On a razor's edge: Status and prospects of the critically endangered Bellinger River snapping turtle, *Myuchelys georgesi*

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**Abstract**

1. In the summer and autumn of 2015, the Bellinger River snapping turtle (*Myuchelys georgesi*), a narrow-range endemic of eastern New South Wales, Australia, suffered mass mortality from epidemic disease, apparently caused by a previously unknown virus. Information on the current population size and structure of *M. georgesi*, and the body condition and growth of the surviving individuals, is needed to support planning of conservation actions. Population estimates are also needed for a sympatric population of the widely distributed Macquarie turtle (*Emydura macquarii*), which has probably been introduced to the Bellinger River and may threaten the persistence of *M. georgesi* through hybridization, competition, and disease transmission.

2. Data from five turtle surveys between November 2015 and November 2018 were used to estimate populations of the two species in the Bellinger River by an analysis based on habitat extent and turtle detectability. Changes in the body condition of *M. georgesi* and the body growth of both species were also assessed.

3. Current populations of ~150 *M. georgesi* and ~500 *E. macquarii* are indicated, although the uncertainty of these estimates is high. The estimate for *M. georgesi* represents a decline of >90% from the historical population. Moreover, about 88% of the surviving *M. georgesi* are immature, and only about 5% are mature females. However, the body condition of the survivors has improved recently. Growth models suggest that *M. georgesi* matures later than *E. macquarii*, which may provide the latter with a competitive advantage.

4. Evidence presented here does not support a previous hypothesis that *M. georgesi* were predisposed to disease through malnutrition and consequently reduced immune competence caused by high water temperatures and low river flows. Continuing disease, hybridization, and interspecific competition are probably the greatest threats to the persistence of the species.

**Keywords**

alien species, disease, growth, reptiles, river
In recent decades, disease has increasingly been recognized as a cause of declines and extinctions of wildlife species globally (Daszak, Cunningham, & Hyatt, 2000; Fisher et al., 2012; Smith, Sax, & Lafferty, 2006). The most notorious example is chytridiomycosis caused by the fungi *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans*, which have devastated or exterminated numerous amphibian species around the world (Bower, Lips, Schwarzkopf, Georges, & Clulow, 2017; Fisher, Garner, & Walker, 2009; Kilpatrick, Briggs, & Daszak, 2010). However, viral infections can also afflict vulnerable species and populations, including aquatic species. For example, a ranavirus causes seasonally recurrent epidemics in populations of tiger salamanders (*Ambystoma tigrinum*) in the USA and Canada (Epstein & Storfer, 2016), and viral fibropapillomatosis has become increasingly prevalent in endangered green turtles (*Chelonia mydas*) worldwide (Jones, Ariel, Burgess, & Read, 2016). Viral infection can also cause severe mortality in captive populations of aquatic species, as with haemorrhagic disease in farmed Chinese softshell turtles (*Pelodiscus sinensis*; Liu, Cao, Lin, Ye, & Xu, 2015).

In the case of turtles and tortoises, arguably the world’s most threatened vertebrate group (Lovich, Ennen, Agha, & Gibbons, 2018), disease adds to the hazards posed by massive over-exploitation, habitat destruction, invasive species, pollution, and climate change (Klemens, 2000; Turtle Conservation Fund, 2002). The global decline of turtle and tortoise populations is greatly reducing their contributions to food webs and ecological processes, for example, as predators, herbivores, prey, disturbers of soils, and vectors and germination enhancers of seeds (Lovich et al., 2018).

In 2015, a novel nidovirus of unknown origin, now named Bellinger River virus (BRV), was apparently responsible for mass mortality of Bellinger River snapping turtles (*Myuchelys georgesi*) in the Bellinger River, New South Wales (NSW), Australia (Jakob-Hoff et al., 2017; Moloney, Britton, & Matthews, 2015; Zhang et al., 2018). *Myuchelys georgesi* (Figure 1a) is a moderate-sized, omnivorous, short-necked chelid turtle (maximum carapace length 240 mm) with an historical distribution confined to approximately 70 km of the Bellinger River and a short length of its major tributary the Kalang River (Allanson & Georges, 1999; Blamires, Spencer, King, & Thompson, 2005; Cann, 1997; Cann, Spencer, Welsh, & Georges, 2015). Since the mass mortality, it has been formally declared critically endangered by the NSW and Australian governments. It is listed as Data Deficient (under the name *Elseya georgesi*) in the IUCN Red List of Threatened Species (www.iucnredlist.org), but Critically Endangered in the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group Draft Red List (Rhodin et al., 2017).

Following the first discovery of diseased *M. georgesi* by kayakers in mid-February 2015 (in the austral summer), about 432 dead or moribund *M. georgesi*, predominantly adults, were retrieved up to April 2015 (Moloney et al., 2015). The initial outbreak was in the downstream part of the range of *M. georgesi* in the Bellinger River, and observations of affected turtles thereafter extended progressively upstream at an average rate of 1.1 km per day (Moloney et al., 2015). No disease was reported in the Kalang River.

