Freshwater turtle assemblages of the Mary River (Queensland, Australia), with a focus on the population of the endangered *Elusor macrurus*.

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Declaration by author

This work contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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A McDougall HA Campbell

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List of Abbreviations

AMTD	Adopted Middle Thread Distance
BMRG	Burnett Mary Regional Group
CI	Confidence interval
EPBC	The Environment Protection and Biodiversity Conservation Act 1999
KS	Kolmogorov-Smirnov
MRCCC	Mary River Catchment Co-ordinating Committee
MRT	Mary River turtle
PIT	Passive integrated transponder
QLD	Queensland
SCL	Straight carapace length
TCF	Turtle Conservation Fund
TDLG	Tiaro and District Landcare Group
TE	Trapping Episode
TSA	Turtle Survival Alliance

Abstract

Reversing the consequences of anthropogenic impacts on species decline and assemblage modifications is a pressing environmental challenge. Often, community-based conservation projects aim to redress these issues; however, the efficacy of the chosen technique(s) is rarely tested. In response to the decline in the nesting population of the endangered Mary River turtle (*Elusor macrurus*), a community led nest protection program has resulted in at least 2,843 hatchlings entering the river over the past 15 years. The aims of the present study were: 1) identify the composition of turtle assemblages along a river continuum, and 2) assess the effects of the conservation program on the population of *E. macrurus*. I hypothesised that the river reach where the nest protection program operated would contain a greater number of immature and adult female *E. macrurus* compared to other reaches. A passive capture technique - set-nets - was employed. Sampling was repeated identically every six months over two years at four study reaches, arraying over 180 km of the Mary River (QLD, Australia). Three discrete assemblages comprised of the six-turtle species inhabiting the river were identified. The observed spatial variance in species assemblage was consistent over time and unaffected by the season, illustrating the robustness of the sampling technique. Mark and recapture methodology was used to estimate the population of *E. macrurus*. A total of 268 individuals were captured, measured, and marked. Twenty-nine were recaptured in subsequent sampling episodes. Contrary to my hypothesis, the reach where the nest protection program operated contained no immature *E. macrurus*, and the adult female population was least within that river reach. The results of this study suggest that while the nest protection program successfully produced hatchlings, it has not translated into the recruitment of reproductive females to ensure an increase and preservation of the population.

Keywords

Fyke net, mark and recapture, monitoring community-based conservation, nest protection, turtle sampling methods, turtle assemblage.

Chapter 1

General Introduction

"Protection of freshwater biodiversity is perhaps the ultimate conservation challenge because it is influenced by the upstream drainage network, the surrounding land, the riparian zone and – in the case of migrating aquatic fauna – downstream reaches."

(modified from Dudgeon et al. 2006)

Community-based conservation is one of the approaches that has been widely promoted to address biodiversity loss. Substantial monies are invested in conservation programs by governments, philanthropists, and the not-for profit sector (Catterall et al. 2004; Field et al. 2007). Since 1997, the Australian Government has invested more than AUD$3.7 billion in programs aimed at environmental restoration. The philanthropic organisation, Mohammed bin Zayed Species Conservation Fund has invested US$15.9 million in threatened species conservation (Mohamed bin Zayed Species Conservation Fund 2017). Since 2003, the Turtle Conservation Fund has invested almost US$1 million in turtle and tortoise conservation (Turtle Conservation Fund 2017). Each fund mandates indicators to be monitored, which assures the grantor that the funds were used by the grantee in accordance with the project objectives.

