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The efficacy of protecting turtle nests as a conservation strategy to reverse population decline

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ABSTRACT

A common strategy used to conserve turtles is to increase the number of hatchlings entering the population by protecting the nests. Typically, nest protection programs require regular and systematic abundance surveys conducted over lengthy periods between oviposition and sexual maturity (20+ years) to assess if this conservation strategy is effective in recovering the population. Here, we describe a methodology that dramatically reduced the monitoring period (3 years) required to assess the impact of nest protection upon a threatened freshwater turtle (*Elusor macrurus*). An age-at-length curve was developed to define the size of turtles produced within the nest protection program period (15 years) and mark-recapture models were used to compare population demographics between areas with and without a nest protection program. Unexpectedly, the study revealed substantially fewer turtles younger than 15-years of age in areas with nest protection compared to areas where no nest protection program existed. The reason for the lack of recovery was due to a significantly lower number of nesting females and a much lower rate of survival from hatching through to the sub-adult stage within the area of the nest protection. Consequently, for *E. macrurus,* the nest protection program did not result in localised population recovery and in-stream mortality may be an equal if not greater threat to *E. macrurus* in this part of their geographical range. The study presents a relatively rapid methodology to assess the effectiveness of turtle nest protection programs and diagnose the reason for ongoing population declines.

1. Introduction

Monitoring is fundamental to assess the efficacy of conservation actions and evaluate the success of an intervention (Legge et al., 2018). It is integral for ensuring that actions are appropriate for improving the trajectory of the population and contribute to ongoing refinement (Woinarski et al., 2017). For long-lived species, the lengthy period between birth/oviposition and sexual maturity makes the assessment of population trends logistically and financially challenging, and instead for these species, measuring a change in number of individuals at a particular life-stage is the most commonly used approach (Bennett et al., 2017; Legge et al., 2018).

Turtles are long-lived animals and whilst this makes it challenging to accurately assess population trends (Balazs and Chaloupka, 2004), there is a general consensus that the taxon is in a state of decline across the globe (Hoffmann et al., 2010; TCC, 2018) despite widespread conservation efforts (TCF, 2002). The International Union for Conservation of Nature (IUCN) reports that 45% of the 263 identified species of freshwater and terrestrial turtles are either threatened or endangered (IUCN, 2016), and as a group, Chelonians are at higher risk of extinction than birds, amphibians, elasmobranches, and mammals (Hoffmann et al., 2010). The reasons for turtle declines have been attributed to pollution, habitat change, bycatch, and overharvesting (Pritchard, 2007), with high rates of egg predation been documented as one of the most pressing causes of population decline for many species (Thompson, 1983; Tomillo et al., 2008; Spencer et al., 2016; Van Dyke et al., 2019).

A commonly used turtle conservation strategy is to protect the nests

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from predation (Wibbels, 1990; Bowen et al., 1994; Haskell et al., 1996; Yerli et al., 1997; Bell et al., 2005; Mitrus, 2005; Haegen et al., 2009; Moncada et al., 2010; Bona et al., 2012; Chan, 2013; Burke, 2015). The assumption being that a greater number of hatchlings being recruited into the population will result in more turtles reaching reproductive age and ultimately halt or reverse ongoing population declines. Whilst there is good data to support that nest protection programs have direct impacts upon increasing the number of hatchlings entering the environment, there is far less information about how these programs influence the number of individuals reaching reproductive age, and reduce chronic rates of decline (Crouse et al., 1987; Dodd and Seigel, 1991; Bennett et al., 2017). The studies that have assessed the efficacy of nest protection upon the breeding population have required data to be frequently collected over very long ($>$ 30 years) periods (Balazs and Chaloupka, 2004; Dutton et al., 2005; Bell et al., 2020). Collecting, storing, and maintaining the integrity of datasets over such long time periods is challenging, and therefore, they don't exist for many species of turtle where nest protection is being used as the primary conservation strategy.

Here, we aimed to assess the efficacy of nest protection as a conservation strategy for a threatened species of freshwater turtle using a much shorter and manageable monitoring window (3 years). For this, we developed an age-at-length curve to define the size of turtles produced within a nest protection program period ($<$ 15 years of age) and integrated this within a mark-recapture population model to compare areas within a long-term nest protection program against areas without a similar strategy in place. The species of study was the Mary River turtle (*Elusor macrurus*), currently listed as endangered by the IUCN red list and under Australia's EPBC Act (EPBC, 1999; IUCN, 2016). This species is one of the largest Australian short-necked Chelids (Cann, 2017) and is endemic to the Mary River catchment (Queensland, Australia). *Elusor macrurus* eggs were collected in high numbers (~2000 per year; Flakus, 2002) throughout the lower reach of the Mary River during the 1960 and 70's for the pet trade, but ceased following the introduction of the Fauna Conservation Act (Cann, 2017). Over the intervening years, there has been a lack of population recovery and managers have attributed this to predation of the clutches by native and introduced predators (e.g. monitor lizards, foxes, wild dogs) and the trampling of nests by livestock (Flakus, 2002; Limpus, 2008). In direct response, a community-driven nest protection program was initiated in 2001. This nest protection scheme was focused in the lower reach of the Mary River, whilst the species also inhabits the upper catchment, with little opportunity for movement between the two river stretches during baseflow. This provided the opportunity to test the efficacy of protecting turtle nests as a conservation strategy to reverse population decline. We hypothesized that in the lower reach of the Mary River, a significantly greater proportion of the population would be comprised of young turtles compared to areas not protected by the program. However, the study findings were unexpected and highlight the value of assessing population demographics for refining conservation actions.

