata*) that died with hepatopathy characterised by cytoplasmic vacuolation, nuclear pyknosis, and multifocal basophilic intranuclear inclusion bodies within hepatocytes. This animal was co-infected with *Mycoplasma* sp. (Farkas & Gál, 2009)
- Sulawesi tortoise adenovirus 1 was detected in a group of 105 confiscated Sulawesi tortoises (*Indotestudo forsteni*) where 82% of the animals died with severe systemic disease. Pathological findings in infected tortoises included hepatic lipidosis, myeloid necrosis and necrotising enterocolitis (Rivera, et al., 2009)
- Two impressed tortoises (*Manoiria impressa*) and a Burmese star tortoise (*Geochelone platynota*) that had been in contact with animals that survived the outbreak described above were found to be infected with Sulawesi tortoise adenovirus 1. It is interesting to note that the Burmese star tortoise died 19 months after first exposure and 13 months after the removal of Sulawesi tortoises from the collection. This finding is, consistent with viral persistence or latency in asymptomatic turtles. One impressed tortoise had facial

dermatitis, diphtheritic stomatitis, ulcerative and pseudomembranous duodenitis and colitis, interstitial pneumonia, myocarditis, fibrinous splenitis, renal tubular necrosis, and multifocal bone marrow necrosis. Intranuclear basophilic inclusion bodies were evident within the biliary epithelium, hepatocytes, hepatic endothelial cells and splenic reticuloendothelial cells. The second impressed tortoise had no gross lesions, but had microscopic evidence of heterophilic and histiocytic enteritis with submucosal oedema, heterophilic and necrotising interstitial nephritis, cholangiohepatitis, mild non-suppurative meningoencephalitis. Serous atrophy of fat and myocardial degeneration in the second animal was interpreted to be a result of inanition. This animal was co-infected with multisystemic intranuclear coccidia, which made identification of viral inclusion bodies more difficult. Rare intranuclear inclusion bodies may have been present in the splenic and colonic endothelial cells (Schumacher, et al., 2012)

- Although pathology is not described, a novel adenovirus, possibly of a novel lineage, were detected in tissues of captive pancake tortoise (*Malacochersus tornieri*), four eastern box turtles (*Terrapene carolina carolina*) and two red-eared sliders (*Trachemys scripta elegans*) and yellow-bellied sliders (*T. scripta scripta*) (Doszpoly, et al., 2013)
- More recently an adenovirus has been associated with hyperplastic stomatitis and oesophagitus in a spur-thighed tortoise (*Testudo graeca*) (Garcia-Morante, et al., 2016).

Transmission

Adenoviruses tend to be host-specific and are transmitted by the faecal-oral route, or through contact with oronasal secretion, and horizontal transmission among infected reptiles has been documented for Sulawesi tortoise adenovirus 1 (Schumacher et al., 2012). The adenoviral events, described above, were most often associated with young, immunocompromised animals, suffering co-infection, or subject to poor husbandry conditions (Gibbons & Steffes, 2013).

Diagnosis

A diagnosis of adenoviral infection is often based on a combination of clinical signs, gross and microscopic post-mortem examination findings in conjunction with electron microscopy, viral culture or specific PCR tests.

Histological findings include interstitial pneumonia, myocarditis, renal tubular necrosis, ulcerative stomatitis, facial dermatitis, and non-suppurative meningoencephalitis. Basophilic intranuclear inclusions are most consistently found in the liver of infected animals, but may or may not be identified in a variety of other tissues.

Pan-adenoviral PCR for reptiles is available through Murdoch University.

Control

Adenoviruses can be persistent in the environment. As adenoviral disease in turtles has been limited to captive animals, quarantine and disinfection protocols are important to prevent disease spread with the introduction of new animals into a collection (Gibbons & Steffes, 2013).

Treatment

A variety of treatments have been provided to adenoviral infected turtles, but the prognosis for adenoviral disease is poor (Gibbons & Steffes, 2013). Supportive care and prevention, or treatment of secondary infections, are recommended.

Papillomavirus

Clinical Signs and Pathology

Papillomavirus are non-enveloped ancient circular DNA viruses that are known to be highly host and tissue specific. These viruses replicate only in the deep layer of epithelial surfaces causing warts (papillomas). Papillomavirus associated lesions are most commonly around the mouth, urogenital sinus, anus, airways and sometimes the conjunctiva.

Papillomaviruses have been described in many reptiles, but only a small number of chelonian cases are documented. Electron microscopy was used to demonstrate the presence or papillomavirus in Bolivian side-necked turtles (*Platemys platycephala*). These lesions were focal or coalescing, flat, white, oval skin lesions on the head or plastron. Plastron lesions were prone to ulceration and secondary infection (Jacobson, et al., 1982b). Similarly electron microscopy was used to identify papillomavirus particles within the lung wash of a Russian tortoise (*Testudo horsfieldii*) that had chronic stomatitis (Drury, et al., 1998). Papillomavirus was also identified in small white oral papules in a green turtle (*Chelonia mydas*) and a loggerhead turtle (*Caretta caretta*). These lesions resolved spontaneously after several months (Manire, et al., 2008). Partial sequence analysis of a single gene revealed that the papillomaviruses found in the marine turtles were distinct from each other and from known papillomaviruses (Manire, et al., 2008).

Papillomavirus has also been detected on electron microscopy of a lung wash from a Horsfield's tortoise (*Testudo horsfieldii*) that had stomatitis (Divers, S.J. and Mader, D.R. eds., 2005).

Transmission

Transmission of most papillomaviruses occurs through direct and indirect contact with infected epithelium.

Diagnosis

Diagnosis of papillomavirus infection is generally achieved through a combination of consistent clinical signs, histopathology, and either electron microscopy or molecular identification. The microscopic changes associated with papillomavirus infection tend to include a thickened epithelium, which can form raised plaques, finger-like projections (papillae), or raised, rugose (undulating) lesions. The epithelium is thickened primarily within the stratum spinosum, which contains hyperplastic cells with large nuclei and abundant basophilic cytoplasmic fibrillar material. Basophilic intranuclear inclusion bodies and peripheralisation of nuclear chromatin are less commonly evident within lesions. The stratum corneum is often thickened. There may be mild lymphoplasmacytic inflammation within the dermis underlying epithelial lesions. Secondary ulceration, inflammation and infection may occur, particularly where lesions are prone to physical trauma.

Electron microscopy of active lesions may illustrate the presence of 45 – 50 nm viral particles, particularly if the beam is focused on intranuclear inclusion bodies.

Papillomavirus-specific PCR tests are available on a research basis at several Australian universities. Rolling circle PCR testing strategies may be more sensitive than conventional PCR, as they have a high capacity to amplify very small quantities of DNA.

Treatment

Many papillomas will not require treatment unless they affect mobility, the ability to prehend food, or they are secondarily infected with bacteria or fungi. Surgical removal of lesions may be undertaken, but lesions that are surgically removed usually recur.

Hygiene, husbandry and nursing care may be applied to generally improve immune system function in affected animals in the hopes that this may contribute to favourable outcomes.

Control

Infected animals should be isolated to reduce the risk or spread to other animals.

Togavirus

Togaviruses, within the genus Alphavirus, have been identified through serological and molecular surveillance in a broad range or reptiles across North and South America, Europe, Asia, and Australia. Eastern and Western equine encephalitis viruses are Alphaviruses that have the capacity to infect a broad range of mammals and birds, and cause serious illness in livestock and humans. Reptiles may play an ecologically significant role as reservoir hosts for some Alphaviruses, helping them to persist over temperate region winters in the absence of mosquito vectors. Experimental infection of reptiles with Eastern and Western equine encephalitis viruses demonstrated the capacity of reptile hosts to sustain persistently high viral titres, but no clinical signs have been observed in infected reptiles - reviewed by (Marschang, 2011b).

Pox-like Virus

Pox-like virus infections have been detected in individual reptiles by electron microscopy. Papular skin lesions around the eyes of a Hermann's tortoise (*Testudo hermanni*) were found to contain pox-like viruses (Oros, et al., 1998).

Reovirus

A reovirus has been identified in a single cachectic spur-thighed tortoise (*Testudo graeca*) with lingual necrosis (Marschang, 2001; Kugler, et al., 2016).

Paramyxoviruses

Paramyxoviruses (PMV) are rarely described in chelonians, and are associated with dermatitis (Zangger, et al., 1991) and pneumonia (Marschang, et al., 2009b; Papp, et al., 2010).

PMV, distinct from ferlaviruses of snakes and lizards, was isolated from a single Hermann's tortoise (*Testudo hermanni*) with pneumonia (Marschang, et al., 2009b).

A PMV most closely related to snake ferlavirus was identified in tissues from a leopard tortoise (*Geochelone pardalis*) with pneumonia. The significance of viral detection in this animal was

uncertain, because PMV was not detected in lung tissue from the infected animal (Papp, et al., 2010).

Bunyavirus

A single, wild-caught Texas soft-shelled turtle (*Trionyx spinifer emoryi*) was found to be infected with bunyavirus, which was detected in blood. Intracerebral inoculation of the virus into suckling mice proved invariably fatal. Serological studies found cross-reactivity with two mosquito-borne viruses of the genus *Orthobunyavirus* (Tordo, et al., 2005).

Picornovirus

Infection with a picornovirus, known as Virus X, has been documented in a broad range of reptiles. Preliminary sequence analysis indicates that Virus X belongs to a novel genus of picornoviruses. The virus has been isolated from clinically healthy chelonians (Marschang, 2001; Heuser, 2011; Heuser, et al., 2014), but has also been isolated along with herpesviruses and *Mycoplasma* sp. in tortoises with chronic rhinitis (Marschang, 2011a). Most commonly Virus "X" has been isolated from spur-thighed tortoises (*Testudo graeca*), but they have also been isolated in marginated tortoises (*T. marginata*), Hermann's tortoises (*T. hermanni*), leopard tortoises (*Geochelone pardalis*), Indian star tortoises (*Geochelone elegans*) and Egyptian tortoises (*T. kleinmanni*). More recently, a syndrome of shell softening, skeletal abnormality, nephropathy and osteodystrophy associated with the presence of virus X has been described in a large breeding colony of spur-thighed tortoises and Indian star tortoises (Heuser, et al., 2014).

Flavivirus

Antibodies to a variety of mosquito-borne flaviviruses have been found in reptiles, including chelonians. It may be that reptiles are an important over-wintering host for some of these viruses. Through natural and experimental infection, clinical signs associated with infection are incredibly rare. St. Louis encephalitis virus, Powassan virus, Japanese encephalitis virus, West Nile Virus and other flavivirus-like agents have been detected in chelonians (Whitney, et al., 1968; Shortridge, et al., 1974; Shortridge, et al., 1975; Drury, et al., 2001; Farfán-Ale, et al., 2006). West Nile Virus transmission studies have been conducted in a number of reptile species, including red-eared sliders (*Trachemys scripta elegans*), but none of the animals developed any clinical signs (Klenk & Komar, 2003).

A single report documents a flavi-like virus isolation from a leopard tortoise (*Geochelone pardalis*) with cloacal and nasal haemorrhage, biliverdinuria, and anaemia (Drury, et al., 2001; Wilkinson, 2004).

Appendix IV: Disease Risk Analysis

Disease Risk Analysis Goals:

Using the knowledge and specialist expertise of key stakeholders and wildlife disease specialists, develop a disease risk management strategy for BRST held in captivity, those extant in the Bellinger River and animals to be reintroduced back into the river under a reintroduction program. Identify and prioritise key knowledge gaps related to recovering the BRST population. Identify, consider and prioritise mitigation strategies for potential population level threats.

Disease Risk Analysis Scope:

Conduct a qualitative analysis of relevant published and unpublished information relating to historical and potential future health threats to the BRST population and consider methods to establish, monitor and maintain individual and population health.

Disease Risk Analysis Focus:

This analysis will focus on the identification, assessment and mitigation of all significant health risks to the BRST associated with captivity, reintroduction and fitness in the Bellinger River. The process will focus upon, but, not be limited to, consideration of the BRV.

Disease Risk Analysis Questions:

1) What is the risk of disease in BRST extant in the Bellinger River and how can these disease risks be minimised?
2) What is the risk of disease arising in BRST from identified health hazards associated with their reintroduction and how can these disease risks be minimised?
3) What are the risks of disease in BRST held in captivity and how can these disease risks be minimised?