A major challenge for conservation projects is to monitor and evaluate changes to ecological variables (Catterall et al. 2004; Field et al. 2007; Lindenmayer & Likens 2010). Most monitoring programmes have no realistic chance of detecting these changes, partly because detecting change in ecological systems can be quite challenging (Field et al. 2007; Legg & Nagy 2006; Reynolds et al. 2011). Inherent obstacles such as the lack of long-term funding for projects, the skills required, the loss of key personnel, poor data management, and poor sampling design are compounded by the time required for an ecological variable to show significant change in relation to the time-frames of the program (Field et al. 2007; Lindenmayer & Likens 2010). In addition, the cost of ecological monitoring is, on occasions, considered a disincentive by conservation practitioners, as it is seen to be diverting limited funds from direct conservation actions (Lindenmayer & Likens 2010; Tulloch et al. 2011). Yet, without monitoring the impact of conservation actions, it is unknown if the funds may have been more effectively used if they were allocated to alternative actions, which may

subsequently have led to an improvement in conservation outcomes (Ferraro & Pattanayak 2006; Lindenmayer et al. 2012; Moll & Moll 2004). To address some of these challenges, it has been proposed that conservation managers, researchers, and bureaucrats collaborate as a way forward to achieve meaningful ecological monitoring (Field 2007, Lindenmayer 2010).

The dissemination of ecological monitoring data from community conservation projects is limited. The results of these projects are often published in 'grey' literature, as perhaps they are not considered of sufficiently high impact for many peer-reviewed scientific journals (Gibbons et al. 1997).

In this study, researchers and government personnel collaborate to assess the field component of a community-driven conservation program.

Population monitoring

Monitoring the population of a species has its roots in the 1890s, when researchers began to use techniques to determine how populations of various species changed over time (Stem et al. 2005). Population monitoring is imperative for determining the status of a species, its distribution and for identifying the potential threats to its survival. Although population monitoring has been key to generating conservation knowledge and tracking changes over time, it is generally considered too time-consuming and expensive to be a feasible approach for conservation programs and site-level status assessments (Stem et al. 2005).

However, the mark-recapture method is a powerful method for estimating abundance and makes a population study more practical (Lettink & Armstrong 2003). The recent development of easy-to-use software such as Program MARK, provides a

unified approach to analysing the count data. However, this method has limitations. When recapture rates are low, mark-recapture analyses quickly lose power and generate imprecise parameter estimates (Kendall 1999).

Population assessments can be very expensive in terms of resources and personnel required, thus a clear set of objectives are required at the outset, together with adequate resources (Witmer 2005). Estimates of population size have been found to vary widely according to the sampling methodology employed, due to the inherent bias of each type (Tesche & Hodges 2015; Witmer 2005). The aim of the study was to estimate the size of the population at a point in time, thus sampling efficiency becomes a critical consideration. Environmental Impact Assessments often require an assessment of species abundance in a particular location at a specific time, and thus sampling efficiency is critical to the results (Hill 2005). However, for long-term studies whose objective is to detect trends in the population, rather than sampling efficiency, the critical factor is standardisation of the methodology, which maintains probability of capture over space and time constant and by different personnel. Considering the time-lag required to detect ecological changes, there is a high degree of the likelihood that different personnel will be conducting future assessments (Gibbons et al. 1997; Witmer 2005).

While the question, ‘how many animals are there?’ is frequently asked, in the case of evaluating the effectiveness of conservation actions, the question should be, ‘is the population increasing or decreasing?’ Detecting population trends can reflect the efficacy of management actions and thus trigger appropriate changes to management actions should they be required (Jackson et al. 2008; Lettink & Armstrong 2003; Lindenmayer et al. 2012). Even so, a rigorous baseline study is required to detect population trends.

Typically, many projects rely on an array of funding bodies for the necessary finances. Most of these programs concentrate on monitoring project achievements such as length of fencing erected, rather than detecting changes in the variables of interest (Field et al. 2007). Short-term funding cycles typically produce brief surveys that may lead to erroneous conclusions on species abundance, distribution, and diversity (Gibbons et al. 1997). For long-lived species such as turtles, it may require one to several decades for substantial increases in the population to become apparent, thus the changes occur outside the timeframe of funding cycles (Moll & Moll 2004). Here, I assess the impact of recovery actions on the population of an endangered turtle species, 15 years since the commencement of the conservation program.