2. Methods

2.1. Nest protection program

The nest protection program (2001 to 2016) was carried out by a community group (Tiaro & District Landcare Group) throughout the lower reach of the Mary River (QLD, Australia; Fig. 1). The protocol involved early morning patrols during nesting season along the riverbank in areas where female *E. macrurus* were known to nest (Fig. 1; Flakus, 2002; Micheli-Campbell et al., 2013a; Cann, 2017; Espinoza et al., 2018). *Elusor macrurus* nesting occurs annually (October to January) under the darkness of the night following rainfall events (Micheli-Campbell et al., 2013a; Cann, 2017). Once a nest was visually located, a small excavation was conducted to confirm the presence of eggs, causing as minimal disturbance as possible. Once eggs were confirmed, each nest was protected with a flat plastic mesh (1 × 1 × 0.005 m; mesh size 10 × 10 cm) secured by 8 × 300 mm polypropylene sand pegs. Each nest was then numbered sequentially. The mesh size ensured that emerging hatchlings could pass through it. Nests were excavated following hatching (December–February). The number of hatched eggshells was then used to estimate the number of hatchlings that emerged from protected nests. Hatched eggs were determined as shells that were empty and had evidence of hatching (i.e. ribboning effect of the shell from the turtle egg tooth) and that lacked evidence of a deceased embryo or egg yolk.

2.2. Mark-recapture

Turtle capture was undertaken in two separate study areas, each stretch was ~50 km in length. Study area 1 was located in the lower reach (nest protection program area) and study area 2 in the upper reach of the Mary River (Fig. 1). Approximately 65 km linear river distance and a stream gauging weir (Gympie, QLD) separate the two study areas (Fig. 1). Six sampling events (6 months apart) were conducted within each study area during April (spring) and October (autumn) from 2015 to 2017. Turtles were captured in unbaited set nets. For each sampling event, ten nets were set over four consecutive nights within each sampling area (total 480 trap nights). Sampling protocols were replicated identically across all sites and sampling events, and an effort was made to standardise trapping protocol, and net placement and spacing for both study areas. Nets were set upstream of riffle zones, in areas of similar river topography and where water depth and flow rate were comparable. Each net consisted of a 4 m funnel with a 0.4 m opening and two 10 m long wings. Nets were set facing upstream and parallel to the riverbank. Location and time-period were replicated on subsequent sampling episodes so that biases were consistent throughout the study (for details of trapping methodology see Connell et al., 2018).

The nets were checked each morning, the bycatch recorded and released, and turtles processed on the adjacent riverbank. Morphological measurements (straight carapace length - SCL and weight) were taken for each captured *E. macrurus*. The sex of each individual was visually determined (male, female, or juvenile/unsexed). *Elusor macrurus* exhibits visually distinctive sexual dimorphism, as the tail of the male is substantially larger than the female in relation to carapace length (Cann and Legler, 1994; Flakus, 2002). The individuals that could not be confidently sexed in this manner due to their small sizes were considered as juveniles in the analysis (Table 1).

Each turtle was scanned with a Pocket EX reader (Biomark Inc., Idaho, USA). Unmarked individuals were tagged with individually identifiable tags. A single-use All-In-One applicator (Trovan® Ltd., UK) was used to insert an FDX-B PIT-tag (1.8 mm × 30 mm) through the muscle layer into the right-anterior inguinal region (Buhlmann and Tuberville, 1998; Hamann et al., 2008). Supplementary identification methods were employed to ensure that a recaptured turtle was positively identified. First, a 2 mm^2 section of tissue was taken from the webbing on the hind right foot. This did not grow back during the 3-year study and ensured identification that a turtle was a recapture in the absence or failure of the PIT tag. Second, high-resolution photographs were taken of the carapace and plastron of each turtle. These were stored with the turtle PIT-tag ID, location, and date of capture to verify a recaptured individual's identity in case of a PIT-tag loss/failure. Less than 3% of all recaptures required this supplementary method of identification. Following processing, each turtle was released downstream of the set-net in which they were captured to minimise the chances of immediate recapture.