Assumptions and Limitations

All decision making involves some assumptions and various constraints. Making these assumptions and limitations explicit is an essential part of any wildlife disease risk analysis as information is often scarce and resources limited. This transparency enables conclusions and recommendations arising to be considered within a 'real world' context. The following points were identified by the organisers and were subject to further discussion in the workshop.

Assumptions

- BRST are susceptible to the full range of health hazards recorded to date in Chelidae
- BRST are susceptible to pathogens that have been demonstrated to have a broad host range across reptiles
- The available data combined with the analytical and decision-making processes used by the experts involved in this DRA will enable reasonable decisions to be made to minimise health risks to BRST in captivity, extant in the Bellinger River and in reintroduction.

Limitations

- The epidemiology of the BRV is still largely unknown

- Compared to disease knowledge available for domestic animals and humans, the understanding of the range of potential pathogens of BRST and the epidemiology of these pathogens is poor
- There have been no systematic studies that have proactively screened for potential pathogens and assessed the health of free-ranging BRST prior to the 2015 disease outbreak
- The pharmacokinetics and pharmacodynamics of drugs that may be used for disease treatment has not been conducted for this species and extrapolation from other species is necessary.

Acceptable Risk

- A zero risk scenario is not feasible in the real world. Consequently, to enable decisions on realistic risk mitigation measures to be made, the level of acceptable risk should be determined for each hazard (part of the workshop discussion).

Source of Bellinger River Snapping Turtles for Future Reintroduction

First Captive Population

In April 2015, 17 animals were secured from one waterhole section in the upper reaches of the Bellinger River of which 16 survive as at November 2016. In April 2016 these (16) BRST were transferred from WSU quarantine facilities to Taronga Zoo to commence a captive breeding program within the zoo system. This consists of nine males and seven females.

Proposed Second Captive Population

A proposal for a second captive population of juvenile/sub-adult animals sourced from the Bellinger River (and potentially supplemented with adult animals sourced from private collections) has been made and will be pursued soon after the workshop. Animals from different sources will need to be quarantined separately.

Role of Captive Populations

The role of the captive populations are as insurance against extinction of the species in the wild, particularly should another mass mortality event occur, and to provide animals/offspring for release to augment the wild population.

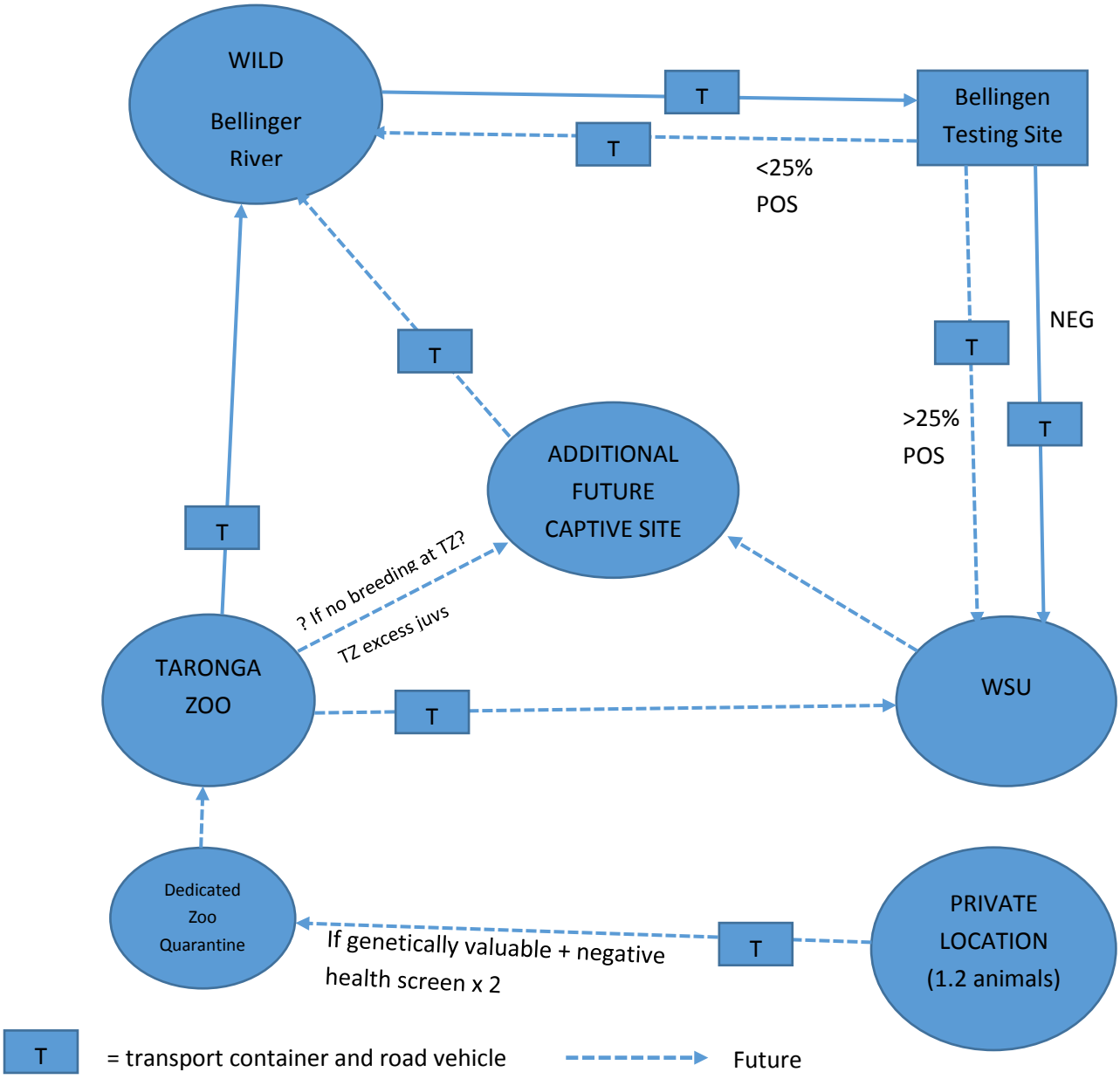
Animals in captivity remain in permanent quarantine from other animals as per best practice for reintroduction programs.

HAZARD IDENTIFICATION AND PRIORITISATION

Translocation pathway:

To clarify the current and potential future plans for wild-captive, captive-wild and captive-captive translocation of BRST and identify potential points of disease transmission the following graphic representation of the translocation pathways was developed.

FIGURE 8: PROJECTED BRST TRANSLOCATION PATHWAYS 2016-2021



WSU = Western Sydney University quarantine centre

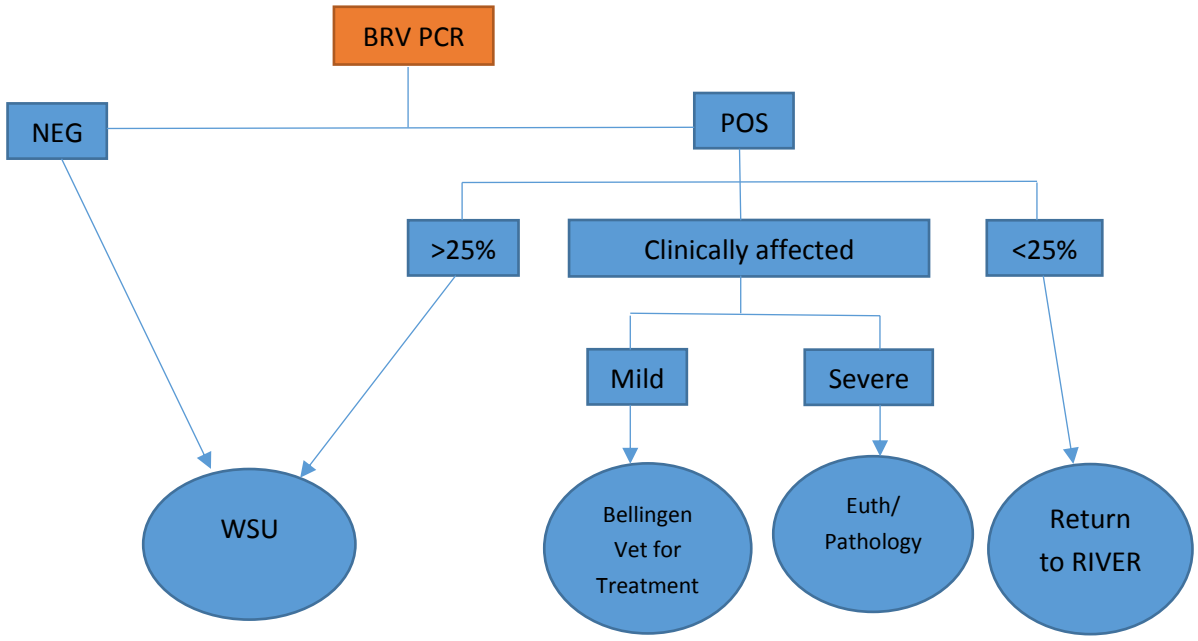
Notes to Figure 8:

- The timeframe for the translocations considered and graphically represented in Figure 8 is five years
- Assumes some release of hatchlings and some grown in captivity up to two years prior to release
- Taronga Zoo currently has capacity to house up to 60 – 70 juveniles (13 is the average per clutch, and survival of 10 of these is considered good). If production exceeds this would need to liaise with WSU re possible accommodation of excess juveniles
- The additional future captive site will only be stocked with 3-4 year old juveniles so it will take at least 3-5 years for them to breed (although this may be earlier under captive conditions). Taronga may move some animals to this site if holding capacity is exceeded or they do not breed here
- There are plans to capture juvenile turtles from the wild to establish a second population at WSU in November 2016. These animals will initially spend 48 hours at the testing station in Bellingen for genetic testing and BRV PCR screening. If animals test positive, current advice is to return them to the river. Another option (if WSU and DPI are agreeable) is that BRV PCR positive animals could go to WSU. This would complicate management (essentially establishing an additional quarantine population to keep BVR PCR negative and BVR PCR positives animals separate). If genetically unsuitable for breeding some BVR PCR negative animals could be returned to the Bellinger River although that is not the preferred option
- If any newly captured animals are returned to the Bellinger River, DPI would require them to be released at their site of origin

Recommendation

The preference is to take only BRV PCR negatives into captivity for now. However, in the event that there are a considerable number of BRV PCR positive animals in the next cohort of 25 – 30 animals to be captured (all of which are marked as having been previously tested negative for BRV by PCR) the following decision tree should be followed (see also Figure 8):

FIGURE 9: DECISION TREE FOR DISTRIBUTION OF BRV POSITIVE ANIMALS FROM THE WILD



Notes to Figure 9:

- If <25% are BRV-PCR positive, return these to river; if > 25% are positive move to WSU and keep separate if WSU and DPI are agreeable. All negatives should go to WSU (Figure 9).[3]
- Ricky Spencer advised that WSU would be able to effectively manage the separation of negative and positive animals. However this decision would need to consider that holding BRV-positive turtles could limit the ability to distribute animals from WSU to some sites such as Taronga Zoo with its 'negatives only' policy.
- While the holding of positive animals would involve more complex quarantine procedures, it would also provide useful opportunities to learn about the epidemiology and investigate other key research questions such as 'are hybrids more resistant to BRV?'

ACTION: Actioned by OEH (see footnote below).

Disease Hazard Prioritisation

Three populations of interest were identified: Captive, Wild and Private. The expert group used their combined knowledge and expertise to allocate each of the infectious disease hazards (Table 5) identified from published and unpublished sources (Appendix II & III) to one of the spaces in a Likelihood x Consequence matrix for each of these populations (Tables 7-9) based on their assessment of likelihood of exposure and consequence if exposed under the specified circumstances.

The non-infectious hazards identified in Table 6 were not considered in the prioritisations exercise, as it was considered that these are generally associated with captivity and, under the high quality of

[3] As at 23/2/17 a second captive population is already in quarantine. Nineteen animals were transferred to WSU on 26/11/16.

captive husbandry and health monitoring provided at current (and required of future) captive facilities they will either be prevented or detected and appropriately treated at an early stage.