External disease symptoms of affected turtles (Figure 1b) included lethargy, emaciation, conjunctivitis, anterior uveitis, nasal discharge, hind-limb paresis, and plaque-like skin lesions. Histopathology revealed inflammation of the eyes, oral cavity, and internal organs, including the spleen, kidneys, and liver (Moloney et al., 2015; Zhang et al., 2018). Diseased turtles were initially given veterinary care, but this failed to prevent death, and severely ill turtles were thereafter euthanized. The disease was not observed in any other species, including the Macquarie turtle (*Emydura macquarii*; Figure 1c), which also

**FIGURE 1** Photographs of (a) a healthy *Myuchelys georgesi*, (b) a diseased *M. georgesi*, (c) a healthy *Emydura macquarii*, and (d) turtle habitat in the Bellinger River. Credits: (a) Arthur Georges, (b) Rowan Simon, (c) Bruce Chessman, and (d) Gerry McGilvray.
inhabits the Bellinger and Kalang rivers. However, low levels of BRV RNA were detected in swabs from two *E. macquarii* sampled in November 2015 (Zhang et al., 2018). Although BRV appears to have been the principal aetiological agent, confirmation of its lethality to *M. georgesi* through experimental transmission studies has not been possible because *M. georgesi* is Critically Endangered (Zhang et al., 2018).

By April 2015, it was apparent that *M. georgesi* was at imminent risk of extinction in the wild. At this time the former NSW Office of Environment and Heritage (OEH) and partners removed 17 healthy mature and immature *M. georgesi* from the upper reaches of the Bellinger River, where the disease had not yet reached, to form a captive insurance population, now housed at Taronga Zoo, Mosman, NSW. An additional turtle removed from the Kalang River in April 2015 was identified as a hybrid of *M. georgesi* and *E. macquarii* and therefore was not included in the insurance population. Subsequently, in November 2016, a batch of 19 immature *M. georgesi* was removed from lower reaches of the Bellinger River to form a second insurance population at the Symbio Wildlife Park, Helensburgh, NSW. The first population is breeding, and 10 of its progeny were released into the Bellinger River in November 2018.

Historically, the population size of *M. georgesi* in the Bellinger River was estimated at 4,468 ± 1,409 individuals (mean ± SE) by capture-mark-recapture (CMR) analysis of data obtained in 1988–1991 and 2000–2004 (Blamires et al., 2005). The population size of *E. macquarii* was not estimated at this time but was probably about 100, as only 11 *E. macquarii* were captured compared with 466 *M. georgesi*. Subsequently, *E. macquarii* apparently became more common, with Spencer, Georges, and Welsh (2007) recording 360 *M. georgesi* and 76 *E. macquarii* in a survey in 2007. However, 26 of the *E. macquarii* were caught by dip netting from a boat at night, a capture method not employed by Blamires et al. (2005). Density estimates from surveys in 2007 and 2014 suggested an *M. georgesi* population half the size calculated by Blamires et al. (2005); that is, about 2,200 individuals (Spencer et al., 2018). The population size of *M. georgesi* immediately before the disease outbreak is unknown.

Knowledge of the size of the residual post-disease population of *M. georgesi* in the Bellinger River, and the body condition and growth of the survivors, is critical for planning conservation actions. For example, poor body condition as a result of malnutrition can reduce immune competence in freshwater turtles and render them more susceptible to infection (Borysenko & Lewis, 1979, though also see Polo-Cavia, Engstrom, López, & Martín, 2010). Here, data from multiple sources are used to estimate the current populations of both *M. georgesi* and *E. macquarii* in the Bellinger River, and to document the growth rates of both species and changes in the body condition of *M. georgesi* over time. Relationships between body condition and environmental variables are also explored to test the hypothesis that the body condition of *M. georgesi* is adversely affected by high temperatures and low river flows (Spencer et al., 2018).

## METHODS

### Study area

The Bellinger River (Figures 1d, 2) rises on the Great Dividing Range in eastern NSW (30.5°S, 152.4°E), flows generally eastward for 109 km to the Tasman Sea, and is not impounded. Its catchment area, including that of its main tributary the Kalang River, is 1,100 km² (Reinfelds, Cohen, Batten, & Brierley, 2004). The two rivers meet in the saline estuary, so freshwater turtle movements between them are unlikely at present sea levels. The upper catchment and steeper slopes in the lower catchment are mostly covered with native forest, whereas the lower slopes and river flats have mainly been converted to grazing pasture. Upstream of the tidal limit in the town of Bellingen, the Bellinger River consists of alternating rocky rilles and pools with both stony and sand–silt substrata. Since European settlement of the valley commencing in the 1840s, and associated deforestation, the river has undergone some channel straightening, bed incision, reconfiguration of rilles and pools, and up to a trebling of width (Cohen, 2003). The basin has a subtropical climate with average annual rainfall ranging from 1,500 to 2,200 mm (Reinfelds et al., 2004). Mean maximum air temperatures at Bellingen range from 20.0°C in July to 29.8°C in January and mean minima from 4.8°C in July to 18.3°C in February (www.bom.gov.au).

### Data sources

No information on water temperatures before or during the disease outbreak could be located. Air temperature data for Dorrigo (19 km north-east of the upstream limit of *M. georgesi* in the Bellinger River) and Coffs Harbour (27 km north-east of the downstream limit) were obtained from the Australian Bureau of Meteorology (www.bom.gov.au). These are the closest stations to the Bellinger River for which climate data are available for the entire period of interest. Data on flow of the Bellinger River at Thora (about midway along the distribution of *M. georgesi*) were obtained from WaterNSW (realtimedata.waternsw.com.au/water.stm).