Turtle assemblages

Turtle assemblages tend to be comprised of a relatively low number of species and are seldom as diverse as fishes (Moll & Moll 2004). One of the more diverse turtle assemblages (seventeen species) is found in the lower Ganges and Brahmaputra river basins (Moll & Moll 2004). Species presence and abundance are influenced by water depth, strength of current, type of substrate, food sources, availability of basking and nesting sites, competing species and predators. Strong relationships have been found between the gradients in stream morphology and the structuring of turtle assemblages (Bluett et al. 2013; DonnerWright et al. 1999). The composition of assemblages may be constant, however, longitudinal variation in relative species abundance may occur within a waterway.

The Mary River supports one of the most diverse assemblages of freshwater turtles in Australia, with six species representing five of the eight genera that are found in Australia: *Elusor*, *Elseya*, *Emydura*, *Wollumbinia*, and *Chelodina* (Cann & Sadlier 2017; Moll & Moll 2004). However, until 1994, it was long thought that only four genera inhabited the river (Cann & Legler 1994). In 1870, a team from the Australian Museum led by George Masters made their first QLD trip to the Mary River, and the neighbouring Burnett River, where they collected 47 freshwater turtles (Cann & Sadlier 2017). Gerard Krefft later identified five species belonging to four genera, *Chelodina expansa*, *Chelodina sulcata*, *Chelymys macquaria*, *Elseya latisternum*, and *Elseya dentata*. While the taxonomy and nomenclature of the later four species have undergone changes, none of the specimens belonged to the genus *Elusor*. This species and its habitat remained a mystery for the following 120 years until its native habitat was confirmed by John Cann in 1990 (Cann & Legler 1994). The *Elusor* genus is restricted to the Mary River, and *Elseya albagula* is endemic to this and the neighbouring Burnett and Fitzroy River catchments.

Turtle populations

Turtles have exhibited extraordinary resilience over millions of years unlike numerous creatures such as dinosaurs, numerous megafauna, and other vertebrate species. Yet, they are an unlikely candidate for survival given they offer predators substantial quantities of meat and they lack all offensive capability (Pritchard 2007). However, this persistence is under concerted assault and turtles have become casualties of human exploitation and climate change (Klemens 2000b; Rhodin et al. 2015; Rhodin et al. 2011). Since the beginning of the Pleistocene, 25% are estimated to have gone extinct (Rhodin et al. 2015) and ca. 42% of all known chelonian species are

considered threatened (Turtle Conservation Coalition 2018). This extinction rate is expected to increase over the next century unless directed strategic conservation actions forestall the present global turtle survival crisis. Australian chelonian populations are also in decline (Cann & Sadlier 2017). Seven percent of Australia's turtles are in the top 25 most endangered tortoises and turtles of the world (Turtle Conservation Coalition 2018).

However, many consider them a commonplace animal, as turtles are frequently seen. Many folks have a story to tell about an encounter with a turtle, which reinforces this perception (Klemens 2000b). The possibility of an encounter is greater within suburban modified water habitats than in nature reserves, due to the greater abundance levels of some species, particularly ecological generalists (Roe et al. 2011). Human-induced environmental changes act as a non-selective filter favouring those species that are best able to survive within a modified ecosystem (Devictor et al. 2008). Thus, the composition of a community changes systematically through time (Dornelas et al. 2014), with generalist species replacing specialist species (Eskew et al. 2010; Kennett & Tory 1996; Roe et al. 2011).

Unlike other reptiles, turtles have an appeal that endears them to humans and translates into their popularity as pets (Tisdell 2004). Collection of animals from the wild to meet the demands of the pet trade has contributed to population declines (Rhodin et al. 2011). In the mid-1960s, Goode decried the 95% death rate of the 10,000 Australian freshwater tortoises sold every year by dealers (Goode 1967). Although *E. macrurus* was misidentified, the species was targeted for commercial egg harvest from 1962 to 1974 with the pet trade annually taking thousands of hatchlings from the banks of the Mary River (Cann 1998; Flakus 2002).