TABLE 5: BRST INFECTIOUS DISEASE HAZARDS

Infectious Hazards
Bellinger River Virus (BRV)
Ranavirus/Other Iridoviruses
Herpesviruses (Hepatic necrosis, Grey patch disease, Lung-eye-trachea disease, Fibropapillomatosis)
Adenoviruses
Paramyxovirus (including ferlavirus)
Papillomavirus
Pox-like virus
Reovirus
Picornavirus
Togaviruses, Flaviviruses, Bunyaviruses
SCUD (Septicaemic Cutaneous Ulcerative Disease)
Aeromonas septicaemia
Mycoplasma spp. (Upper respiratory tract disease, rhinitis)
Enteric bacteria (*Salmonella* spp, *E. coli*, *Clostridium* spp)
Chlamydia spp.
Mycobacteria (*M. ulcerans*, *M. chelonae*, *M. haemophilum*, *M. marinum*)
Aeromonas spp
Morganella morganii
Gram negative rods
Paecilomyces lilacinus
Dermatophilus congolensis
Fusarium incarnatum, Trichosporon sp. Murcor spp.
Nematodes (Spirurida, Serpinema (freshwater turtles), Spirurida, Spiroxys (freshwater turtles), Strongyloida, Chapiniella (tortoise). Oxyurids, various (cheloniae))
Ectoparasites (Cloacaridae – *mites,* Hirudinea – *leeches,* Neopolystoma spp - *mongenean trematodes,* Ticks – variety)
Entamoeba spp. (Amoebiasis)
Hexamitia parva
Intranuclear coccidiosis
Cryptosporidium spp.

Haemogregarine parasites
Pentastomids (*Diesingia* spp)

TABLE 6: BRST NON-INFECTIOUS DISEASE HAZARDS

Non-Infectious Hazards
Lead toxicity
Ivermectin toxicity
Hypovitaminosis A
Hypothiaminosis (leukoencephalopathy)
Chlorhexidine toxicity

TABLE 7: RISK PRIORITISATION MATRIX OF INFECTIOUS DISEASE HAZARDS FOR CAPTIVE BRST

		Consequence			
		High (3)	Medium (2)	Low (1)	Negligible (0)
Likelihood	High (3)				Haemogregarine
	Medium (2)	*Aeromonas* septicaemia Mycobacteria spp		Nematodes	Enteric bacteria if incidental
	Low (1)	Bellinger River Virus Ranavirus/ Other Iridoviruses Enteric bacterial disease Entameba Intranuclear coccidia	SCUD Pseudomonas *Morganella morganii* *Paecilomyces*	*Fusarium incarnatum, Trichosporon* sp. Mucormycosis Cryptosporidiosis	
	Negligible (0)		Herpesviruses Adenoviruses; Paramyxovirus	Papillomavirus Pox-like virus Chlamydiosis Ectoparasites Pentastomes	Pox-like virus Picornavirus Togaviruses, Flaviviruses, Bunyaviruses Mycoplamosis Necrotic stomatitis Dermatophilosis Hexamita

Management Criteria

This population is maintained in permanent quarantine with appropriate barriers and biosecurity precautions applied to translocations in and out of the facility with particular attention to physical hazards as these are of greatest concern during transit

Consequence Assessment Criteria Applied to Table 7 with reference to INDIVIDUALS:

High: Mortality, unable to breed; **Medium**: Morbidity, short term reproductive deficiency; **Low**: Mild illness, short term

TABLE 8: RISK PRIORITISATION MATRIX OF INFECTIOUS DISEASE HAZARDS FOR FREE LIVING BRST

		Consequence			
		High (3)	Medium (2)	Low (1)	Negligible (0)
Likelihood	High (3)	Bellinger River Virus			Haemogregarine Ectoparasites
	Medium (2)				Enteric bacteria if incidental
	Low (1)	Ranavirus/ Other Iridoviruses	Herpesviruses Adenoviruses; Paramyxovirus	Aeromonas septicaemia Enteric bacterial disease Mycobacteriosis Entameba Intranuclear coccidia Nematodes Chlamydiosis Pentastomes	SCUD Pseudomonas Morganella morganii *Paecilomyces* *Fusarium incarnatum, Trichosporon sp.* Mucormycosis Cryptosporidiosis
	Negligible (0)			Papillomavirus Pox-like virus	Pox-like virus Picornavirus Togaviruses, Flaviviruses, Bunyaviruses Mycoplamosis Necrotic stomatitis Dermatophilosis Hexamita

Management Criteria

Free-ranging so monitoring only

Consequence Assessment Criteria applied to Table 8 with reference to POPULATIONS:

High: Population decline, mortality; widespread, frequent; **Medium**: Moderate morbidity, localized, periodically; **Low**: Mild morbidity, single site, rare

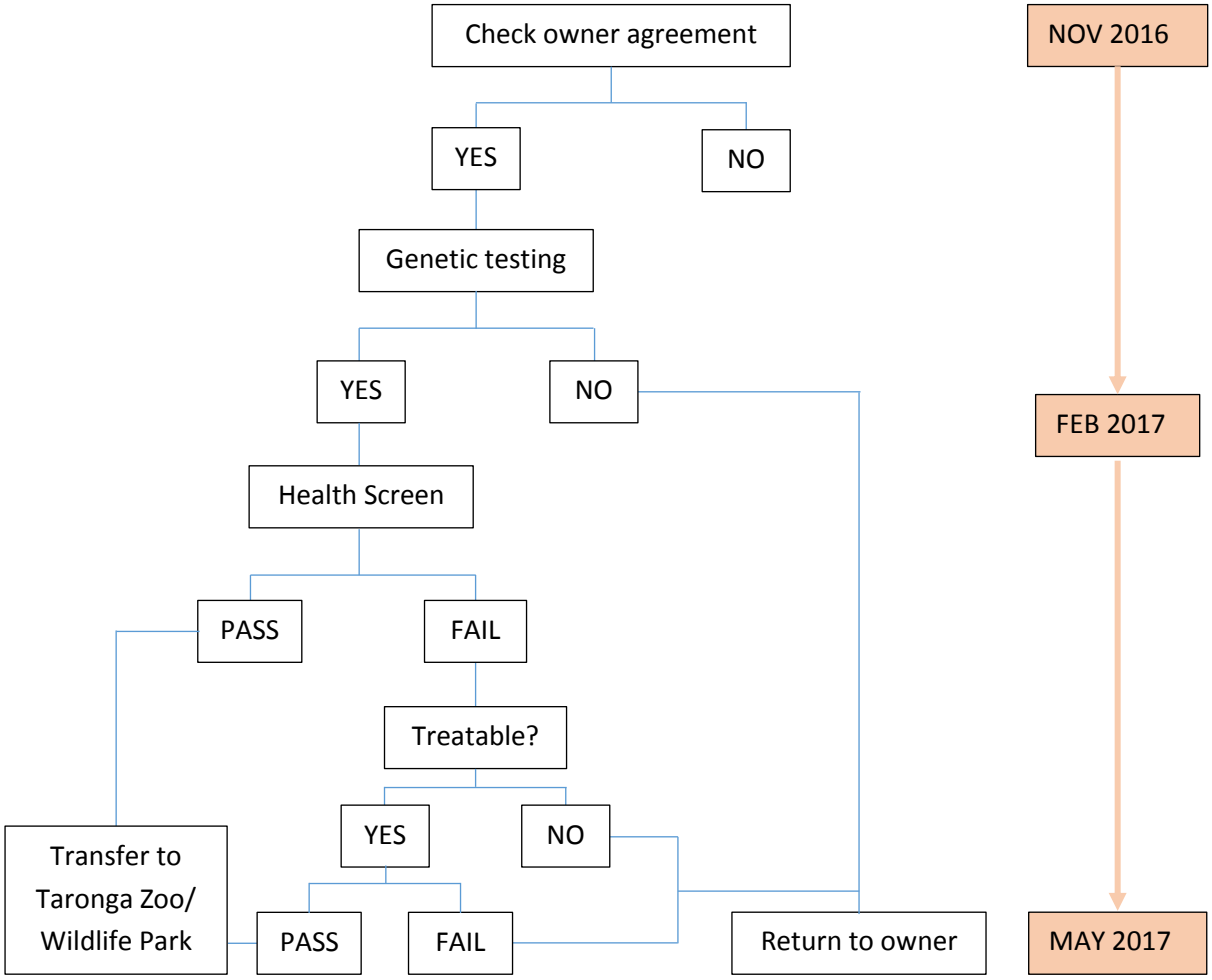
TABLE 9: RISK PRIORITISATION MATRIX OF INFECTIOUS DISEASE HAZARDS FOR PRIVATELY HELD BRST

		Consequence			
		High (3)	Medium (2)	Low (1)	Negligible (0)
Likelihood	High (3)	Mycobacteriosis	Adenoviruses; Paramyxovirus		Haemogregarine
	Medium (2)	Aeromonas septicaemia Bellinger River Virus Ranavirus/ Other Iridoviruses Enteric bacterial disease Entameba	SCUD Pseudomonas *Morganella morganii* *Paecilomyces* Herpesviruses	Nematodes Chlamydiosis *Fusarium incarnatum, Trichosporon sp.* Mucormycosis Cryptosporidiosis Pentastomes Mycoplamosis	Enteric bacteria if incidental
	Low (1)	Intranuclear coccidia			
	Negligible (0)			Papillomavirus Pox-like virus Ectoparasites	Pox-like virus Picornavirus Togaviruses, Flaviviruses, Bunyaviruses Dermatophilosis Hexamita

Management Criteria

Privately held BRST could be valuable to the existing captive breeding program. However, when assessing disease risk, any animals held outside the managed program should be considered potentially **high risk**. If incorporating privately held animals into the captive breeding program is to be considered, mitigation of the potential risks to the current captive populations would require any private animals to be health screened rigorously and quarantined for 6-12 months in a separate quarantine from resident BRST (See Figures 8 & 10).

FIGURE 10: DECISION TREE – ACQUISITION OF PRIVATELY HELD BRST



Any privately held BRST could be valuable to the existing captive breeding program. However, several procedures would be required before incorporation into the program could be considered. Inclusion of any legally held animals in the program would firstly require an owner's agreement. Animals would also need to undergo genetic evaluation (i.e. check hybrid status) and preliminary health screening.

Paired Ranking of Medium – High Disease Hazards

Table 10 shows the results of a paired ranking exercise in which the Expert Panel sequentially assessed the relative consequence to the wild population of BRST of each disease hazard against each other hazard to come up with a priority ranking.

TABLE 10: PAIRED RANKING OF MEDIUM – HIGH PRIORITY DISEASE HAZARDS FOR WILD BRST POPULATIONS

Hazard	Score	Rank
Bellinger River Virus	XXXXXXXXXXX (11)	1
Ranavirus	XXXXXXXXXXX (11)	1
Paramyxovirus	XXXXXXXXX (9)	2
Aeromonas (septicaemia)	XXXXXXXX (8)	3
Enteric bacteria	XXXXXXX (7)	4
Gram –ve Rods	XXXXXX (6)	5
Amoebiasis	XXXXX (5)	6
Mycobacteria	XXXX (4)	7
SCUD	XXX (3)	8
Adenovirus	XX (2)	9
Paeciliomyces	X (1)	10
Herpesvirus	(0)	11

Discussion

It was recognized that the list of hazards was heavily skewed by the inclusion of the disease concerns associated with the potential incorporation of the privately held animals into the managed captive population. Because of the unknown health history of these animals and their contact with other reptiles, these animals constitute by far the highest risk of disease introduction into the managed population. As these animals will only be acquired if they meet the stringent testing and quarantine protocols that will be instituted (including testing for the medium to high risk hazards identified in Table 9) it was agreed that applying the paired ranking process to the full list of hazards in Table 5 would bias the prioritisation of true disease hazards as systematically assessed in the hazard prioritisation matrices for wild and captive populations (Tables 7 & 8). Consequently the following three **Medium to High** priority hazards for these populations were selected for detailed risk assessment:

- **Bellinger River Virus (BRV)**
- ***Aeromonas* (septicaemia)**
- ***Mycobacterium* spp.**

BRV was clearly the highest priority and, given workshop time constraints and imperatives for progressing the response to the mass mortality associated with this organism, it was agreed that this

would be the only disease hazard considered in detail during the workshop. The value of further risk assessment of the other two hazards listed above is to be assessed by the management group at a later date.