Turtle data and associated environmental measurements were obtained mainly from surveying in the Bellinger and Kalang rivers by OEH and supporting organizations and individuals between November 2015 and April 2019. For survey purposes, the length of the Bellinger River from which turtles have historically been recorded has been divided into four sections (Table 1; Figure 2). The distribution of known and possible turtle habitat in each of these sections has been mapped from a combination of survey experience, aerial imagery, and on-ground reconnaissance of selected areas. This habitat excludes areas that are considered too fast-flowing and shallow for turtles to occupy except transiently when migrating. The known and possible habitat has been divided into 143 reaches of varying sizes, mostly delimited by the upstream and downstream ends of deep pools.
OEH led five major surveys in November 2015, March 2016, November–December 2016, November 2017, and November 2018. The second of these included six reaches on the Kalang River, whereas the remainder were confined to the Bellinger River. Initially, the survey objective was to locate as many epidemic survivors as possible with available resources. Therefore, for the first survey, in November 2015, the survey reaches were selected primarily according to accessibility and local knowledge of areas where turtles had been observed in the past. For the next two surveys, in March 2016 and November–December 2016, coverage of the Bellinger River was progressively expanded by resurveying many reaches surveyed previously and including additional ones where landowners made access available. For the surveys in November 2017 and November 2018, planned survey reaches were selected randomly in the Darkwood and Thora sections from reaches of known or possible habitat, whereas in the Bellingen section the aim was to survey all habitat reaches because previous surveys had shown that turtle densities were higher there. However, planned reaches were not always surveyed because of access constraints, time limitation, poor underwater visibility, presence of sharks, or lack of suitable habitat. In many of these cases, alternative reaches were substituted for those originally selected. The upstream Brinerville section was included in the major surveys only in November 2015 (Table 1). In addition to the five major surveys, turtles were captured for various purposes in October 2016, February 2017, April 2018, November–December 2018, and March–April 2019.

Of the turtle captures between November 2015 and April 2019, 97% were by divers equipped with masks, snorkels, and fins. The number of divers and dive times varied among reaches according to available personnel, time constraints, and divers’ perceptions of the time needed to search a reach thoroughly. In a few cases, multiple reaches were surveyed jointly and the catches were combined. The remaining turtles were caught with ‘cathedral’ traps baited with sheep liver (telescoping vertical cylindrical nets ~1 m wide and 2 m high when fully extended, constructed of 25 and 50 mm stretched mesh, with three entrance funnels near the base) and unbaited fyke nets (~1 m high and 3 m long, with two wings 5 m long, constructed of 25 mm stretched mesh). Commencing in November 2017, water transparency in surveyed reaches was measured with a Secchi disc extended horizontally.

Captured turtles were identified morphologically as *M. georgesi*, *E. macquarii*, or apparent hybrids of the two species. Mature and near-mature males were recognized by their enlarged tails. Before being released near the point of capture, turtles were examined for

<table>
<thead>
<tr>
<th>Section</th>
<th>Length (km)</th>
<th>Reaches</th>
<th>Reaches surveyed</th>
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<tbody>
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<td>Brinerville</td>
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<td>Darkwood</td>
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<td>Thora</td>
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<tr>
<td>Bellingen</td>
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**Table 1** Sections of the Bellinger River surveyed for turtles, listed from upstream to downstream. The total number of known or possible habitat reaches and the number of reaches included in each major survey are also shown.

**Figure 2** Map of the Bellinger River and surrounding areas showing the survey sections of the Bellinger River (Brinerville, Darkwood, Thora, and Bellingen) and the Kalang River (Kalang)
external abnormalities, measured with vernier calipers for straight-line medial carapace length (SCL) and plastron length (PL) to the nearest 0.1 or 1 mm, and weighed with digital scales to the nearest 1 or 5 g. In some cases, the inguinal cavities of large females were palpated to determine whether shelled eggs were present in the oviducts. Most turtles were marked with unique combinations of notches in marginal scutes so that they could be identified if recaptured, but in November 2015 the turtles <100 mm SCL were not marked. From March 2016 onwards, passive integrated transponder tags were injected into the body cavities of turtles >100 mm SCL. Captured turtles were scanned for tags in subsequent surveys to supplement scute marking as a means of recognizing recaptures.

Additional data, including turtle sex, SCL, PL and mass M were obtained from surveys in the Bellinger River in March–April and October 2007 as part of a doctoral study of *Myuchelys* spp. (Fielder, 2010). Curved carapace length, PL, and M were recorded in March 2015 for numerous diseased turtles collected under licence issued by OEH and measured at a control centre established in Bellingen to manage responses to the outbreak. SCL, PL, and M of the healthy turtles removed from the Bellinger River in April 2015 for the first insurance population were determined by personnel at Western Sydney University.

Approvals for turtle capture and handling at various times were issued by the NSW National Parks and Wildlife Service, the NSW Department of Primary Industries, and the animal ethics committees of the NSW Office of Environment and Heritage, Taronga Conservation Society Australia, University of New England, University of New South Wales, and Western Sydney University.

### 2.3 Size at maturation

Turtles that were likely to be mature were distinguished from immatures by body size and the presence or absence of enlarged tails. However, recognition of mature turtles was constrained by the limited available information on size at maturation. Blamires et al. (2005) reported a minimum SCL of 154 mm for gravid female *M. georgesi*, but the maximum size of immature females, the mean size at female maturation, and the minimum, mean, and maximum sizes at male maturation are unknown for this species. In the absence of such information, *M. georgesi* with SCL ≥ 154 mm and without enlarged tails were assigned as mature females. *Myuchelys georgesi* with SCL ≥ 140 mm and with enlarged tails were assigned as mature males, because all but one *M. georgesi* with enlarged tails captured between November 2015 and April 2019 had an SCL ≥ 130 mm, and enlarged tails develop before full maturation in Australian chelid turtles (Chessman, 1978).