2.3. Age estimation

In many reptile species, age is often accurately estimated using skeleton-chronological approaches by means of analysing annuli on epidermal scutes (Spencer, 2002). However, without proper validation,

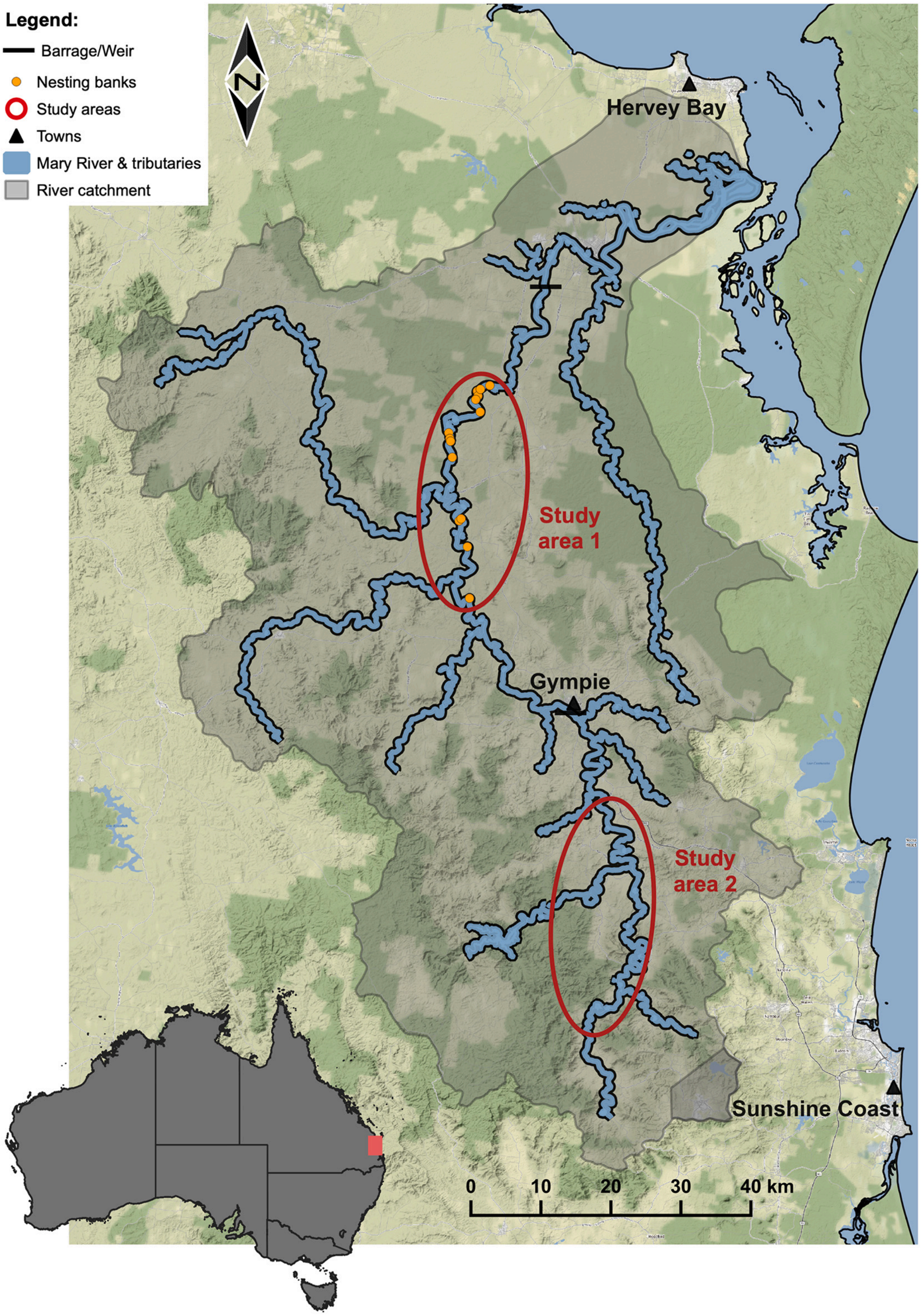

Fig. 1. Location of the two study areas (red circles) within the Mary River catchment (Queensland, Australia). Ten set nets were deployed above riffles throughout each study area per trapping/sampling episode (exact netting locations are not displayed at the request of landholders). Orange dots indicate the location of *E. macrurus* nesting areas (sandy riverbanks), where the community-driven *Elusor macrurus* nest protection program operated between 2001 and 2016. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

growth annuli can be unreliable in providing accurate age estimates in turtles and have been considered an inaccurate means of estimating age for *E. macrurus* (Cann, 2017). Instead, we established a length-at-age relationship for this species using repeated records of age and length from known aged individuals (n = 43; Table S1 in Supplementary Data). Sufficient replicate data was not available to construct sex-specific length-at-age relationships; therefore, a single species-level relationship was constructed. Length-at-age relationships were formulated using three well established models: von Bertalanffy, Gompertz, and Logistic growth models (Table 2). A multi-model approach was used to compare the three formulations with best fitting models assessed using an information theoretic approach (i.e. Akaike

Table 1
Number of captured (mark) and recaptured (recapture) *Elusor macrurus* per sex and study area (study area 1 = lower reach – nest protection area; study area 2 = upper reach of the Mary River, QLD, Australia).