RISK ASSESSMENT

Disease Hazard: Bellinger River Virus (BRV)

Justification for Hazard Status:

The Bellinger River Snapping Turtle (BRST) (*Myuchelys georgesi*) is currently listed as Critically Endangered under the Threatened Species Conservation Act NSW (1995) and is endemic to a 60km stretch of the Bellinger River, NSW and possibly a portion of the nearby Kalang River in coastal north eastern New South Wales. The species has cultural significance as a locally iconic species and is considered to be an indicator species reflecting the state of river ecosystem health.

Prior to 2015, the BRST was described as locally abundant with a population estimated to range between 1,500 and 4,000 individuals in the Bellinger River. Potential threats to the population were considered to be vulnerabilities associated with limited distribution and specific habitat requirements, predation, alteration to water quality, and possible hybridisation and competition with introduced *E. macquarii*.

The natural history of the BRST reflects a pattern of low fecundity, decreasing mortality rates with increased age, high adult survival rates, and a long lifespan (approximately 28 years). Male BRST are estimated to mature at 5-6 years of age. Females mature at 8-10 years, are gravid between September and November and nest between October and December. Clutch size varies between 10 to 25 eggs, averaging between 15 and20. Hatchlings appear after 72 days of incubation.

In mid-February, 2015 a significant mass mortality event was observed in BRSTs. Affected turtles displayed symptoms such as severe swelling or ulceration of the eyelids, cloudy corneas, lethargy, reluctance to move, and some animals dragged their hind legs behind them. Most affected animals died within a short time of being found, and animals that were brought into rehabilitation care were euthanased within a few days due to progression of the debilitating disease despite nursing care.

More than 430 turtle deaths were recorded in the period until June 2016. This consisted of dead bodies collected and affected BRST that were collected and euthanased by a local veterinarian under the direction of DPI. This is likely to be an underestimate of total deaths due to lack of detection of bodies on river bottom and washed away by floods.

A virus, previously not known to science was isolated in a pattern consistent with it being the likely agent responsible for the mortality event. This virus, referred to as Bellinger River Virus (BRV), has been identified as the greatest threat to the survival of the BRST.

To facilitate a detailed risk assessment of the BRV the workshop group initially considered the host, pathogen and environmental factors that may influence the persistence of this virus, its transmission to Bellinger River snapping turtles and susceptibility of the turtles to the disease (Table 11).

TABLE 11: HOST, PATHOGEN AND ENVIRONMENTAL FACTORS THAT MAY INFLUENCE OUTBREAK OF DISEASE

Host	BRV	Environment
Age Sex Genetics Species Diet Body condition Seasonality: • Breeding • Brumation • Food selection Population density Population dynamics Compromised health Immune status	Vectors Pathogencity (variation in strains?) Mutation rate Source/ecology	Location Food availability Water quality Toxin exposure Temperature Season – including food availability Vectors?

Release Assessment:

The Bellinger River system is the only site from which animals affected by BRV has been isolated. Although the current prevalence of BRV in this river system is not known the last BRV positive BRST in the Bellinger River was identified in March, 2016 but at levels much lower than comparable animals during the mortality event (P. Kirkland, et al., unpublished data). On this basis the likelihood of exposure of the wild population is estimated as **MODERATE** to **HIGH**.

Exposure Assessment:

The current prevalence of BRV in this river system is not known but, as stated above, the last BRV positive BRST in the Bellinger River was identified in March 2016[4]. On this basis the likelihood of exposure of the wild population is estimated as **MODERATE** to **HIGH**.

Only animals repeatedly tested as BRV PCR negative are held in the only current captive site within the recovery program (Taronga Zoo) and these animals are held in a dedicated, purpose-built facility maintained with a high level of biosecurity, health monitoring and husbandry. For this captive population the likelihood of exposure, while these conditions are maintained, is assessed as **LOW** to **NEGLIGIBLE**.

Consequence Assessment:

As noted above, BRV was associated with a peracute mass mortality event affecting, as far as is known, mostly only adult BRST. Initial presenting signs included swollen to ulcerated eyelids sometimes with corneal oedema, and the turtles were thin. Many affected turtles had a slight clear nasal discharge. A small proportion of animals exhibited hind limb paresis. At necropsy, animals

[4] As noted above, 6/25 (24%) of animals captured in November 2016 returned a positive PCR test for BRV.

were thin, had bilateral swollen eyelids and anterior uveitis, and some animals had tan foci on the skin of the ventral thighs (Moloney, et al., 2015).

Histopathology showed inflammation extending from the eyelids, peri-orbital tissues, and sinuses, sometimes extending along the olfactory/optic nerve into the meninges. There was also histological evidence of splenitis, nephritis and multisystemic fibrinoid vasculopathy. All turtles had acute lesions which seemed insufficient in duration to account for the animals' thin body condition. It was suggested that the nutritional plane of the animals may have been poor in advance of the outbreak, perhaps predisposing the animals to the severe consequences of infection by a novel pathogen (Moloney, et al., 2015).

On this basis the consequence assessment for both the wild and captive BRST populations is assessed as **HIGH**.

Risk Estimation:

On the basis of the above the overall risk is estimated as **HIGH** for both wild and captive populations and appropriate risk mitigation actions are recommended.

Level of Uncertainty (information gaps):

The level of uncertainty for this hazard is **HIGH** and measures to reduce this are listed in Table 12.

TABLE 12: MEASURES NEEDED TO REDUCE UNCERTAINTY (IN PRIORITY ORDER):

Knowledge Gaps	Measures needed to reduce uncertainty
Mode of transmission	Experimental Infection trials – transmission, incubation, shedding, age sex, pathogenesis (mechanism of disease), reservoir species (e.g. *E. macquarii*, eels, fish) Ongoing epidemiological river surveys Associations between and diet and exposure
Current prevalence and distribution of BRV	Develop serological capability On-going surveillance of river
Species Susceptibility	(see Appendix II)

RISK MANAGEMENT

Potential transmission pathways and critical control points at which mitigation actions could be taken to prevent infection of wild and captive populations respectively are listed in Table 13 and graphically represented in Figures 11 and 12.

TABLE 13: POTENTIAL TRANSMISSION PATHWAYS FOR THE BRV

Pathway	CCP (wild population)	CCP (captive population)
1. Direct (animal to animal or via food)	Potential reservoir species	Introduction of new animals Food
2. Vector (as amplifiers)	Biting insects/leeches	Biting insects
3. Fomites	Vessels (e.g. kayaks) Vehicles People Arthropods Fishing bait	People Arthropods Food
4. Environment	Free e.g. water Air	Water Air
5. Vertical		Breeding management i.e. mate selection (BRV neg with neg)

CCP = Critical Control Points

FIGURE 11: POTENTIAL TRANSMISSION PATHWAYS FOR BRV IN THE WILD

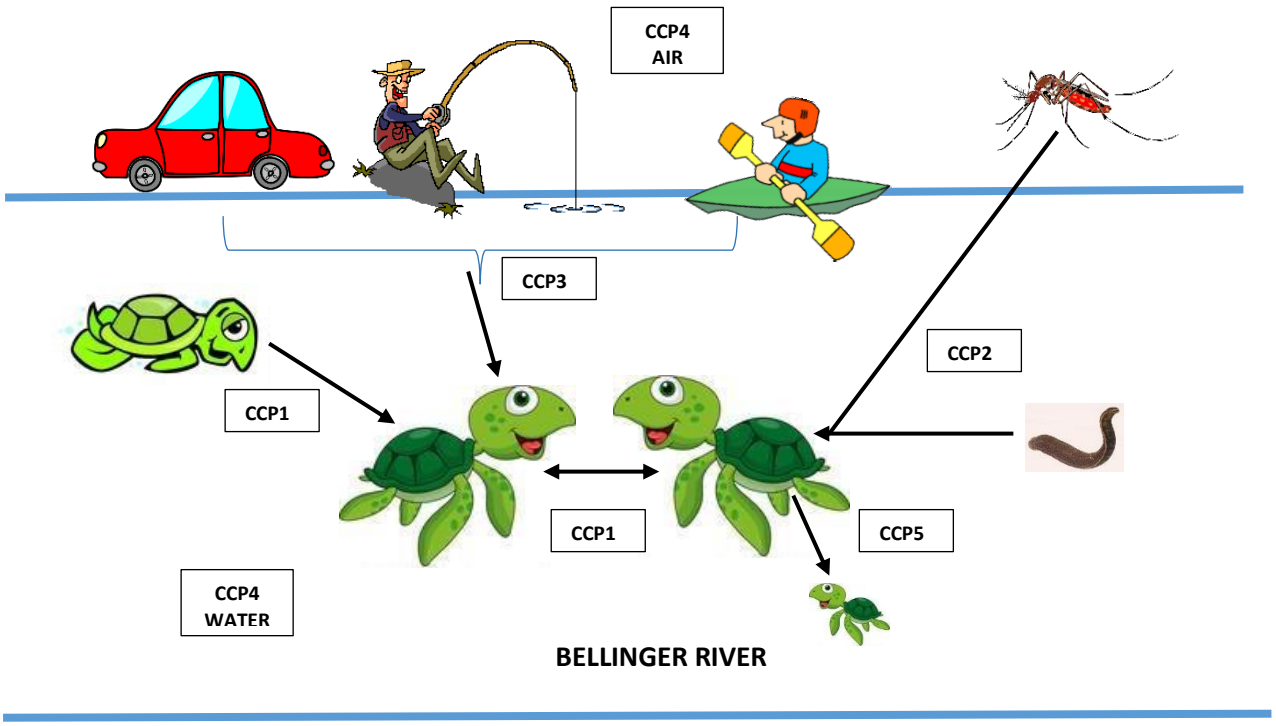
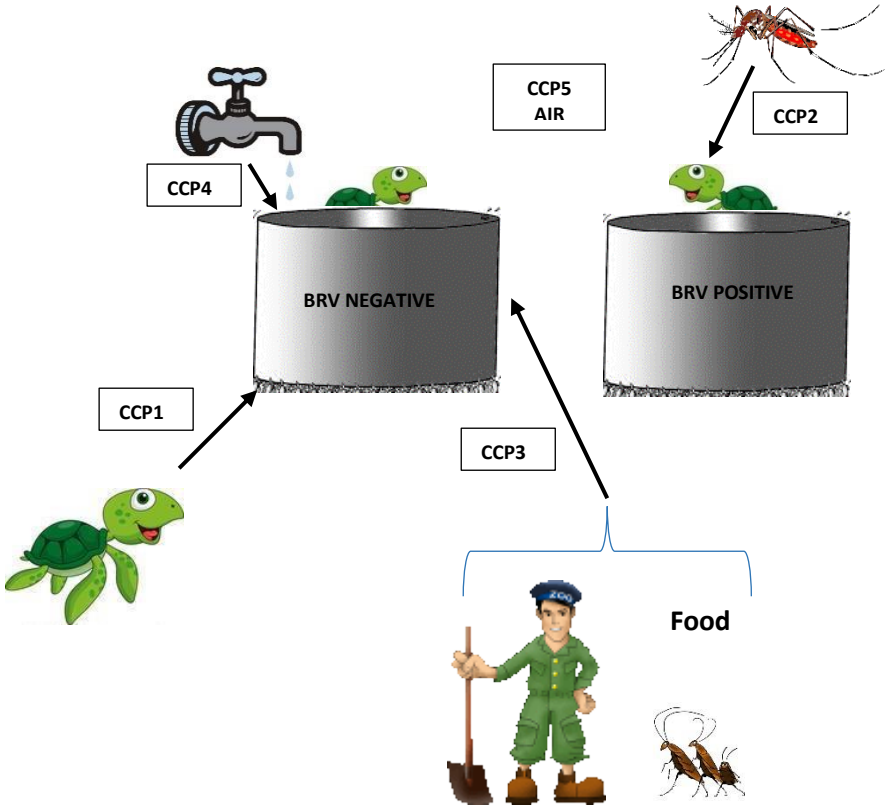


FIGURE 12: POTENTIAL TRANSMISSION PATHWAYS FOR BRV IN CAPTIVITY



As there is currently very limited information on the epidemiology, biological characteristics and pathogenesis (mechanism of disease) of BRV the transmission pathways listed are speculative and the level of uncertainty high. The actions listed in Table 14 would assist in reducing the level of uncertainty:

TABLE 14: METHODS TO REDUCE UNCERTAINTY IN THE TRANSMISSION PATHWAYS FOR BRV

Method	Explanation	Would inform
Experimental transmission trials	Unable to extrapolate from other viruses as BRV is novel and unlike any currently described virus	• Transmission routes • Pathogenesis • Susceptibilities of potential reservoir species and age and sex predispositions • Incubation period • Virus shedding patterns
Develop serological test	Enables surveillance of animals for virus exposure	• Prevalence and geographic distribution • Previous exposure to virus • Identification of reservoirs species • Assessment of immune response
On-going epidemiological river surveys	Continuing to monitor for presence of virus and affected animals using a	• Prevalence and geographic distribution of BRV over time • Release sites and timing

	combination of PCR, clinical health assessment and, in the future, serology*	
Food	Investigation of range of turtle foods that would constitute a low risk of contamination with BRV	• Food selection for captive turtles to provide as varied a diet as possible

Potential options for mitigating disease risks of BRV to wild and captive populations and, ultimately, for captive bred animals to be reintroduced to the Bellinger River, are listed in Table 15. Each option was then evaluated according to its feasibility and effectiveness by the expert group during the DRA workshop (Table 16).