Data are also unavailable on size at maturation for male and female *E. macquarii* in the Bellinger River. However, linear regression of data in Judge (2001) showed that, among river systems, SCL of *E. macquarii* at maturation is strongly related to maximum SCL for both males and females ($R^2 = 0.92$ and 0.95 respectively). These relationships were used, therefore, to estimate SCL at maturation for Bellinger River *E. macquarii* as 151 mm for males and 167 mm for females. Of all captures between November 2015 and April 2019, the smallest Bellinger River *E. macquarii* with an enlarged tail had an SCL of 129 mm, but recapture data showed that tail enlargement did not occur until an SCL above 145 mm in some males. The smallest Bellinger River *E. macquarii* with oviducal eggs detected by palpation in all captures between November 2015 and April 2019 had an SCL of 174 mm. These findings are compatible with the maturation sizes estimated by regression.

For turtles without SCL measurements, SCL was estimated from a linear regression of SCL on PL for each species ($R^2 = 0.99$ in both cases), or from the SCL:PL ratio of the same turtle when captured on another occasion.

### 2.4 Population estimates

Post-disease population sizes for *M. georgesi* and *E. macquarii* were estimated for each major survey. Turtles were assigned as either *M. georgesi*, *E. macquarii*, or apparent hybrids according to the morphological identification at the time of surveying. However, some turtles identified morphologically as *M. georgesi* or *E. macquarii* may actually be F1 hybrids or back-crosses of F1 hybrids to either species (Georges, Spencer, Kilian, Welsh, & Zhang, 2018).

Population estimation by CMR methods was not appropriate because sampling was not random, even in the later surveys. The same reaches were resurveyed substantially more often than expected by chance; consequently, marked turtles had a greater probability of recapture than unmarked turtles, because turtle movements among reaches were limited. Thus, the critical assumption underlying CMR methods of population estimation, that marked and unmarked animals have equal probability of capture, was not met.

Instead, population estimation was based on habitat extent and turtle detectability; that is, the probability that a turtle is captured by divers if it is present in a survey reach. Probability of capture was estimated as follows. In March 2016, one reach (chosen subjectively) was resurveyed 1 day after the initial survey. In November 2017, two reaches (also chosen subjectively) were resurveyed 8–9 days after the initial survey. In April 2018, seven reaches (chosen randomly from reaches in which *M. georgesi* had previously been detected) were resurveyed 1–2 days after the initial survey. Data from these resurveying exercises were combined and used to estimate probability of capture $\hat{p}$ of each species with the following equation (Thompson, 2012, p. 271):

$$\hat{p} = \frac{X}{\bar{X}}$$

where $X$ is the number of turtles captured and marked in the initial surveys and $\bar{X}$ is the number of those marked turtles that were reca-
 captured in the repeat surveys. The variance (var) of $\hat{p}$ was estimated with the following equation (Thompson, 2012, p. 271):
\[ \text{var}(\hat{\tau}) = \frac{N^2}{p^2} \left( \frac{N-n}{N} \right)^2 \left( \frac{1-p}{N} \right) \bar{y} + \frac{y^2}{p^2} \text{var}(\hat{p}) \]

where \( y \) is the total number of turtles captured on resurveying and \( \hat{\tau} = y/p \).

Population estimates for each species, survey, and river section were made with the following equation (Thompson, 2012, p. 221):

\[ \hat{\tau} = \frac{N\bar{y}}{p} \]

where \( \hat{\tau} \) is the estimated population in the river section, \( N \) is the number of known or possible habitat reaches in the river section, \( \bar{y} \) is the mean number of turtles captured in those reaches that were surveyed (snorkelling captures only), and \( \hat{p} \) is the estimated probability of capture, calculated as already described.

The variance (var) of \( \hat{\tau} \) was estimated with the following equation (Thompson, 2012, p. 221):

\[ \text{var}(\hat{\tau}) = \frac{N^2}{p^2} \left( \frac{N-n}{N} \right)^2 \left( \frac{1-p}{N} \right) \bar{y} + \frac{y^2}{p^2} \text{var}(\hat{p}) \]

where \( n \) is the number of habitat reaches surveyed in the river section, \( s \) is the standard deviation of the number of turtles recorded per reach surveyed, and other symbols are as already described. This variance was used to derive 95% confidence limits for \( \hat{\tau} \). In occasional cases when multiple reaches were surveyed jointly, the catches were divided as equally as possible among the constituent reaches before calculation of the standard deviation. This practice probably resulted in a slight underestimation of the true standard deviation.

### 2.5 | Body condition

The relative body condition (RBC) of individual \( M. \) georgesi was calculated by dividing observed \( M \) by the \( M \) value predicted from a regression of \( M \) on PL, of the form \( M = aPL^b \), where \( a \) and \( b \) are constants. A condition value >1 thus signified an \( M \) value higher than expected for the turtle’s PL. Separate regressions were calculated for mature males, mature females, and immatures, considering all available data for turtles that appeared healthy. A few turtles with outlying positions on scatter plots of \( M \) versus PL were excluded from the analysis because they were strongly suspected to have measurement errors. PL was used rather than SCL because the latter was not available for the diseased turtles.