The precipitous decline of many turtle populations from their former levels, and the complete extirpation of some species from large areas of their former ranges, are causes for alarm both for the turtles, as well as for the health of the waterways on which turtles and humans depend upon (Buhlmann et al. 2009; Moll & Moll 2004; Turtle Conservation Fund 2002). Stream modification was found to lower the species richness, a reduction in dietary variability and prey abundance (Bodie 2001; Tucker et al 2012; Vandewalle & Christiansen 1996). Impoundments have resulted in the loss of free-flowing riffle zones in the Fitzroy and Burnett Rivers in Queensland. The loss of this microhabitat has been suggested as a contributing factor to the declining recruitment for the Fitzroy River turtle, *Rheodytes leukops* and the white-throated snapping turtle, *Elseya albagula* (Cann & Sadlier 2017). In the summer of 2014/15 in Australia, two species of turtles, Johnstone River snapping turtle, *Elseya irwini* and the Bellinger River snapping turtle, *Muchelys georgesi*, suffered mass mortality events (Spencer et al 2018). A mystery disease almost drove the Bellinger River snapping turtle in the North Coast of New South Wales, Australia to extinction in less than a month (Spencer et al 2018). Environmental changes such as regional warming and localised drying which reduced water levels and the number of flooding events, may have exacerbated the virulence and contagiousness of this disease (Spencer et al 2018). For Tiaro and District Landcare Group (TDLG), the connection between *E. macrurus* and the health of the river is a key motivation for their Mary River turtle conservation program.

Conservation management

The persistence of the ancient and iconic vertebrate group, chelonians, is under concerted assault, and without directed, strategic conservation planning, a significant

portion of turtle diversity could be lost over the next century (Buhlmann et al. 2009). Conservation programs have been initiated globally to reverse these trends (Spencer et al. 2017). Nevertheless, while these programs may have good intentions, without a critical appraisal, it is unknown if they are achieving their objective (Pritchard 1980). Frequently, conservation programs target the early life stage without consideration of the species' life history traits (Frazer 1992; Heppell et al. 1996; Klemens 2000a). An innate human response to the presence of predated eggs strewn on nesting beaches is to target this life stage (Pritchard 1980), rather than consider the less visual threats to other life stages that maybe occurring beneath the surface of the water. The loss of individuals during the later life stages can be subtler and is more likely to lead to the demise of the species (Crouse et al. 1987). While these issues similarly relate to marine and freshwater turtles, this study focuses on freshwater turtles.

There is an emphasis on the interventionist style of management within turtle conservation programs (Seigel & Dodd 2000). This style of management aims to maintain, increase, or restore the numbers of a target species, rather than the alternative approach that focuses on the landscape and maintaining functional ecosystems. Interventionist management measures include: 1) in-situ protection of freshly laid nests, 2) head-starting, and 3) repatriation or translocation (Seigel & Dodd 2000). Head-starting focuses on raising hatchlings in captivity until they reach a certain size when they are released into the wild. Repatriation or translocation programs include moving individual turtles to a locality where the population has either been extirpated or in need of augmentation. While these interventionist methods have an intrinsic anthropogenic appeal as they produce hatchlings, they are not without critics (Burke 2015; Frazer 1992; Paez et al. 2015; Seigel & Dodd 2000). The predominant criticism is that these actions address the symptoms rather than the causes (Frazer 1992). Some

would argue that one of the consequences of the life history traits of turtles is that they are poor candidates for this approach (Congdon et al. 1993; Crouse et al. 1987; Heppell et al. 1996; Seigel & Dodd 2000). Conversely, others advocate that head-starting should be a primary tool for managing freshwater turtles under threats that affect multiple life stages, but only at very high supplementation rates (Spencer et al. 2017).

Despite the controversies, interventionist methods are widely used in conservation programs for marine and freshwater turtles (Burke 2015). The Turtle Survival Alliance manages captive assurance colonies for 30 critically endangered chelonians at their facilities in South Carolina USA (Rhodin et al. 2011). Captive breeding, head-starting, and reintroductions brought the population of the western swamp turtle, *Pseudemydura umbrina* in Western Australia, back from the brink of extinction, though the population remains precarious at 300 animals (Rhodin et al. 2011). A Malaysian community turtle conservation program achieved a mean hatching success of 67.8% of head-started Southern River terrapin hatchlings, *Batagur affinis,* in the Kemaman River (Chen 2017). This achievement would not have been possible without intervention and the involvement of the local community. In Brazil and Venezuela, numerous turtle conservation projects focus their efforts on head-starting (Paez et al. 2015).