TABLE 15: RISK MITIGATION OPTIONS FOR CRITICAL CONTROL POINTS

CCP	**Wild BRST**	**Captive BRST**	**Reintroduction (assume susceptibility)**
1. Direct	• Remove sick animals • Remove known carriers (e.g. *E. macquarii*) • Vaccine • Mandate only tested and negative fishing bait is used throughout the Bellinger River	• Isolation and quarantine • Vaccine • Food: irradiate, heat, feed only tested and negative food sources	• Depends on whether released animals are to be exposed or naïve to BRV • Vaccine • Select sites of low incidence
2. Vector	• Establish response protocol to detection of BRV through arbovirus surveillance program	• Insect screens • Insect control program	• Establish response protocol to detection of BRV through arbovirus surveillance program
3. Fomite	• "Keep it clean" hygiene • Mandate only tested and negative fishing bait is used throughout the Bellinger River	• "Keep it clean" hygiene	• "Keep it clean" hygiene • Mandate only tested and negative fishing bait is used throughout the Bellinger River
4. Environment	• Improve river water quality where indicated by on-going river health monitoring • BRST population health management where indicated through on-going population health monitoring	• Hygiene and sanitation • Water filtration systems	• Monitor river health • Monitor BRST population health • Sites of low incidence
5. Vertical		• Breed with BRV negative animals • Test and quarantine any positive animals	• Only release negative animals

TABLE 16: MITIGATION OPTION EVALUATION AND RECOMMENDATIONS

CCP	Mitigation Options	Effectiveness	Feasibility	Explanation	Recommendation
1. Direct	Population control of *E. macquarii*	High (if reservoir)*	L	Only effective if they are a key reservoir. Significant ethical and political issue and need to consider impacts on river ecology	Pending identification of reservoir status. Would also prevent hybridisation
	Restrict movement of potential reservoir species upstream e.g. eels, fish	High (if reservoirs)*	L	As above and logistically challenging	Pending identification of reservoir status
	Remove sick animals	High	M	Would reduce environmental load of virus but recognise will not get every animal	Yes
	Stop release of non-endemic species	High (if reservoir)*	M	Would need major community support	Pending identification of reservoir status
	Stop use of potentially contaminated fishing bait e.g. prawns	High	L	Ensuring public compliance would be hard; there are also multiple sources of bait	Pending identification of reservoir status
2. Vector	Control of vectors	High	L	BRST found to have haemoparasites vectored by biting insects. There are a number of arboviruses in Australia but to date none identified in turtles. Leeches are commonly found in turtles and vector some pathogens including *Aeromonas*	Not now. This is more a research path than a mitigation option. Not currently a high priority
3. Fomites	Education to encourage hygienic practices for people, vessels and vehicles	Low	M	Possibility of low compliance. Evidence to date indicates fomites alone are not responsible for transmission	Yes

*One healthy *E. macquarii* has tested positive for the BRV but there is currently no evidence that other species may act as reservoirs for BRV. The apparent movement of the disease upstream during the 2015 outbreak would be consistent with involvement of eels or fish that travel upstream but this is currently speculative. There is also no current evidence that native or non-native animals released into the river can act as reservoirs for this virus

Evidence to support the assumption that the disease moved upstream is shown by what was observed in the area from Dardanelles Creek upstream to Brinerville (in the New England National Park) not readily accessible to the public. Surveillance conducted in early March, 2015 in the area detected no sick animals. In mid-April, during the emergency transfer, healthy animals were collected in Brinerville which have never tested positive for the virus at any time. During the collection, an area approximately 5 km downstream of the collection point was observed and sick animals were detected. Subsequently, approximately 6 weeks after the collection, dead BRST were found in the same location from which the healthy animals were collected in April.

Appendix V. Workshop Participants

Name	Affiliation	Email address
1) Professor Arthur Georges	Member of the executive of the Institute for Applied Ecology – University of Canberra	georges@aerg.canberra.edu.au; arthur.georges@iae.canberra.edu.au
2) Associate Professor Ricky Spencer	Senior Lecturer in Zoology – Western Sydney University	R.spencer@westernsydney.edu.au
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4) Michael McFadden	Supervisor, Herpetofauna Department, Taronga Conservation Society Australia	mmcfadden@zoo.nsw.gov.au
5) Dr Karrie Rose	Manager, Australian Registry of Wildlife Health; Taronga Conservation Society Australia	krose@zoo.nsw.gov.au
6) Jane Hall	Wildlife Health Project Officer, Australian Registry of Wildlife Health; Taronga Conservation Society Australia	JHall@zoo.nsw.gov.au
7) Dr Sam Gilchrist	Project Officer, Wildlife Health Australia	sgilchrist@wildlifehealthaustralia.com.au
8) Dr Bruce Chessman	Freshwater Ecologist. Consultant	brucechessman@gmail.com
9) Dr Larry Vogelnest	Senior Veterinarian, Taronga Zoo	lvogelnest@zoo.nsw.gov.au
10) Kristen Petrov	PhD Student, Western Sydney University. Project on BRST (supervised by Ricky Spencer and Karrie Rose)	K.petrov@westernsydney.edu.au
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12) Gerry McGilvray	Project Officer, Ecosystems and Threatened Species Unit, OEH, Coffs Harbour	gerry.mcgilvray@environment.nsw.gov.au
13) Bruno Ferronato	PhD Ecology, Zoology, University of Canberra	brunoferronato@hotmail.com
14) Adam Skidmore	Senior Herpetological Keeper, Taronga Zoo	askidmore@zoo.nsw.gov.au
15) Caroline Lees	Facilitator and modeller, Co-convenor CBSG Australasia	caroline@cbsgaustralasia.org
16) Dr Richard Jakob-Hoff	Facilitator and DRA analyst, Co-convenor CBSG Australasia	richard@cbsgaustralasia.org

Appendix VI. What does success look like?

The following statements were created as part of a visioning exercise. Participants were invited to imagine a future for BRSTs, one in which the plan being developed had been implemented with success. They were asked to describe one or more elements of this success. These statements were used by a small group to craft the vision statement.

- Management goals were achieved.
- Program has been set up as a model for managing freshwater turtles throughout Australia – a benchmark program.
- If ongoing intervention is required, it is community driven habitat restoration.
- I have retired up there!
- A self-sustaining population was built.
- The program was very collaborative, with multiple partners working towards the same goal.
- The program was dynamic and shifted strategies with increased knowledge.
- Resources were available to undertake vital actions.
- A vast knowledge base was quickly built to guide the management strategies.
- The Bellinger River is widely known for its health and biodiversity. Rehabilitation of channel and riparian areas have been massively successful, with community and agency involvement. The turtles survive and thrive.
- Have identified the cause of decline and taken measures to ameliorate future declines
- Have been taking field trips of volunteers to continually monitor and know nesting sites, since reintroduction, with increasing numbers every year.
- Swimming with turtles.
- Turtles are an iconic species to the community.
- Community groups and land owners manage riparian environments, but turtle populations are self-sustaining.
- Model used for recovery of other freshwater environments.
- BRST population is self-sustaining, no longer in need of manipulations like captive breeding and head-starting.
- The habitat is restored and *M. georgesi* is thriving. The population is stable and represents all sizes and age classes.
- Regular river surveys have shown that the BRST population has continued to increase, is stable and minimum ongoing effort is needed to maintain this status.
- Bellingen community continues to thrive and is proud of its River.
- BRST population is currently estimated at 1500.
- BRST are often seen by the community in the River.
- The River has been restored to full health.
- No outbreaks of BRV since 2015.
- Invasive *Emydura macquarii* is eradicated.
- Re-population of the Bellinger River with BRSTs to a level that is sustainable as a result of releases of juveniles from captivity.
- Rehabilitation of the riverbanks

- Reflection from members of the community that the work we achieved has made them happy and has restored the BRST to a point where they are content.
- Ownership – there was a long list of stakeholders who considered the project worthwhile and appropriately dedicated resources that led to success.
- Population size is high and composition is balanced.
- Number of hybrids is low.
- Disease is rare.
- Community is aware and supportive.
- The BRST is fulfilling an ecological role in maintaining river health.
- The wild BRST population is self-sustaining.

Appendix VII. Local Stakeholders Group

Name	Organisation	Role
Gerry McGilvray	Project Officer, Ecosystems and Threatened Species Unit, OEH, Coffs Harbour	Project officer - Coordinator
Shane Ruming	Threatened Species Officer, Ecosystems and Threatened Species Unit, OEH, Coffs Harbour	Coordinator
John Schmidt	Senior Natural Resources Officer, OEH, Kempsey	Member
Scott Filmer	Ranger, New England National Park, National Parks and Wildlife Service	Member
Natasha English, Kylie Brooks	Bellinger Landcare	Member
Jane Eales	Bellingen Shire Council River and Biodiversity Projects Officer	Member
Piers Harper	Senior Land Services Officer, North Coast Local Land Services	Member
Gary Williams, Michael Jarrett	Muurbay – Aboriginal Language and Culture Cooperative	Member
Leif Lemke	Orama River Care Association (ORCA)	Member
Ben Perrim	Bellinger River Turtle Festival Director	Member
Sue Lennox	OzGreen CEO and Co-founder	Member
Darcy Browning	Upper Bellinger River Residents Association – Chair	Member
Tim Thorncraft, Rowan Simon	Community member	Member
Representatives	Bellingen Environment Centre	Member

Appendix VIII: Extract from Kristen Petrov's PhD Research Proposal

Objective: Determine whether competition and niche overlap occurs between remaining *M. georgesi* and *E. macquarii*.

Non-native *E. macquarii* in the Bellinger River pose a threat to the survival of the remaining *M. georgesi*. *Emydura macquarii* were abundant in the recent surveys of the Bellinger River and may compete with *M. georgesi* for food and space. Radio transmitters will be fitted on up to 25 *E. macquarii* and 25 *M. georgesi* and will be used to monitor movement patterns and geographic overlap between the two species. I will also compare the diets of *M. georgesi* to those of *E. macquarii* (and hybrids), using stable isotopes and stomach flushing to determine whether interspecific competition for food might limit food availability for *M. georgesi.* Stable isotope ($\delta^{13}C$ and $\delta^{15}N$) compositions of animals generally reflect the mean compositions of their diets and can be used to broadly demonstrate what animals eat, how individuals overlap in their diet and how diets change over time (Post, 2002). If there are overlaps in the home ranges and diets of *M. georgesi* and *E. macquarii*, an experiment will be implemented on the Bellinger River to test the following hypotheses:

1. *Myuchelys georgesi* change their home ranges in the presence/absence of *E. macquarii.*
2. *Myuchelys georgesi* consume different prey items in the presence/absence of *E. macquarii.*

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, method and assumptions. *Ecology* 83(3), 703-718.

References

Allanson, M. and Georges, A., 1999. Diet of *Elseya purvisi* and *Elseya georgesi* (Testudines: Chelidae), a sibling species pair of freshwater turtles from eastern Australia. *Chelonian Conservation and Biology* 3, 473-477.