Sex	Study area 1		Study area 2		Total	
	Mark	Recapture	Mark	Recapture	Mark	Recapture
Juvenile	1	0	17	0	18	0
Female	35	9	78	11	113	20
Male	103	17	124	24	227	41
Total	139	26	219	35	358	61

Information Criterion, AIC; Burnham and Anderson, 2002). Model comparisons were conducted in the R statistical environment (version 3.6.1; R Core Team, 2019) using the '*AquaticLifeHistory*' package (Smart, 2019a). The best fitting model (von Bertalanffy) was used to construct the length-at-age curve with confidence bands (Fig. 2) using a Bayesian Markov chain Monte Carlo process using the '*BayesGrowth*' R package (Smart, 2019b). The best fitting length-at-age model was used to estimate the age of all individuals encountered during the mark-recapture program. The estimated ages were subsequently used to classify individuals that were likely hatched prior to (i.e. > 15 years) and during (i.e. < 15 years) the period when the nest protection program was operating.

2.4. Population estimates

Mark-recapture data for *E. macrurus* was used to generate population estimates using the '*RMark*' package (Laake, 2013) in R (R Core Team, 2019). The POPAN formulation of the Jolly-Seber model for a closed population was used to estimate *N* (the size of super-population, i.e. total number of individuals present within the population during the entire study period) for 4 attribute groups (Pollock et al., 1990). The assumption of closed populations (i.e. turtles have an invariable chance of being recaptured within the study area of initial capture, unless they die) was based on previous telemetry studies of *E. macrurus* showing that both the adults and early life-stages do not travel long distances (Micheli-Campbell et al., 2013b, 2017). The linear mid-stream distance between the study areas was ~65 km and a weir across the river presented a further barrier for movement. The four groups were males and females greater than or less than 15 years of age. The age of a turtle based on its size was determined from the length-at-age curve and 15 years was chosen as the cut-off because this was the period that the nest-protection program had been in place. Juveniles (sex not determined) were excluded from the model.

A set of candidate models was tested for each study area. The AIC_c weight (i.e. AIC index corrected for small sample sizes) of each model was used as an objective means to select the most parsimonious model of those tested (Burnham and Anderson, 2002). Models assumed either constant survival (phi) or allowed 'phi' between sampling events to vary with size, sex, and/or study area (Table 3). All models assumed constant probabilities of capture (p) and entry into the population (pent). Models also hypothesized super-population size (*N*) to vary with sex (or age) and study area, either as independent effects or as an interaction. Models with $\Delta\text{AICc} \leq 2$ were considered competing models, and model averaged estimates for phi, p, pent, and *N* were estimated using all competing models for age or sex. Once the most likely POPAN model was identified, population estimates (*N*) were generated for each attribute group for each study area. Identical sampling protocols were used for each sampling event, so the effect of sampling effort on the results was not assessed.

3. Results

3.1. Protected nests

The community driven nest protection program protected 631 *Elusor macrurus* nests over a period of 15 years (number of nests per annum ranged from 11 to 87 between 2001 and 2016; Table S2 in Supplementary Data). Considering an average clutch size of 15 eggs (Micheli-Campbell et al., 2013a), we estimate that the program resulted in 9465 hatchlings emerging from the eggs in the lower reach (nest protection program area) of the Mary River over 15 years prior to this study.

3.2. Mark-recapture

A total of 358 *E. macrurus* were captured and marked over the three years of sampling: 227 males, 113 females, and 18 juveniles (Table 1). Sixty-one turtles (41 males and 20 females) were recaptured, three were recaptured twice, and one recaptured three times. Minimum trap awareness and site fidelity were observed. All recaptures occurred within the same study area as initial capture, with 47% of those occurring in the exact same trap as first capture, and 36% within 2 consecutive traps (0.2–4 km). Fourteen turtles that were captured and marked as hatchlings during a prior study between 1998 and 2001 (Flakus, 2002) were recaptured in this study. These fourteen individuals were recaptured within a 1 km radius of initial capture location, and time since initial capture was used to calculate age and growth parameters of individuals. Data from these individuals was used in the length-at-age model.

The sex ratio of captured turtles was 1:2.9 (females:males) within nest protection area (study area 1; Table 1, Fig. 3) and the bias towards the capture of male turtles over females was significant across all ten netting locations in this study area (Welch Two Sample *t*-test, $t = -3.1$, df = 12.8, *p*-value = 0.009). The sex ratio in the study area 2 (upper reach) was 1:1.6 but the bias towards the capture of male turtles was not significant across all ten netting locations (Welch Two Sample t-test, $t = -1.6$, df = 12.3, *p*-value = 0.123). Apart from one juvenile (136 mm SCL), no other turtles < 215 mm SCL were captured in the study area 1 (lower reach – nest protection area), whilst 17 juveniles (91–172 mm SCL) were captured in the study area 2 (upper reach).

3.3. Age and population estimations

The length-at-age relationship curve showed that a 15-year-old *E. macrurus* had an SCL of 317 ± 9 mm (Fig. 2).

The Jolly-Seber POPAN model showed that the total *E. macrurus* population was larger (1074) in the study area 2 (upper reach) compared to the similar length of river surveyed in the study area 1 (714; nest protection area within the lower reach). The population of *E. macrurus* younger than 15 years of age was almost 3-fold greater in the

Table 2
Summary of multi-model comparisons to identify best fitting growth curve used to define length-at-age relationship for *Elusor macrurus*. L_a is length-at-age *a*, L_∞ is the asympotic length, and L_0 is the length-at-birth. Growth coefficients are model specific (k = von Bertalanffy; g = Gompertz; and g = Logistic).