Comparison of the mean and variance of PL and RBC between diseased and healthy \( M. \) georgesi measured and weighed in March–April 2015 was undertaken using Student’s t-tests and Fisher’s F-tests. Analysis of variance (ANOVA) was used to compare the RBC of \( M. \) georgesi among all periods with data for at least 15 individuals, excluding diseased turtles in March 2015. To test the hypothesis that body condition is depressed by high temperatures and low flows, the average RBC for healthy \( M. \) georgesi in each period with data for at least 15 individuals was correlated with the mean and maximum daily maximum air temperatures at Dorrigo and Coffs Harbour for intervals of 30, 90, and 180 days before each period’s starting date, as well as with the minimum and mean river flow at Thora for the same antecedent periods. Flow statistics were transformed to natural logarithms to reduce skew. Statistical tests were performed with XLSTAT (Addinsoft, 2019).

### 2.6 | Growth

Differences in SCL between the first and last captures of recaptured Bellinger River \( M. \) georgesi and \( E. \) macquarii, with a separation of at least 90 days, were used to fit von Bertalanffy growth models to each species by Fabens’ method with Growth II software (Henderson & Seaby, 2006). Growth was assumed to cease during winter (June–August) because activity and feeding of freshwater turtles in south-eastern Australia are greatly reduced at that time (Chessman, 1988; Fielder, 2012). SCL at age 0 (hatching) was set to 30 mm for \( M. \) georgesi, based on data in Cann (1997), and 27 mm for \( E. \) macquarii, based on data in Judge (2001). Separate models were constructed for males and females, with immatures of unknown sex included in both models. Data were insufficient to fit credible models for \( M. \) georgesi if immatures were excluded. These models were used to estimate the ages at which male and female turtles mature with the following equation:

\[ y = \frac{\ln(1 - \frac{a}{k}) - \ln(1 - \frac{1}{k})}{h} \]

where \( y \) is age in years, \( a \) is asymptotic SCL fitted by the model, \( h \) is SCL at hatching, \( L \) is SCL at maturity, and \( k \) is the von Bertalanffy growth parameter fitted by the model.

### 3 | RESULTS

#### 3.1 | Environmental conditions

Mean daily maximum air temperatures at Dorrigo and Coffs Harbour were higher than the average for the time of year in the spring and early summer of 2014–2015, although below the highest values on record (Figure 3). These temperatures were near average during the period when dead and moribund turtles were observed in the late summer and early autumn of 2015.

Daily mean flow at the Thora gauge on the Bellinger River was sometimes low in late 2014, reaching a minimum of 0.24 m³ s⁻¹ in November (Figure 4). Lower flows occurred previously at this gauge in 1991, 1992, 1993, 1994, 2002, 2003, and 2004, but flow has never ceased since measurements began in 1982. Flows were substantially higher from December 2014 onwards, with a series of peaks before and during the period when dead and moribund turtles were discovered (Figure 4). Flows in late 2016 reached lower levels than those in 2014.
Secchi transparency averaged 3.8 m (range 2.0–6.5 m) during the November 2017 survey and 4.2 m (range 1.4–6.0 m) during the November 2018 survey. Transparency at the sites resurveyed at various times for detectability estimation averaged 4.7 m (range 2.3–8.0 m).

### 3.2 | Population composition

In 2007, before the disease outbreak, four of 112 *M. georgesi* (4%) were immature (Figure 5a). In March–April 2015, during the disease outbreak, 11 of 137 diseased *M. georgesi* (8%) were immature (Figure 5b), and one of the 17 healthy *M. georgesi* removed to form the first insurance population (6%) was immature.

The survey of the Kalang River in April 2016 captured no *M. georgesi* and 14 *E. macquarii*. For the Bellinger River, 178 turtles identified morphologically as *M. georgesi* (including the 19 removed in November 2016 for the second insurance population), 426 as *E. macquarii*, and five as apparent hybrids were marked between November 2015 and April 2019. This total may include some of 18 *M. georgesi* and three *E. macquarii* with SCL < 100 mm that were not marked when initially captured and may have been recaptured later.

Population structure in the Bellinger River as revealed by all captures between November 2015 and April 2019 was contrasting for the two species. For *M. georgesi*, there was a strong skew toward smaller turtles, with 88% of captures being immatures, 7% mature males, and 5% mature females (Figure 5c). For *E. macquarii*, the skew was toward larger turtles, with 43% immatures, 27% mature males, and 30% mature females (Figure 5d).

### 3.3 | Population size

A complication arose in the estimation of capture probability. An *E. macquarii* captured in the initial survey of one of the reaches selected for resurveying was recaptured in an adjacent reach not selected for resurveying on the same day that the first reach was resurveyed. For calculation purposes, this turtle was treated as though it had been recaptured in the first reach. The estimated capture probability was 0.59 (var = 0.01) for *M. georgesi* and 0.57 (var = 0.01) for *E. macquarii*.

No population estimates were derived for the upstream Brinerville section of the Bellinger River because, excluding released captive-bred turtles, only one individual (an *M. georgesi*) was captured from that section between November 2015 and April 2019. Estimates for the other sections (Figure 6) varied widely among surveys and often had broad confidence intervals because variability in catches among reaches was high. The earlier estimates (November 2015 and March 2016) suggest total Bellinger River populations of ~300–400 *M. georgesi* and ~600–700 *E. macquarii*, whereas the more recent estimates (November 2017 and November 2018) suggest ~150 *M. georgesi* and ~500 *E. macquarii*. 