Ariel, E., 2011. Viruses in reptiles. *Veterinary Research,* 21 September, 42(1), p. 100.

Ariel, E. and Owens, L., 2011. Challenge studies of Australian native reptiles with a ranavirus isolated from a native Amphibian. In: Sutton, W.B. (Eds.), Proceedings of the 1st International Symposium on Ranaviruses: 08 July 2011, Minneapolis, Minnesota, USA, p. 90. Available: http://fwf.ag.utk.edu/mgray/ranavirus/RanavirusProgram_LongVersion_FinalDraft.pdf

Australian Wildlife Health Network, 2009. Adenovirus infection in bearded dragons - Factsheet. [Online] Available at: https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/Adenovirus%20(Bearded%20Dragons)%2015%20Aug%202009%20(1.0).pdf

Bellinger Landcare, 2016. Bellinger River System Landholder Booklet, Bellinger Landcare Inc., Bellingen.

Bicknese, E., Childress, A. and Wellehan, J.J., 2010. A novel herpesvirus of the proposed genus Chelonivirus from an asymptomatic bowsprit tortoise (*Chersina angulata*). *J. Zoo Wildl. Med*. Volume 41, p. 353–8.

Blahak, S. and Uhlenbrok, C., 2010. Ranavirus infections in European terrestrial tortoises in Germany. In: Öfner S., Weinzierl F. (Eds.), Proceedings of the 1st International Conference on Reptile and Amphibian Medicine: 04–07 March 2010, Munich, p. 17–23.

Blamires, S., Spencer, R-J., King, P. and Thompson, M., 2005. Population parameters and life-table analysis of two coexisting freshwater turtles: Are the Bellinger River turtle populations threatened? *Wildlife Research* 32, 339-347.

Blamires, S. and Spencer, R-J., 2013. Influence of habitat and predation on population dynamics of the freshwater turtle *Myuchelys georgesi*. *Herpetelogica* 69, 46-57.

Bodetti, T.J., Jacobson, E., Wan, C., Hafner, L., Pospischil, A., Rose, K. and Timms, P., 2002. Molecular evidence to support the expansion of the hostrange of Chlamydophila pneumoniae to include reptiles as well as humans, horses, koalas and amphibians. *Systematic and Applied Microbiology* 25.1*,* pp. 146-152.

Cann, J., 1993. The Bellinger and Orara Rivers water supply scheme. An aquatic study of the freshwater turtles. Consultancy carried out for Mitchell McCotter on behalf of the Coffs Harbour City Council and Department of Public Works.

Cann, J., 1997. Georges short-neck turtle. *Monitor* 9(1), 19-23.

Cann, J., 1998. *Australian Freshwater Turtles*. Beaumont Publ., Singapore.

Cann, J., Spencer, R-J., Welsh, M. and Georges, A., 2015. *Myuchelys georgesi* (Cann 1997) – Bellinger River Turtle. In: Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Iverson, J.B. and Mittermeier, R.A. (eds.). Conservation Biology of Freshwater Turtles and Tortoises. *Chelonian Research Monographs* 5(8), 091.1–9.

Chessman, B.C., 2011. Declines of freshwater turtles associated with climatic drying in Australia's Murray-Darling Basin. *Wildlife Research* 38, 664-671.

Chessman, B.C., 2015. Observations on turtles collected from the Bellinger River in November 2015 – Unpub. report to Office of Environment and Heritage, Coffs Harbour.

Cohen, T., Reinfelds, I. and Brierley, G.J. 1998. River styles in the Bellinger-Kalang catchment. Department of Land and Water Conservation, Sydney.

Cope, I., Wheelhouse, N., Pocknell, A., Dagleish, M. and Summers B., 2014. An unusual presentation of Chlamydia pneumoniae infection in a Royal Python (*Python regius*). Veterinary Record Case Reports 2.1, p. e000086.

Cowan, M., Raidal, S. and Peters, A., 2015. Herpesvirus in a captive Australian Krefft's river turtle (*Emydura macquarii krefftii*). *Australian Veterinary Journal* 93(1-2), pp. 46-49.

Cox, W., Rapley, W. and Barker, I., 1980. Herpesvirus-like infection in a painted turtle (*Chrysemys picta*). *J. Wildl. Dis.* Volume 16, p. 445–449.

Curry, S., Brown, D. and Gaskin, J. e. a., 2000. Persistent infectivity of a disease associated herpesvirus in green turtles after exposure to seawater. *J Wildl Dis* Volume 364, p. 792–797.

Department of Environment, Climate Change and Water NSW (DECCW), 2010. NSW Climate Impact Profile: The impacts of climate change on the biophysical environment of New South Wales. Department of Environment, Climate Change and Water NSW, Sydney South. http://climatechange.environment.nsw.gov.au/Impacts-of-climate-change/2010-NSW-climate-impact-reporting.

Department of Primary Industries, 2015. Keep a 'clean' routine: Bellinger River Snapping Turtle mortality. PrimeFact 1401, October 2015

Divers, S.J. and Mader, D.R. eds., 2005. *Reptile medicine and surgery.* s.l.:Elsevier Health Sciences.

Doszpoly, A. W. J. C. A., Tarján, Z., Kovács, E. and Harrach, B. a. B. M., 2013. Partial characterization of a new adenovirus lineage discovered in testudinoid turtles. *Infection, Genetics and Evolution* Volume 17, pp. 106-112.

Drury, S., Gough, R. and McArthur, S., 2001. Detection and isolation of a flavivirus-like agent from a leopard tortoise (*Geochelone paradalis*) in the United Kingdom. *Vet. Rec.* Volume 148, p. 452.

Drury, S., Gough, R., McArthur, S. and Jessop, M., 1998. Detection of herpesvirus-like and papillomavirus-like particles associated with diseases of tortoises. *Vet. Rec.* Volume 143, p. 639.

Farfán-Ale, J., Blitvich, B.J., Marlenee, N.L., Loroño-Pino, M.A., Puerto-Manzano, F., García-Rejón, J.E., Rosado-Paredes, E.P., Flores-Flores, L.F., Ortega-Salazar, A., Chávez-Medina, J., Cremieux-Grimaldi, J.C., Correa-Morales, F., Hernández-Gaona, G., Méndez-Galván, J.F. and Beaty, B.J., 2006. Antibodies to West Nile virus in asymptomatic mammals, birds, and reptiles in the Yucatan Peninsula of Mexico. *Am. J. Trop. Med. Hyg.* Volume 74, p. 908–914.

Farkas, S. and Gál, J., 2009. Adenovirus and mycoplasma infection in an ornate box turtle (*Terrapene ornata ornata*) in Hungary. *Vet. Microbiol.* Volume 138, p. 169–73.

Flint, M., 2010. *Freshwater turtle disease investigation for the Burnett Catchment Central Queensland.* Brisbane: Veterinary Marine Animal Research, Teaching and Investigation Unit, The University of Queensland.

Flint, M., Limpus, D., Limpus, C. , Patterson-Kane, J.C., Eales, J.A., and Mills, P.C., 2011. Biochemical and hematological reference intervals for Krefft's turtles [*Emydura macquarii krefftii*] from the Burnett River Catchment, Australia. *Dis Aquat Org* Volume 95, p. 43–48.

Frye, F., Oshiro, L., Dutra, F. and Carney, J., 1977. Herpesvirus-like infection in two Pacific pond turtles. *J. Am. Vet. Med. A.* Volume 171, p. 882–884.

García-Díaz, P., Ross, J., Woolnough, A. and Cassey, P., 2016. Managing the risk of wildlife disease introduction: pathway-level biosecurity for preventing the introduction of alien ranaviruses. *Journal of Applied Ecology* 54, 234-241.

Garcia-Morante, B., Pénzes, J., Costa, T. and Martorell, J., Martínez, J., 2016. Hyperplastic stomatitis and esophagitis in a tortoise (*Testudo graeca*) associated with an adenovirus infection. *Journal of Veterinary Diagnostic Investigation* 28(5), pp. 579-583.

Garner, M., Gardiner, C., Wellehan, J., Johnson, A.J., McNamara, T., Linn, M., Terrell, S.P., Childress, A. and Jacobson, E.R., 2006. Intranuclear coccidiosis in tortoises: nine cases. *Veterinary Pathology Online* 43(3), pp. 311-320.

Georges, A., Limpus, C.J. and Parmenter, C.J., 1993. Natural history of the Chelonia. In: Glasby, C.J., Ross, G.J.B. and Beesley, P.L. (eds.), *Fauna of Australia: Amphibia and Reptilia* Vol. 2a, pp. 120-128. Australian Government Publishing Service, Canberra.

Georges, A., Walsh, R., Spencer, R-J., Welsh, M. and Shaffer, H.B., 2007. The Bellinger Emydura. Challenges for management. Unpublished report to NSW National Parks and Wildlife Service by the Institute for Applied Ecology, University of Canberra.

Georges, A. and Spencer R-J., 2015. Bellinger River turtles: assessment of genetic diversity and hybridization in a species under threat. Report for NSW OEH by Institute for Applied Ecology, University of Canberra, Canberra.

Georges, A., Spencer, R-J., Welsh, M., Schaffer, H.B., Walsh, R. and Zhang, X., 2011. Application of the precautionary principle to taxa of uncertain status: the case of the Bellinger River turtle. *Endangered Species Research* 14, 127-134.

Geyer, J., Kiefer, I., Kreft, S., Chavez, V., Salafsky, N., Jeltsch, F. and Ibisch, P.L., 2011. Classification of climate-change-induced stresses on biological diversity. *Conservation Biology* 25, 708–715.

Gibbons, J.W., Greene, J.L. and Congdon, J.D., 1983. Drought-related responses of aquatic turtle populations. *Journal of Herpetology* 17, 242–246.

Gibbons, P. and Steffes, Z., 2013. Emerging Infectious Diseases of Chelonians. *Veterinary Clinics of North America: Exotic Animal Practice* 16(2), pp. 303-317.

Gilligan, D., 2010. *The condition of freshwater fish assemblages in the Bellinger Catchment - A Report to the Northern Rivers Catchment Management Authority.* NSW Department of Industry and Investment, Batemans Bay.

Greenblatt, R., Quackenbush, S.L., Casey, R.N., Rovnak, J., Balazs, G.H., Work, T.M., Casey, J.W. and Sutton, C.A., 2005. Genomic variation of the fibropapillomas-associated marine turtle

herpesvirusacross seven geographic areas and three host species. *J. Virol*. Volume 79, p. 1125–1132.

Haines, H. and Kleese, W., 1977. Effect of water temperature on a herpesvirus infection of sea turtles. *Infect. Immun.* Volume 15, p. 756–759.

Heckers, K., Rüschoff, B. and Stöhr, A. e. a., 2013. *Detection of a new herpesvirus in European pond turtles (Emys orbicularis) in Germany.* Germany, s.n., p. 137.

Heuser, W., 2011. *Untersuchungen zu Virus "X", ein aus Landschildkröten isoliertes Picornavirus..* Justus-Liebig-Universität Giessen, Germany: Ph.D. Dissertation. Klinik für Vögel, Reptilien, Amphibien und Fische.

Heuser, W., Pendl, H., Knowles, N.J, Keil, G., Herbst, W, Lierz, M. and Kaleta, E.F., 2014. Soft plastron, soft carapace with skeletal abnormality in juvenile tortoises. *Tierärztliche Praxis Kleintiere* 42(5), pp. 310-320.

Hotzel, H., Blahak, S., Diller, R. and Sachse, K., 2005. Evidence of infection in tortoises by Chlamydia-like organisms that are genetically distinct from known Chlamydiaceae species. *Veterinary Research Communications,* pp. 71-80.

Hunt, C., 2006. Herpesvirus outbreak in a group of mediterranean tortoises (*Testudo* spp). *Vet Clin North Am Exot Anim Pract,* Volume 93, p. 569–574.

Innis, C.G., Garner, M.M., Johnson, A.J., Wellehan, J.F., Tabaka, C., Marschang, R.E., Nordhausen, R.W. and Jacobson, E.R., 2007. Antemortem diagnosis and characterization of nasal intranuclear coccidiosis in Sulawesi tortoises (*Indotestudo forsteni*). *Journal of Veterinary Diagnostic Investigation* 19(6), pp. 660-667.