Model	Formulation	AIC	ΔAIC	R^2
von Bertalanffy	$L_a = L_\infty(1 - e^{-k(a-L_0)})$	1339.16	0	0.97
Gompertz	$L_a = L_0 e^{\log(L_\infty/L_0)(1-e^{-ga})}$	1475.57	136.41	0.92
Logistic	$L_a = (L_\infty * L_0 * e^{ga})/(L_\infty + L_0 * e^{ga-1})$	1530.77	191.61	0.94

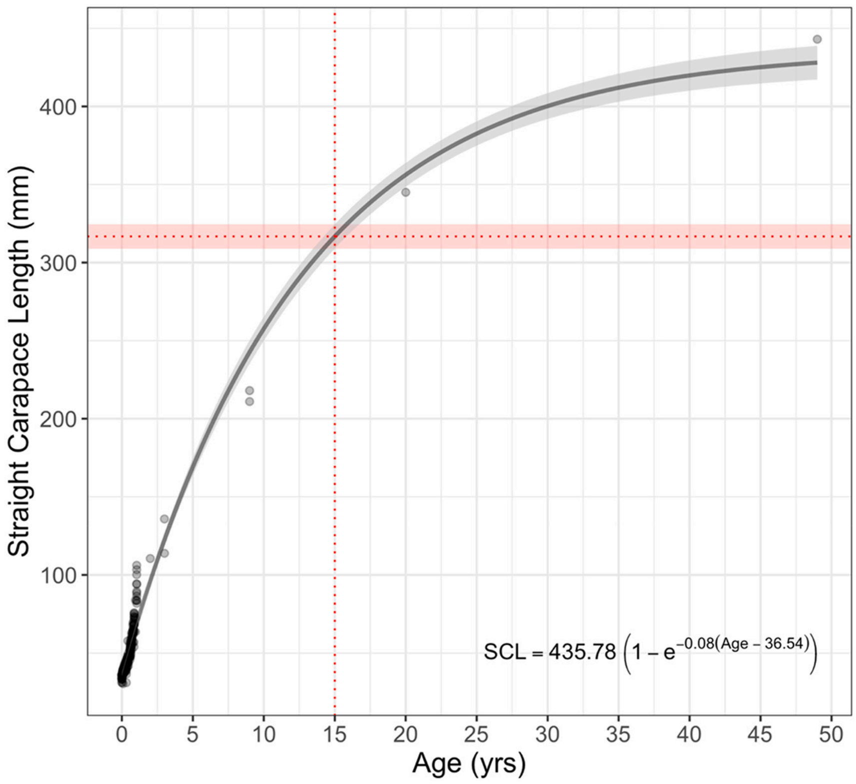

Fig. 2. Best-fitting von Bertalanffy growth curve for *Elusor macrurus* using verified length-at-age data collected from 43 individuals from captive and wild populations (see Table S1 in Supplementary Data). Black line indicates predicted age-length relationship with grey shaded area indicating confidence band generated by a Bayesian Markov chain Monte Carlo process. Red dotted lines indicate the SCL estimated for a 15-year-old turtle, and the red shaded area the level of confidence in the modelled age-length estimate at 15 years of age (317 ± 9 mm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

upper reach (study area 2) compared to the lower reach (study area 1, Table 4). The ratio of individuals below and above 15 years of age was 1:2.9 in the study area 2 (upper reach) compared to 1:6.0 in the study area 1 (lower reach). This translated into only 14% of the *E. macrurus* population being below 15 years of age in the study area 1 (lower reach – nest protection area) compared to 25% in the study are 2 (upper reach).

There was a substantially greater number of reproductive females in the study area 2 (upper reach; Table 5). If we assume that each reproductive female produced an average of 15 eggs per year (this varies from year to year due to rainfall but annual variations would be similar across the two study areas, Micheli-Campbell et al., 2013a), then the number of eggs laid per kilometre of river would be ~2-fold greater in the study area 2 (upper reach) compared to the study area 1 (lower reach – nest protection area). Importantly, the model estimates revealed that not only there were more eggs laid in the upper reach (study area 2) over the past 15 years, but that a greater proportion of laid eggs hatched and were recruited into the population (Table 5).

4. Discussion

Protecting the nests of the freshwater turtle *Elusor macrurus* from predation resulted in thousands of additional hatchlings annually entering the lower reach of the Mary River over 15 years. Using only hatching rate to quantify the effectiveness of this conservation strategy would deem it to have been a great success and worthwhile investment. However, our integrative modelling technique suggested that a very small percentage of the hatchlings protected under this conservation strategy had survived. Further, a higher level of turtle recruitment occurred over the past 15 years in a stretch of the river without a long-term nest protection program in place. This has substantial implications for the ongoing management of *E. macrurus* because it demonstrates that the predation of eggs from the nests may not be the singular factor hampering population recovery, and therefore nest protection alone is not a sufficient strategy to reverse ongoing decline. These findings have significance for any threatened turtle species where nest protection is the primary long-term conservation strategy and where success has been measured by the number of hatchlings entering the wild population.