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**FIGURE 3** Mean daily maximum air temperatures at (a) Dorrigo and (b) Coffs Harbour for each month from January 2014 to May 2015 (thick solid lines), compared with long-term averages (thin solid lines), and highest recorded values (thin dashed lines). Data from www.bom.gov.au

**FIGURE 4** Daily mean flow of the Bellinger River at Thora (logarithmic scale) from 2011 to 2019. Data from realtimedata.waternsw.com.au/water.stm
3.4 | Body condition

The following regression relationships were calculated for *M. georgesi* between *M* (g) and PL (cm) for mature males, $M = 0.2650PL^{2.9175}$ ($n = 76; R^2 = 0.92$); for mature females, $M = 0.0798PL^{3.3543}$ ($n = 81; R^2 = 0.95$); and for immatures, $M = 0.5588PL^{2.5940}$ ($n = 245; R^2 = 0.98$).

In March–April 2015, during the disease outbreak, the variance of PL did not differ significantly between diseased and healthy *M. georgesi* ($F_{1,35} = 1.11; P = 0.38$); nor did mean PL (pooled-variance $t$-test: $t_{151} = 0.71$). Diseased *M. georgesi* had a significantly greater variance of RBC ($s^2 = 0.0034$) than healthy *M. georgesi* ($s^2 = 0.0012$; $F_{95,16} = 2.77; P = 0.024$). Mean RBC was significantly lower for diseased *M. georgesi* (0.94) than for healthy *M. georgesi* (1.00; separate-variance $t$-test, $t_{34.2} = 5.40, P < 0.001$). However, 10 of 96 diseased *M. georgesi* for which *M* was available had RBC > 1.

Excluding diseased turtles, mean RBC of *M. georgesi* differed significantly among periods with data for at least 15 individuals (ANOVA: $F_{8,372} = 2.57; P = 0.010$; Figure 7). Tukey post-hoc tests showed that mean RBC was significantly higher in April 2018 than in November 2015 ($P = 0.046$). However, mean RBC of healthy turtles at the time of the disease outbreak was not significantly different from mean RBC at any other time (Tukey post-hoc tests: $P > 0.31$). Excluding diseased turtles, Pearson’s correlations between mean RBC per period and antecedent air temperatures and river flows were not statistically significant ($P > 0.23$).

3.5 | Growth

Age at maturation estimated from the fitted von Bertalanffy growth models was greater for *M. georgesi* than for *E. macquarii* for both sexes (Table 2).
FIGURE 6  Estimated population sizes of Myuchelys georgesi and Emydura macquarii in the (a, b) Darkwood, (c, d) Thora, and (e, f) Bellingen sections of the Bellinger River over five surveys (mean ± 95% confidence limits)
**Figure 7** Relative body condition of (a) immature, (b) mature male, and (c) mature female *Myuchelys georgesi* in various survey periods (mean ± SD). Black symbols are for unhealthy turtles at the time of the disease outbreak, and white symbols are for healthy turtles. Numbers are sample sizes.

**Table 2** Numbers of turtles included (*n*) and parameters (α and k) for fitted von Bertalanffy growth models (means with 95% confidence intervals in parentheses) and age at maturation estimated from the models.

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>n</th>
<th>α (mm)</th>
<th>k</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Myuchelys georgesi</em></td>
<td>Male</td>
<td>58</td>
<td>162 (133–192)</td>
<td>0.17 (0.08–0.26)</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>52</td>
<td>227 (155–300)</td>
<td>0.09 (0.03–0.14)</td>
<td>11.3</td>
</tr>
<tr>
<td><em>Emydura macquarii</em></td>
<td>Male</td>
<td>59</td>
<td>183 (175–190)</td>
<td>0.21 (0.18–0.25)</td>
<td>7.4</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>80</td>
<td>223 (211–236)</td>
<td>0.14 (0.11–0.17)</td>
<td>9.1</td>
</tr>
</tbody>
</table>
Historically, the *M. georgesi* population in the Bellinger River was dominated by mature individuals. Immatures made up 16% of the *M. georgesi* captured in 1988–2004 (Blamires et al., 2005), 4–5% in 2007 (Spencer et al., 2007; present study), and 23% in the river upstream of Thora in 1988–2008 (Blamires & Spencer, 2013). The proportion of immatures was also low among diseased turtles in 2015 (8%). The much higher proportion of immatures since the disease outbreak ended (88%) suggests differential mortality of juveniles and adults. Spencer et al. (2018) suggested that differences in diets between juveniles and adults may have resulted in different infection rates. Alternatively, juveniles may have been more resistant. It is also unknown how the disease was able to progress upstream at a rate of more than 1 km per day. It seems unlikely that infected turtles would travel upstream at this rate, but no other virus vectors have been confirmed (Zhang et al., 2018).

The method used here for population estimation relies on several assumptions: (a) that detectability in the reaches that were resurveyed to estimate capture probability was representative of detectability in all inhabited reaches; (b) that the populations of the resurveyed reaches were closed between survey and resurvey occasions; (c) that all turtles present in resurveyed reaches were equally likely to be captured; (d) that the average number of each turtle species in the habitat reaches surveyed was the same as the average number in all habitat reaches within the same river section; and (e) that numbers of habitat reaches in each river section were estimated accurately.

With regard to assumption (a), detectability probably depends substantially on water transparency, and average transparency was a little higher in the resurveyed reaches than in all reaches surveyed in November 2017 and November 2018. Consequently, average detectability may have been slightly overestimated, which would have resulted in underestimation of population sizes. Assumption (b) was clearly not always met, at least for *E. macquarii*, because one individual of that species was recaptured in a different reach on the day that the reach in which it was first captured was resurveyed. In this case the two surveys were 8 days apart, which allowed some time for turtles to move between reaches. In most cases, however, surveying and resurveying of the same reach were only 1–2 days apart. Recaptures, in general, have shown that turtles sometimes move considerable distances within the river, particularly male *E. macquarii*. However, recaptures of *M. georgesi* have generally been in the same reach or a nearby reach, especially for immatures. Hence, potential violation of assumption (b) may be of less concern for *M. georgesi*.