IUCN Standards and Petitions Subcommittee 2014. Guidelines for Using the IUCN Red List Categories and Criteria. Version 11. Standards and Petitions Subcommittee.

Jacobson, E.R. ed., 2007. *Infectious diseases and pathology of reptiles: color atlas and text.* CRC Press ISBN 9780849323218 - CAT# 2321.

Jacobson, E., Buergelt, C., Williams, B. and Harris, R., 1991. Herpesvirus in cutaneous fibropapillomas of the green turtle *Chelonia mydas*. *Dis Aquat Org* Volume 12, p. 1–6.

Jacobson, E., Gaskin, G. and Wahlquist, H., 1982a. Herpesvirus-like infection in map turtles. *J. Am. Vet. Med. Assoc.* Volume 181, p. 1322–1324.

Jacobson, E., Gaskin, J., Clubb, S. and Calderwood, A., 1982b. Papilloma-like virus infection in Bolivian side-neck turtles. *J. Am. Vet. Med. Assoc.* Volume 181, p. 1325–1328.

Jacobson, E., Gaskin, J.M., Roelke, M., Greiner, E.C. and Allen, J., 1986. Conjunctivitis, tracheitis, and pneumonia associated with herpesvirus infection in green sea turtles. *J. Am. Vet. Med. Assoc.* Volume 189, p. 1020–1023.

Johnson, A., Pessier, A. and Jacobson, E., 2007. Experimental transmission and induction of ranaviral disease in western ornate box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*). *Vet. Pathol.* Volume 44, p. 285–297.

Johnson, A., Pessier, A.P., Wellehan, J.F., Brown, R. and Jacobson, E.R., 2005. Identification of a novel herpesvirus from a California desert tortoise (*Gopherus agassizii*). *Vet. Microbiol.* Volume 111, p. 107–116.

Johnson, A., Pessier, A.P., Wellehan, J.F., Childress, A., Norton, T.M., Stedman, N.L., Bloom, D.C., Belzer, W., Titus, V.R., Wagner, R., Brooks, J.W., Spratt, J. and Jacobson, E.R., 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. *Journal of Wildlife Diseases* 44(4), pp. 851-863.

Klenk, K. and Komar, N., 2003. Poor replication of West Nile virus (New York 1999 strain) in three reptilian and one amphibian species. *Am. J. Trop. Med. Hyg*. Volume 69, p. 260–262.

Kugler, R., Marschang, R.E., Ihász, K., Lengyel, G., Jakab, F., Bányai, K. and Farkas, S.L, 2016. Whole genome characterization of a chelonian orthoreovirus strain identifies significant genetic diversity and may classify reptile orthoreoviruses into distinct species. *Virus Research* Volume 215, pp. 94-98.

Lackovich, J., Brown, D.R., Homer, B.L., Garber, R.L., Mader, D.R., Moretti, R.H., Patterson, A.D., Herbst, L.H., Oros, J., Jacobson, E.R., Curry, S.S. and Klein, P.A., 1999. Association of herpesvirus with fibropapillomatosis of the green turtle *Chelonia mydas* and the loggerhead turtle *Caretta caretta* in Florida. *Dis Aquat Organ* Volume 37, p. 89–97.

Manire, C., Stacy, B.A., Kinsel, M.J., Daniel, H.T., Anderson, E.T. and Wellehan, J.F. Jr, 2008. Proliferative dermatitis in a loggerhead turtle, *Caretta caretta*, and a green turtle, *Chelonia mydas*, associated with novel papillomaviruses. *Vet. Microbiol*. Volume 130, p. 227–237.

Marschang, R., 2001. Isolierung und Charakterisierung von Irido-, Herpes- und Reoviren aus Landschildkröten sowie Beschreibung eines nicht charakterisierten zytopathogenen Agens. Giessen: Justus-Liebig-Universität, Giessen.

Marschang, R., 2011a. Diagnosis of infectious agents in tortoises. s.l.: Institut für Umwelt und Tierhygiene, Universit of Hohenheim, Stuttgart, Germany.

Marschang, R., 2011b. Viruses infecting reptiles. *Viruses* 3(11), pp. 2087-2126.

Marschang, R., Gleiser, C.B., Papp, T., Pfitzner, A.J., Böhm, R. and Roth, B.N., 2006. Comparison of eleven herpesvirus isolates from tortoises using partial sequences from three conserved genes. *Vet. Microbiol*. Volume 117, p. 258–266.

Marschang, R., Gravendyck, M. and Kaleta, E., 1997. Herpesvirus in tortoises: investigations into virus isolation and the treatment of viral stomatitis in *Testudo hermanni* and *T. graeca*. *Z Vet B* Volume 44, p. 385–394.

Marschang, R., Papp, T., Ferretti, L., Hochscheid, S. and Bentivegna, F., 2009a. Detection and partial characterization of herpesviruses from Egyptian tortoises (*Testudo kleinmanni*) imported into Italy from Libya. *J Zoo Wildl Med* Volume 401, p. 211–213.

Marschang, R., Papp, T. and Frost, J., 2009b. Comparison of paramyxovirus isolates from snakes, lizards and a tortoise. *Virus Res*. Volume 144, p. 272–279.

McArthur, S., Blahak, S., Kölle, P., Jacobson, E.R., Marschang, R.E. and Origgi, F., 2002. Roundtable: Chelonian herpesviruses. *J. Herpetol. Med. Surg*. Volume 12, p. 14–31.

Merck Veterinary Manual, 2015. The Merck Veterinary Manual. [Online] Available at: http://www.merckvetmanual.com/mvm/exotic_and_laboratory_animals/reptiles/bacterial_diseases_of_reptiles.html

Mika, S., Burns, A. and Ryder, D.S., 2016. Water Quality Monitoring of the Bellinger and Kalang Rivers 2015-16. Technical Report for Bellingen Shire Council. University of New England, Armidale.

Ng, T.F.F., Manire, C., Borrowman, K., Langer, T., Ehrhart, L. and Breitbart, M., 2009. Discovery of a novel single-stranded DNA virus from a sea turtle fibropapilloma by using viral metagenomics. *J. Virol*. Volume 83, p. 2500–2509.

Moloney, B., Britton, S. and Matthews, S., 2015. Bellinger River snapping turtle mortality event 2015: Epidemiology report. Unpublished report, Biosecurity NSW, Department of Primary Industries.

NSW Scientific Committee, 2016. Final determination to list the Bellinger River Snapping Turtle *Myuchelys georgesi* (Cann, 1997) as a Critically Endangered species in Part 1 of Schedule 1A of the *Threatened Species Conservation Act 1995*. NSW Scientific Committee, Hurstville.

Office of Environment and Heritage, 2011. NSW threat abatement plan for predation by the red fox (*Vulpes vulpes*) December 2010. Office of Environment and Heritage, Sydney.

Office of Environment and Heritage, 2015. Atlas of NSW Wildlife. Data obtained through the BioNet Atlas of NSW Wildlife website. Viewed 8 May 2015.

Origgi, F., 2006. Herpesvirus in tortoises. In: Reptile medicine and surgery. 2nd ed. Saunders, Missouri: s.n., p. 814–821.

Origgi, F. Romero, C.H., Bloom, D.C., Klein, P.A., Gaskin, J.M., Tucker, S.J. and Jacobson, E.R., 2004. Experimental transmission of a herpesvirus in Greek tortoises (*Testudo graeca*). *Vet. Pathol*. Volume 41, p. 50–61.

Oros, J., Rodríguez, J.L., Déniz, S., Fernández, L. and Fernández, A., 1998. Cutaneous poxvirus-like infection in a captive Hermann's tortoise (*Testudo hermanni*). *Vet. Rec*. Volume 14, p. 508–509.

Papp, T., Seybold, J. and Marschang, R., 2010. Paramyxovirus infection in a leopard tortoise (*Geochelone pardalis babcocki*) with respiratory disease. *J. Herpetol. Med. Surg*. Volume 20, pp. 64-68.

Quackenbush, S., Casey, R.N., Murcek, R.J., Paul, T.A., Work, T.M., Limpus, C.J., Chaves, A., duToit, L., Perez, J.V., Aguirre, A.A., Spraker, T.R., Horrocks, J.A., Vermeer, L.A., Balazs, G.H. and Casey, J.W., 2001. Quantitative analysis of herpesvirus sequences from normal tissue and fibropapillomas of marine turtles with real-time PCR. *Virology* Volume 287, p. 105–111.

Quackenbush, S., Work, T.M., Balazs, G.H., Casey, R.N., Rovnak, J., Chaves, A., duToit, L., Baines, J.D., Parrish, C.R., Bowser, P.R. and Casey J.W., 1998. Three closely related herpesviruses are associated with fibropapillomatosis in marine turtles. *Virology* Volume 246, p. 392–399.

Rebel, G., Rywlin, A. and Haines, H., 1975. A herpesvirus agent associated with skin lesions of green sea turtles in aquaculture. *Am. J. Vet. Res*. Volume 36, p. 1221–1224.

Rivera, S., Wellehan, J.F. Jr, McManamon, R., Innis, C.J., Garner, M.M., Raphael, B.L., Gregory, C.R., Latimer, K.S., Rodriguez, C.E., Diaz-Figueroa, O., Marlar, A.B., Nyaoke, A., Gates, A.E., Gilbert, K., Childress, A.L., Risatti, G.R., Frasca, S. Jr., 2009. Systemic adenovirus infection in Sulawesi tortoises (*Indotestudo forsteni*) caused by a novel siadenovirus. *J. Vet. Diagn. Invest*. Volume 21, p. 415–426.

Ryder, D., Veal, R., Sbrocchi, C. and Schmidt, J., 2011. Bellinger-Kalang Rivers ecohealth project: Assessment of river and estuarine condition 2009-2010. Final Technical Report to the Bellingen Shire Council, University of New England, Armidale.

Schumacher, V., Innis, C.J., Garner, M.M., Risatti, G.R., Nordhausen, R.W., Gilbert-Marcheterre, K., Wellehan, J.F. Jr, Childress, A.L. and Frasca, S. Jr, 2012. Sulawesi tortoise adenovirus-1 in two impressed tortoises (*Manouria impressa*) and a Burmese star tortoise (*Geochelone platynot*. *J. Vet. Diagn. Invest*. Volume 21, pp. 415-426.

Shortridge, K., Ng, M.H., Oya, A., Kobayashi, M., Munro, R., Wong F. and Lance, V., 1974. Arbovirus infections in reptiles: immunological evidence for a high incidence of Japanese encephalitis virus in the cobra *Naja naja*. *Trans. R. Soc. Trop. Med. Hyg*. Volume 68, pp. 454-460.

Shortridge, K., Oya, A., Kobayashi, M. and Yip, D., 1975. Arbovirus infections in reptiles: studies on the presence of Japanese encephalitis virus antibody in the plasma of the turtle, *Trionyx sinensis*. *Southeast Asian J. Trop. Med. Public Health* Volume 6, p. 161–169.

Soldati, G., Lu, Z.H., Vaughan, L., Polkinghorne, A., Zimmermann, D.R., Huder, J.B. and Pospischil, A., 2004. Detection of mycobacteria and chlamydiae in granulomatous inflammation of reptiles: a retrospective study. Veterinary Pathology Online 41.4, pp. 388-397.

Spencer, R-J., 2006. The Bellinger River Emydura survey: Effects of foxes and interspecific competition. Unpublished report to NSW NPWS by Pestat Ltd, March 2006.

Spencer, R-J., Georges, A., Lim, D., Welsh, M. and Reid, A.M., 2014. The risk of inter-specific competition in Australian short-necked turtles. *Ecological Research* 29, 767-777.

Spencer, R-J., Georges, A. and Welsh, M., 2007. The Bellingen River Emydura: Ecology, population status and management. Unpublished report to NSW NPWS by Institute of Applied Ecology, University of Canberra.

Spencer, R-J. and Thompson, M.B., 2000. The ecology and status of *Emydura macquarii* and *Elseya georgesi* in the Bellinger River. Unpublished Report to the NSW NPWS by the Institute of Wildlife Research, University of Sydney.

Spencer, R-J. and Thompson, M.B., 2005. Experimental analysis of the impact of foxes on freshwater turtle populations. *Conservation Biology* 19, 845–854.