Table 3

Models of the various parameterizations of survival (phi) and super-population size (*N*) used in the Jolly-Seber POPAN model in R, where the first 3 lines (bold caps) are the models with ΔAICc ≤2. Probability of capture (p) and probability of entrance into the population (pent) were assumed constant (~1); 'covar' is a factor for either sex (male/female) or age (< or > 15 years of age); and 'area' is a factor for study areas 1 and 2.

Phi	*N*	Model	AICc	
			Age	Sex
~1	**~covar ∗ area**	**Phi(~1)p(~1)pent(~1)N(~covar ∗ area)**	**469.41**	**472.95**
~covar	**~covar ∗ area**	**Phi(~covar)p(~1)pent(~1)N(~covar ∗ area)**	**469.84**	**474.84**
~area	**~covar ∗ area**	**Phi(~area)p(~1)pent(~1)N(~covar ∗ area)**	**470.78**	**474.89**
~area + covar	~covar ∗ area	Phi(~area + covar)p(~1)pent(~1)N(~covar ∗ area)	471.52	475.99
~1	~covar + area	Phi(~1)p(~1)pent(~1)N(~covar + area)	472.88	476.78
~covar	~covar + area	Phi(~covar)p(~1)pent(~1)N(~covar + area)	473.41	477.84
~area	~covar + area	Phi(~area)p(~1)pent(~1)N(~covar + area)	473.59	477.95
~area + covar	~covar + area	Phi(~area + covar)p(~1)pent(~1)N(~covar + area)	474.79	479.90

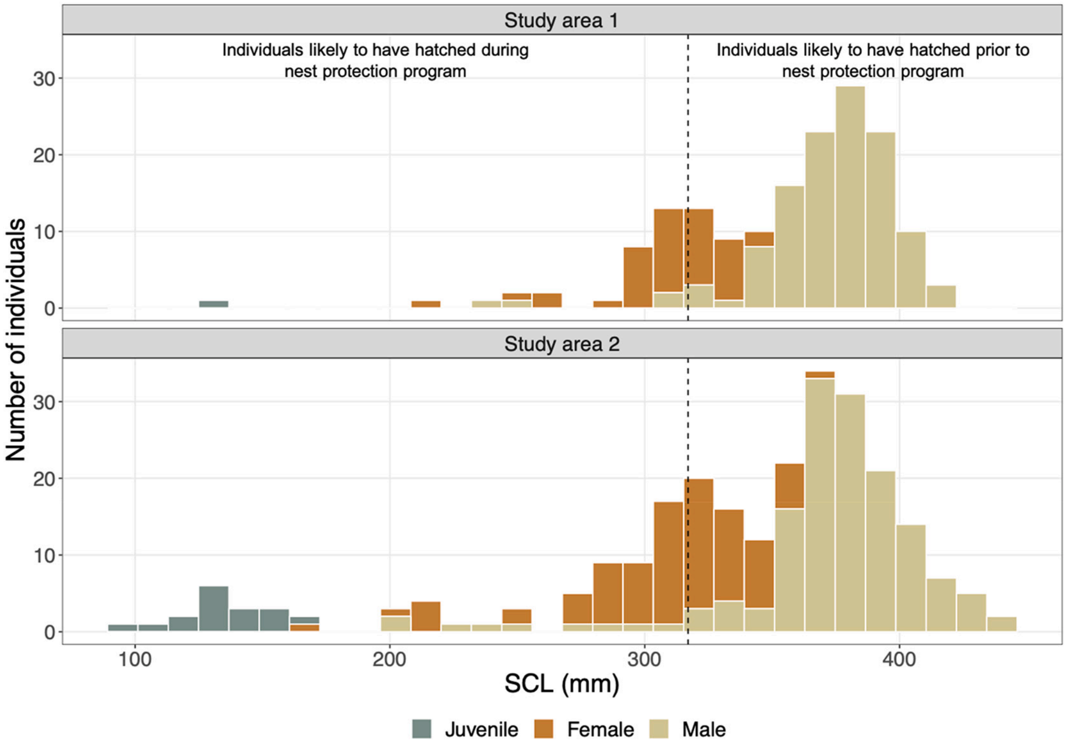

Fig. 3. Frequency distribution of the straight carapace length (SCL, mm) of captured *Elusor macrurus* for each sex within each study area (study area 1 = lower reach – nest protection area; study area 2 = upper reach). Dashed black line represents the 15 years of age used for the population estimate models. This was due to the duration (15 years) of nest protection program undertaken in the lower reach of the Mary River (study area 1) prior to this study.

Table 4
Jolly-Seber POPAN model outputs for super-population estimate (*N*) per 'covar': age (> and < than15 years old) and sex (males and females) of *Elusor macrurus* for the study area 1 (lower reach – nest protection area) and study area 2 (upper reach) in the Mary River (QLD, Australia).