It is also possible that assumption (c) was not met. For example, there may be a bias in capture probability according to body size, or turtles caught on the first occasion may become wary and be more difficult to capture on the second occasion. Some individuals may also be cryptic; for example, some turtles may have hidden in benthic leaf litter, underwater rock crevices, undercut banks, or beds of aquatic vegetation and been undetectable during both surveying and resurveying, resulting in an underestimation of the number of turtles present. Assumption (d) may not have been met because the reaches surveyed were not a random sample of all habitat reaches. Adherence to assumption (e) is difficult to assess because not all reaches have been surveyed yet, and some have not even been inspected on the ground. It is likely that present estimates of the extent of turtle habitat are subject to appreciable error. Given limitations in the extent to which all assumptions were met, the population estimates should be interpreted with considerable caution. The most recent estimates of ~150 *M. georgesi* and 500 *E. macquarii* are probably the most reliable, because the recent surveys covered more reaches and with partly randomized reach selection.

The estimate of the current *M. georgesi* wild population (~150) plus the number removed for the second insurance population in November 2016 (19) totals fewer than the 178 individuals marked between November 2015 and April 2019. Moreover, it is probable that many surviving *M. georgesi* remain unmarked; for example, because many possible habitat reaches have never been surveyed. Therefore, either the estimate of ~150 *M. georgesi* is below the true figure or appreciable mortality has occurred between November 2015 and November 2018. However, even if wild *M. georgesi* currently number as many as 200, the reduction since the disease outbreak is >90% of the historical population. And even if ~400 initially survived, the speed and severity of *M. georgesi* mortality in summer–autumn 2015 is comparable to extreme cases of amphibian mortality from chytridiomycosis (Hudson et al., 2016), vastly exceeds the reported impact of previous disease outbreaks in wild Australian freshwater turtle populations (Ariel et al., 2017; Brooker & Wombey, 1986; Flint et al., 2011; Tucker, Kelly, Limpus, Priest, & Guarino, 2002), and appears to have no counterpart in turtle disease worldwide.

It might be expected that both body condition and growth rates of *M. georgesi* would now be higher than in the past because the reduction in turtle numbers in the river should have diminished competition for food. The mean body condition of *M. georgesi* does appear to have increased recently, but the trend in growth rate is unclear. The age at maturation of female *M. georgesi* estimated here, 11.3 years, is substantially later than the estimate of 7.9 years derived by Blamires et al. (2005) by the same method (von Bertalanffy modeling), implying that female growth is now slower. Blamires et al. (2005) did not report age at maturation for male *M. georgesi*, but assuming that males mature at an SCL of 140 mm, their model parameters predict maturation at about 14 years compared with 10.3 years in the present study, implying that male growth is now faster. However, the present models may have overestimated age at maturation for females and underestimated age for males because unsexed immatures were included in models for both sexes, and faster juvenile growth has been reported for females than for males in other Australian chelid turtles (Kennett, 1996). Averaged for both sexes, the estimated ages at maturation are little different from ages based on Blamires et al. (2005).

Spencer et al. (2018) suggested that *M. georgesi* may have been predisposed to pathogen impact by malnutrition, and consequently reduced immune competence, caused by unusually high temperatures and low river flows in the months before the disease outbreak. They
reported that the healthy \textit{M. georgesi} removed for the first insurance population at the time of the outbreak gained substantial mass after 6 months in captivity, inferring that these animals were in poor condition when captured. However, the analysis reported here shows that the average body condition of these animals when captured was not significantly different from the average condition of wild \textit{M. georgesi} both long before the disease outbreak and subsequent to it. Moreover, it is commonplace for captive animals to have greater mass and fat reserves than their wild equivalents, because captive animals are often provided with abundant nutritious food and do not have to expend energy in foraging (Araújo et al., 2000; Kirkwood, 1991; Turner, Cramer, Nisbett, & Gray, 2016).

The average body condition of diseased \textit{M. georgesi} retrieved during the outbreak was significantly lower than that of the healthy \textit{M. georgesi} removed for the first insurance population, in keeping with reports of diseased turtles being in poor condition or emaciated (Moloney et al., 2015; Zhang et al., 2018). However, the condition of the diseased turtles was more variable, with several being in better condition than expected. These results are consistent with emaciation being a progressive consequence rather than a cause of the disease. Mass loss could have resulted from unhealthy turtles being unable to feed and expending energy in struggling to maintain position in the river, particularly during the high flows that occurred in the summer of 2014–2015. It could also have resulted from evaporative water loss during protracted resting on river banks, where many diseased turtles were found.

The analyses reported here do not lend support to the hypothesis that the body condition of \textit{M. georgesi} is adversely affected by high temperatures and low flows. The body condition of healthy \textit{M. georgesi} was not correlated with antecedent maximum air temperatures, and although regional air temperatures preceding the disease outbreak were high for the time of year, they were not extreme, being within the range of historical variability. Higher temperatures are usually beneficial to freshwater turtles, because warmer waters tend to have greater food productivity, or allow a longer growing season and more rapid food digestion (Ashton, Bettaso, & Welsh, 2015; Frazer, Greene, & Gibbons, 1993; Gibbons, 1970; Thornhill, 1982). However, Spencer et al. (2018) suggested that high temperatures in the Bellinger River in the summer of 2014–2015 might have depressed dissolved oxygen concentrations, which could have had adverse impacts on turtles or their food. It is also conceivable that summer temperatures promoted viral replication (Zhang et al., 2018). Unfortunately, data on water temperatures and dissolved oxygen concentrations before and during the disease outbreak are not available.