Stacy, B., Wellehan, J.F., Foley, A.M., Coberley, S.S., Herbst, L.H., Manire, C.A., Garner, M.M., Brookins, M.D., Childress, A.L. and Jacobson, E.R., 2008. Two herpesviruses associated with disease in wild Atlantic loggerhead sea turtles (*Caretta caretta*). *Vet. Microbiol*. Volume 126, p. 63–73.

Thompson, M.B., 1983. Populations of the Murray River tortoise, *Emydura*: The effect of egg predation by the red fox *Vulpes vulpes*. *Australian Wildlife Research* 10, 363-372.

Thompson, M.B., 1993. Hypothetical considerations of the biomass of chelid tortoises in the River Murray and the possible influences of predation by the introduced fox. In: Lunney, D., Ayres, D. (eds.), *Herpetology in Australia: A Diverse Discipline*, pp. 219-224. Royal Zoological Society of New South Wales, Mosman.

Threatened Species Scientific Committee, 2016. Approved Conservation Advice for *Wollumbinia georgesi* (Bellinger River snapping turtle). Department of the Environment and Energy, Canberra.

Tordo, N., Benmansour, A., Calisher, C., Dietzgen, R.G., Fang, R.X., Jackson, A.O., Kurath, G., Nadin-Davis, S.A., Tesh, R.B. and Walker, P.J., 2005. Rhabdoviridae. In: Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses. Amsterdam: Elsevier Academic Press, p. 623–644.

Tucker, A.D., Kelly, W.R., Limpus, C.J., Priest, T.E. and Guarino, F., 2002. Prevalence of ulcerative disease in free-ranging Krefft's turtle. *Memoirs of the Queensland Museum* 48, 233-238.

U.S. Geological Survey, 2016. Disease Information: Other Diseases: Ranavirus. [Online]. Available at: http://www.nwhc.usgs.gov/disease_information/other_diseases/ranavirus.jsp

Waterson, A.M., Schmidt, D.N., Valdes, P.J., Holroyd, P.A., Nicholson, D.B., Farnsworth, A. and Barrett, P.M., 2016. Modelling the climatic niche of turtles: a deep-time perspective. *Proc. R. Soc. B* 283, 20161408, DOI: 10.1098/rspb.2016.1408.

Whitney, E., Jamnback, H., Means, R. and Watthews, T., 1968. Arthropod-borne-virus survey in St. Lawrence County, New York: Arbovirus reactivity in serum from amphibians, reptiles, birds, and mammals. *Am. J. Trop. Med. Hyg*. Volume 17, p. 645–650.

Wildlife Health Australia, 2009. Ferlavirus (previously OPMV) in wild Australian native reptiles EXOTIC Factsheet. [Online] Available at: https://www.wildlifehealthaustralia.com.au/Portals/0/Documents/FactSheets/EXOTIC%20-%20Ferlavirus%20(previously%20OPMV)%2027%20Jun%202013%20(2.0).pdf

Wilkinson, R., 2004. Clinical pathology. In: Medicine and Surgery of Tortoises and Turtles. Chichester: Blackwell Publishing, p. 141–186.

Wobeser, G., 2006. Essentials of Disease in Wild Animals. Blackwell Publishing, Ames, Iowa.

Yu, Q., Lu, Y., Nerurkar, V. and Yanagihara, R., 2000. Amplification and analysis of DNA flanking known sequences of a novel herpesvirus from green turtles with fibropapilloma: brief report. *Arch Virol* Volume 14512, p. 2669–2676.

Zangger, N., Müller, M. and Pagan, O., 1991. Virale Dermatitis bei der Maurischen (*Testudo graeca*) und der Griechischen (*Testudo hermanni*) Landschildkröte in der Schweiz. Bad Nauheim, Germany, s.n., p. 25–29.

Zwart, P. and Truyens, E., 1975. Hexamitiasis in tortoises. *Veterinary Parasitology* 1(2), pp. 175-183.

Other sources cited

Chessman, B., n.d. Personal communication.

Chessman, B., 2016. Personal communication, 2016, in Threatened Species Scientific Committee, 2016.

Kirkland et al., n.d. Unpublished data.

Kuchling, G., n.d. Personal observation.

Rose, K., n.d. Personal communication.

Spencer, R-J., n.d. Personal communication.

Spencer, R-J., 2015. Personal communication, 11 March 2015, in NSW Scientific Committee, 2016.

Spencer, R-J., 2015. Personal communication, 13 March 2015.

Spencer, R-J., 2016. Personal communication, 2016.

Spencer, R-J., 2017. Unpub. modelled data, May 2017, based on 300 wild juveniles with 85% +/-15% mortality of eggs, and 15 captive females with 50-100% hatching success. Year one survival

at 50% (based on Spencer et al. 2005), increasing 10% per year until adult survival rate of 95%.

Additional reading

ABRS, 2009. Australian Faunal Directory. Australian Biological Resources Study, Canberra. http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/index.html

Artner, H., 2008. The world's extant turtles, Part 1. *Emys* 15, 4-32.

Blamires, S.J., 2003. Review of procedures proposed for population viability analyses for the Bellingen River Emydura 2003-2006. Unpublished report to the NSW NPWS, November 2003.

Blamires, S.J. and Thompson, M.B., 2003. Survey of Bellinger River Turtles, *Elseya georgesi* and *Emydura macquarii* subsp.: an assessment of potential impacts of foxes. Report of surveys conducted September 30-October 10, 2002 and January 8-18, 2003 in conjunction with the New South Wales National Parks and Wildlife Service.

Coberley, S., Condit, R., Herbst, L. and Klein, P., 2002. Identification and expression of immunogenic proteins of a disease-associated marine turtle herpesvirus. *J. Virol*. Volume 7620, p. 10553–10558.

Cogger, H.G., Cameron, E.E., Sadlier, R.A. and Eggler, P., 1993. *Action Plan for Australian Reptiles*. Australian Nature Conservation Agency, Canberra.

Department of Environment, Climate Change and Water NSW, 2010. *NSW Climate Impact Profile Technical Report: potential impacts of climate change on biodiversity.* Department of Environment, Climate Change and Water NSW, Sydney South. http://climatechange.environment.nsw.gov.au/Impacts-of-climate-change/Biodiversity

Department of Primary Industries, 2015. DPI Procedure - Effective decontamination of equipment used in association with a suspected pathogen impacting *Myuchelys georgesi* Bellinger River, Version 3, dated 24/3/15, Unpublished.

Farley, S.M., 2004. Conservation genetics of the snapping turtle (*Elseya* sp.): An assessment of genetic diversity and population structure. Unpublished thesis submitted in partial fulfilment of the requirements for the degree of honours in Applied Science, University of Canberra.

Fielder, D.P., 2010. Population ecology, ecophysiology, phylogenetics and taxonomy of the threatened western sawshelled turtle, *Myuchelys bellii*, from the Murray-Darling Basin of Australia. Unpublished thesis in fulfilment of the requirements for the degree of Doctor of Philosophy, University of New England.

Fielder, D.P., Limpus, D.J. and Limpus, C.J., 2014. Reproduction and population ecology of the vulnerable western sawshelled turtle, *Myuchelys bellii*, in the Murray-Darling Basin, Australia. *Australian Journal of Zoology* 62, 463-476.

Fritz, U. and Havaš, P., 2007. Checklist of chelonians of the world. *Vertebrate Zoology* 57, 149–368.

Georges, A. and Adams, M., 1992. A phylogeny for Australian chelid turtles based on allozyme electrophoresis. *Australian Journal of Zoology* 40, 453-476.

Georges, A. and Adams, M., 1996. Electrophoretic delineation of species boundaries within the short-necked chelid turtles of Australia. *Zoological Journal of the Linnean Society* 118, 241-260.

Georges, A. and Thomson, S., 2010. Diversity of Australasian freshwater turtles, with an annotated synonymy and keys to species. *Zootaxa* 2496, 1-37.

Heppell, S.S., 1998. Application of life history theory and population model analysis to turtle conservation. *Copeia* 1998, 367-375.

Le, M., Reid, B.N., McCord, W.P., Naro-Maciel, E., Raxworthy, C.J., Amato, G. and Georges, A., 2013. Resolving the phylogenetic history of the short-necked turtles, genera *Elseya* and *Myuchelys* (Testudines: Chelidae) from Australia and New Guinea. *Molecular Phylogenetics and Evolution* 68, 251–258.

Legler, J.M. and Georges, A., 1993. Family Chelidae. In: Glasby, C.J., Ross, G.J.B. and Beesley, P.L. (eds.), *Fauna of Australia: Amphibia and Reptilia Fauna of Australia: Amphibia and Reptilia* Vol. 2a, pp. 142-152. Australian Government Publishing Service, Canberra.

Lu, Y., Wang, Y. and Yu, Q., 2000. Detection of herpesviral sequences in tissues of green turtles with fibropapilloma by polymerase chain reaction. *Arch Viro* Volume 1459, p. 1885–1893.

NSW Scientific Committee, 2008. Bellinger River Emydura *Emydura macquarii* (Bellinger River). Review of current information in NSW, May 2008. NSW Scientific Committee, Hurstville.

Office of Environment and Heritage, 2015. Freshwater Turtles. Office of Environment and Heritage, Sydney. http://www.environment.nsw.gov.au/animals/FreshwaterTurtles.htm.

Office of Environment and Heritage, 2015. Bellinger River snapping turtle *Myuchelys georgesi* emergency transfer plan. An emergency plan in response to the Bellinger River mortality event of February-March, 2015, Version 0.3. Unpublished.

Office of Environment and Heritage, 2015. Draft welfare protocol (handling, transport and euthanasia) for the Bellingen River snapping turtle emergency transfer program, Version 0.2, dated 30/03/15. Unpublished.

Office of Environment and Heritage, 2015. Nomination to list *Myuchelys georgesi* under the *Threatened Species Conservation Act 1995.* Unpublished.

Pacioni, C., Eden, P., Reiss, A., Ellis, T., Knowles, G. and Wayne, A.F., 2015. Disease hazard identification and assessment associated with wildlife population declines. *Ecological Management and Restoration* 16, 142-152.

Pike, D.A., Pizzatto, L., Pike, B.A. and Shine, R., 2008. Estimating survival rates of untouchable animals: The myth of high juvenile mortality in reptiles. *Ecology* 89, 607–611.

Spencer, R-J., 2001. The Murray River turtle, *Emydura macquarii*: population dynamics, nesting ecology and the impact of the introduced red fox, *Vulpes vulpes*. Unpublished thesis in fulfilment of the requirements for the degree of Doctor of Philosophy, University of Sydney.

Spencer, R-J., 2002. Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles. *Ecology* 83, 2136-2144.

Spencer, R-J., 2002. Growth patterns of two widely distributed freshwater turtles and a comparison of common methods used to estimate age. *Australian Journal of Zoology* 50, 477–490.

Spencer, R-J. and Thompson, M.B., 2002. The Bellingen River Emydura: assessing the impacts of foxes. Stage 1. October 2001. Unpublished report to NSW NPWS.

Spencer, R-J. and Thompson, M.B., 2003. The significance of predation in nest site selection of turtles: An experimental consideration of macro- and microhabitat selections. *Oikos* 102, 592–600.

Spencer, R-J., Janzen, F.J. and Thompson, M.B. 2006. Counterintuitive density-dependent growth in a long-lived vertebrate after removal of nest predators. *Ecology* 87, 3109–3118.

Tortoise and Freshwater Turtle Specialist Group, 1996. *Elseya georgesi*. The IUCN Red List of Threatened Species. Version 2014.3. www.iucnredlist.org.

Uetz, P. and Hošek, J. eds., 2015. Reptile Database. http://www.environment.gov.au/biodiversity/abrs/online-resources/fauna/afd/index.html.

van Dijk, P.P., Iverson, J., Rhodin, A., Shaffer, H.B. and Bour, R., 2014. Turtles of the world, 7th edition: annotated checklist of taxonomy, synonymy, distribution with maps, and conservation status. *Chelonian Research Monographs* No. 5, doi:10.3854/crm.5.000.checklist.v7.2014.

Wells, R.W., 2007. Some taxonomic and nomenclatural considerations on the Class Reptilia in Australia. A new genus of the family Chelidae from eastern Australia. *Australian Biodiversity Record* 2007(3), 1-12.

Williams, W.D., 1980. An Ecological Basis for Water Resource Management. Australian National University Press, Canberra.