Study area (river reach)	Age/sex 'covar'	N	se[a]	LCL[a]	UCL[a]
1 (lower)	< 15 yo	122	33.44	56.29	187.37
1 (lower)	> 15 yo	734	143.46	452.52	1014.90
2 (upper)	< 15 yo	309	66.17	179.55	438.93
2 (upper)	> 15 yo	912	160.49	597.48	1226.60
1 (lower)	Female	190	53.22	85.43	294.05
1 (lower)	Male	558	134.37	294.82	821.57
2 (upper)	Female	406	98.09	213.84	598.33
2 (upper)	Male	668	152.35	369.32	966.52

[a] se = standard error; LCL = lower confidence limit; UCL = upper confidence limit.

Table 5
Population demographics derived from the age-length growth curve and Jolly-Seber population model outputs for *Elusor macrurus* for the study area 1 (lower reach – nest protection area) and study area 2 (upper reach) in the Mary River (QLD, Australia).

	Study area 1	Study area 2
Proportion of females in population	0.34	0.61
Proportion of females > 15yo	0.83	0.66
N° reproductive females per km[a]	7.88	13.4
Annual N° eggs laid per km[b]	117	200
Proportion of laid embryos that have persisted in the population[c]	0.0035	0.0051

[a] Reproductive females = SCL 317 mm (Limpus, 2008).
[b] Assumed clutch size = 15 eggs (Micheli-Campbell et al., 2013a); assumed net coverage area of 20 km river length (1 km either side of each net).
[c] Estimated number of eggs laid over past 15 years divided by the estimated population of turtles below 15 years of age.

Our assumptions were based upon the following: 1) a species-specific length-at-age curve, which defined the size of individuals that would have been laid during the period of the nest protection program, and 2) population estimates for the cohorts of turtles laid prior to or during the nest protection program. We are confident in our length-at-age estimate because the growth rate estimates were replicable to other freshwater turtle species that occupy similar habitats and had growth data available (Kennett, 1996: *Chelodina rugosa, Elseya dentata*; Fielder et al., 2015: *Myuchelys bellii*; Çiçek et al., 2016: *Emys orbicularis*, *Mauremys rivulata*; Fig. S1 in Supplementary Data). Moreover, our length-at-age curve estimate for a 15-year-old turtle matched the size of *E. macrurus* determined to be around 15-year-old due to the onset of sexual maturity (Flakus, 2002; Limpus, 2008). We are also confident in the validity of our population estimates. We used a passive netting method for turtle capture that has been demonstrated to be successful in capturing freshwater turtles of all size classes (Connell et al., 2018). The net design ensured deployments were comparable and capture probabilities were similar for all trapping episodes. Whilst we cannot be sure that capture probabilities were the same across the size range or sex of the turtles, any biases would have been similar between the areas with and without nest protection. Using this type of experimental design for modelling population demographics has been demonstrated to provide results that reflect actual differences in populations (Amstrup et al., 2005).

We also argue that the model estimates for the abundance of *E. macrurus* below 15 years of age reflects the true population. We discount the possibility of the young turtles moving long distances either down or upstream. Immediately downstream from the lower reach, the river is lotic and deep due to the river being artificially impounded by a saltwater barrage (Johnson et al., 1982; Bodie, 2001; Bunn and Arthington, 2002). This type of environment has been documented to be unfavourable for young *E. macrurus* (Clark et al., 2008). To travel to the upper reach, a young turtle would need to have swum a significant distance (> 65 km) against the water flow, a considerable feat for a small turtle. This conclusion is supported by two biotelemetry studies, which also found no evidence of long-distance river movements by *E. macrurus*. The first study monitored the movements of hatchling and juvenile *E. macrurus*, and the authors recorded a maximum in-stream distance travelled by a juvenile to be 2.50 km (Micheli-Campbell et al., 2013b). Using similar technology (acoustic telemetry array), another study monitored the movements of adult female *E. macrurus*, and their linear home range was recorded between 2.00 and 4.98 km (Micheli-Campbell et al., 2017). Further, there is a stream gauging weir across the river between the two study areas (Gympie) and there have never been reports of mass gatherings of young turtles below this weir.

A partial explanation for the low number of young *E. macrurus* in the lower compared to the upper reach is simply because there are fewer reproductive females, both in absolute and relative terms to the number of males. This translated into an ~2-fold difference in the potential maximum number of eggs laid each year between study areas. Female *E. macrurus* nest at night in sandy banks along the water's edge (Flakus, 2002; Cann, 2017), with a maximum linear distance from nest to water recorded as 43.6 m (Micheli-Campbell et al., 2013a). Because *E. macrurus* females do not need to travel long distances to nest, they are, therefore, not exposed to urbanization threats that may impact other turtle species, such as roads and residential areas (Santori et al., 2018; Steen et al., 2006; Vanek and Glowacki, 2019). Additionally, the sex of an *E. macrurus* embryo is not temperature-dependant, and therefore a close to equal sex ratio at laying is probable, although this has never been assessed (Cann, 2017). If this is the case, then an explanation for the male bias sex ratio is that the mortality rate of females is greater than it is for males, and this is exaggerated in the lower compared to the upper reach of the river. If males live longer than females, then a male biased sex ratio would be an artefact of a population whose death by old age exceeds that of recruitment. This theory is further supported by comparison of the sex ratio observed in this study with one from twenty years ago, which shows that the male bias in this stretch of the river to have doubled (Flakus, 2002). It could be argued that a passive netting capture technique would bias the capture of males if they were more active during either of the trapping seasons and moved greater in-river distances compared to females. However, the capture methodology and spacing between nets were similar in both study areas, suggesting that any bias in the capture of males would be similar across both reaches. Thus, sampling design does not explain the observed reach differences in sex ratio, and the reduction in adult females in the lower catchment. This is worrying because egg collection records from the 1960's and 70's show that the lower reach of the Mary River was the nesting stronghold for the species with abundant nesting females in this part of the river (Flakus, 2002; Limpus, 2008).