The analysis reported here did not find a relationship between body condition of \textit{M. georgesi} and antecedent minimum or mean river discharge. River turtles may have poorer body condition when flows are lower or less frequent because of reduced food supply and opportunities for foraging (Bondi & Marks, 2013; Howard, Beesley, Ward, & Stokeld, 2016). However, high river flows or velocities can also impair turtle populations (Lenhart, Naber, & Nieber, 2013; Usuda, Morita, & Hasegawa, 2012). Although flows in the Bellinger River were sometimes low in spring 2014, the river did not cease to flow or fall below historical low flows. Any loss of turtle habitat in response to flow reduction before the disease outbreak would have been much less than in the studies of Bondi and Marks (2013) and Howard et al. (2016), which respectively involved a spatial comparison between a perennial and an intermittent river and a before–after study of an environmental flow delivered to a receding refuge pool in a floodplain channel during drought.

Recovery of \textit{M. georgesi} in the Bellinger River is a daunting proposition. Natural recruitment is limited by the paucity of adult females (present study) and nest predation rates of ~70% (Blamires et al., 2005). Moreover, in other freshwater turtle populations, natural recovery after major mortality has been slow or negligible, even with substantial numbers of adults remaining. For example, a population of northern map turtles (\textit{Graptemys geographica}) in the USA took 27 years to recover after a period of harvesting in which abundance declined by ~50% (Pitt & Nickerson, 2013), and there was no recovery of a common snapping turtle (\textit{Chelydra serpentina}) population in Canada 23 years after loss of 39% of nesting females to predation by otters (Keevil, Brooks, & Litzgus, 2018). The typical freshwater turtle life-history characteristics of low reproductive success, slow growth rates, and long generation times all militate against rapid population recovery (Howey & Dinkelacker, 2013).

Head-starting (i.e. the release of juvenile turtles that have been bred and reared in captivity to a size at which they are less vulnerable to predators) is a potential means of increasing the wild \textit{M. georgesi} population. Release of head-started \textit{M. georgesi} has commenced, and their post-release survival, health, movements, and growth are being monitored. A crucial question is whether continuing deaths of \textit{M. georgesi} from viral disease or other factors will outweigh population increases through releases of captive-bred individuals and any natural recruitment. Continued monitoring and a better understanding of the aetiology of the disease are needed to answer this question.

\textit{Emydura macquarii} was not known from the Bellinger River system before its discovery in 1990 in a single waterhole, and its diversity of mitochondrial haplotypes in the Bellinger and Kalang rivers, some with apparently restricted distributions at the time of detection, suggests that its presence is due to introduction by humans at multiple locations and times (Georges et al., 2011). This species hybridizes with \textit{M. georgesi} in both the Bellinger and Kalang rivers, and the discovery of back-crosses to both parental species indicates that at least some hybrids are fertile (Georges et al., 2018; Georges, Walsh, Spencer, Welsh, & Shaffer, 2007). Because adult \textit{M. georgesi} are now rare and sporadic, they may tend to mate with the more abundant \textit{E. macquarii} and produce further hybrids, threatening \textit{M. georgesi} with eventual genetic swamping (i.e. replacement by hybrids). It is also possible that \textit{E. macquarii} could act as a vector for disease transmission to \textit{M. georgesi}, because BRV has been detected in \textit{E. macquarii}, although only at low levels that might have resulted from superficial contamination from the environment (Zhang et al., 2018). Furthermore, the ages at maturity estimated here suggest that \textit{E. macquarii} may have a reproductive advantage over \textit{M. georgesi} through earlier adulthood. In the long term, an ever-increasing population of \textit{E. macquarii} may
outcompete M. georgesi for resources, as the two species have similar diets and habitat preferences (Spencer, Georges, Lim, Welsh, & Reid, 2014). The situation in the Kalang River, where E. macquarii greatly dominates and genetically pure M. georgesi have not recently been found, may presage the future in the Bellinger River without management intervention (Georges et al., 2018).

To date, attention to introduced freshwater turtles in Australia has focused on the red-eared slider, Trachemys scripta elegans (Burgin, 2007; Mo, 2019). This sub species is native to the south-eastern USA but is now widely distributed in North America beyond its natural range and throughout the world (Kikillus, Hare, & Hartley, 2010). It is the invasive turtle of greatest concern globally, with a suite of adverse impacts on native turtles and other species (Ficetola, Rödder, & Padoa-Schioppa, 2012; Polo-Cavia, López, & Martin, 2014; Ramsay, Ng, O’Riordan, & Chou, 2007). Removal of non-native slider populations has been achieved or attempted in Australia (O’Keefe, 2009), Europe (Valdeón, Crespo-Díaz, Egaña-Callejo, & Gosá, 2010), and the USA (Drost, Lovich, Madrak, & Monatesti, 2011), but confirming eradication can be difficult (García-Díaz et al., 2017). Though T. scripta elegans is a widely recognized biosecurity risk in Australia, the consequences of translocating turtle species that are native to the continent have received little attention. Consequently, any options for the management of E. macquarii in the Bellinger River and its tributaries would warrant detailed consideration and consultation.

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REFERENCES