The integration of the age-growth curve and population models suggests that in the upper reach not only were a greater number of eggs laid compared to the lower reach, but also a larger proportion of these hatchlings are still alive in the river (Table 5). This was despite the absence of a long-term nest protection program occurring in the upper stretch of the river. There is no available evidence to suggest that the probability of nest predation would be greater in the lower compared to the upper reach. The Mary River is bordered by agricultural land throughout, and the key predators of *E. macrurus* eggs (wild dogs, foxes, monitor lizards, crows) are found throughout the river's length (MRCCC, 2015). Therefore, we argue that post-hatching and/or in-stream mortality of the hatchling and juvenile *E. macrurus* in the lower reach of the Mary River has been a significant cause of turtle mortality over the past 15 years. This could be due to either bottom-up (lack of food) or top-down (predation) processes. Young *E. macrurus* feed upon invertebrates and algae (Flakus, 2002; Cann, 2017), and these have been documented to be as plentiful in the lower as the upper reaches (MRCCC, 2015), and therefore, post-hatching or in-stream predation (top-down) is likely the more threatening process. This could be predation of the hatchlings as they exit the nest and walk across the riverbanks or once they enter the water. A biotelemetry study of hatchling and juvenile *E. macrurus* in the lower reach of the Mary River reported 50% of turtles to have been predated within 6 months of release (Micheli-Campbell et al., 2013b). A possible culprit could be the fork-tailed catfish (*Neoarius graeffei)*. Studies from other river systems have reported young turtles to be one of the most abundant items found in the stomachs of forked tailed catfish (Blamires and Spencer, 2013). During the present study, we captured a high abundance (n = 325) of *N. graeffei* in the lower reach but only low numbers in the upper reach (n = 20) as net bycatch (Table S3 in Supplementary Data). Additionally, the captured *N. graeffei* in the lower reach were very large (mean = 561 mm; max = 710 mm standard length [SL]). In fact, larger than has been previously described for the species (max = 350 mm–600 mm SL; Pusey et al., 2004), and of sizes that could easily predate upon young *E. macrurus*. However, further research is required to determine the underlying process of mortality in hatchling and juvenile *E. macrurus* in the lower reach of the Mary River, and assess alternative management options that will effectively mitigate further declines in the population.

5. Conclusion

The purpose of this study was to assess if fifteen years of protecting *Elusor macrurus* nests from predators had resulted in a significant recovery outcome for the population. Unfortunately, we did not find evidence of conservation success and instead found that over the past 15 years *E. macrurus* has had higher levels of population recruitment in an area where a long-term nest protection program had not occurred. The exact reasons for these findings need to be explored and adopted into new evidence-based conservation strategies. In 2003, *E. macrurus* was included in the Top 25 most threatened species of turtles in the world, remaining on this list after the 2007 assessment (TFTSG, 2007). In 2011, however, *E. macrurus* was downranked to the 41 + group, and this change in status was due to the increased number of hatchling turtles potentially entering the population under the nest protection program (TCC, 2011). This study suggests that this downranking may have been premature and based on insufficient data.

Whilst we do not wish to undermine the utility of nest protection as a strategy for turtle conservation, the study highlights the importance of assessing population demographics to correctly identify and manage the key threating processes causing population decline, and to maximise the effectiveness of conservation actions. We have shown that if aging and mark-recapture studies are possible, then the integration of these techniques can assess population recovery within a relatively short-time window compared to surveying annual abundance over the generation time of the study animal.

CRediT authorship contribution statement

M.A.C.: *Roles* – Conceptualization, Formal analysis, Investigation, Methodology; *Writing* – original draft, review, & editing. **M.J.C.:** *Roles* – Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Resources, *Writing* – original draft & review. **V.U.:** *Roles* – Formal analysis, Methodology; *Writing* – review. **T.L.C.:** *Roles* – Statistical analysis; *Writing* – review; **S.J.C.:** *Roles* – Data curation, Methodology; *Writing* – review. **A.M.:** *Roles* – Conceptualization, Methodology, Supervision; *Writing* – review. **H.A.C.:** *Roles* – Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Resources; Supervision; *Writing* – original draft & review.

The work is all original research carried out by the authors and the manuscript is not being considered for publication elsewhere whilst it is being considered for publication in this journal. All authors agree to the publication of this manuscript within Biological Conservation and have no conflicts of interest.

Declaration of competing interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary Material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.biocon.2020.108769.

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Glossary

EPBC Act: Australian Government Environment Protection and Biodiversity Conservation Act (1999)
IUCN: International Union for Conservation of Nature
QLD: State of Queensland, Australia
SCL: straight carapace length