Conservation programs which target life stages other than the egg-hatchling stage are not as common. Few protected areas have been designated primarily for chelonians. The notable exceptions are the Galapagos Islands National Park which protects giant tortoises, *Chelonoidis niger*, and Huo Cheng nature reserve in Zinjiang, China established specifically for Horsfield's tortoise, *Testudo horsfieldii*. The Trombetas Biological Reserve in Brazil was established primarily to protect the nesting beaches of the giant Amazon River turtle, *Podocnemis expansa* (Rhodin et al.

2011). Turtles make excellent ambassadors to the general public and are frequently used to generate community interest and awareness in the conservation of the species and its habitat (Burke 2015).

The Mary River turtle (MRT) conservation program is one of the longest, continually operating, freshwater turtle conservation programs in Australia. In this study, I assess the outcomes of a turtle conservation program that employed the first of the interventionist approaches, in-situ nest protection. Changes to the population should be detectable given the lengthy period that this program has operated (15 years), thus it presents an ideal opportunity to assess if the results of the conservation actions have transferred to the population. In addition, the oldest data available for any freshwater turtle population in Queensland belongs to this species, making it a suitable candidate for historical comparisons (Limpus 2008).

Study species

Chelodina expansa

The broad-shelled turtle (*C. expansa*; Figure 1.1), is characterised by its extremely long neck (Cann & Sadlier 2017). It occurs broadly through the inland rivers and billabongs of eastern and south-eastern Australia. The species is cryptic in habit yet occupies waters heavily exploited and regulated by humans. Traditionally considered a riverine species, recent studies demonstrate that it is more frequently represented in permanent lakes and billabongs connected to main river channels (Bower & Hodges 2014).

Figure 1.1: Adult broad-shelled turtle, *C. expansa*.

Chelodina longicollis

The eastern long-neck turtle (*C. longicollis;* Figure 1.2) has a wide distribution throughout south-eastern Australia (Cann & Sadlier 2017). It occupies a broad range of freshwater habitats but is more abundant in shallow, ephemeral wetlands often remote from permanent rivers. Its propensity for long distance overland migration enable it to exploit highly-productive ephemeral habitats (Kennett et al. 2009).

Figure 1.2: Adult eastern long-necked turtle, *C. longicollis.*

Elseya albagula

The white-throated snapping turtle (*E. albagula*; Figure 1.3), is one of Australia's largest, short-necked turtle species. It has a limited distribution and is restricted to the Mary, Burnett and Fitzroy Rivers (Thomson et al. 2006). This species is generally found in deep pools (>6 m) either up or down stream from a riffle zone (Hamann et al. 2007). A sexual dimorphism occurs in this species with females being much larger than males (Thomson et al. 2006). It is listed as critically endangered under the Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and endangered under the Queensland Nature Conservation Act 1992.

Figure 1.3: Adult female white-throated snapping turtle, *E. albagula*.

Elusor macrurus

The Mary River turtle (*E. macrurus*; Figure 1.4) is a monotypic species endemic to a single river system (Cann & Legler 1994). A recent review of the phylogenetic relationships of the short-necked turtles of Australia and New Guinea

showed *Elusor* to be one of the most ancient lineages of Australian freshwater turtle species, and the sister lineage to all species of *Elseya*, *Myuchelys,* and *Emydura* (Le et al. 2013). *E. macrurus* was found to have low mtDNA nucleotide variability when compared with other Chelidae (Schmidt et al. 2017). Microsatellite analysis indicated panmixia throughout most of its range with the exception of one tributary, Tinana Creek (Schmidt et al 2017).

Figure 1.4: Adult male, Mary River turtle, *E. macrurus*.

Several distinct morphological traits separate this turtle from its close relatives (Cann & Legler 1994). The enlarged tail of an adult male, a unique feature amongst chelids, can reach approximately 200 mm in length (plastron to tip of tail) and have a circumference of ~260 mm. The function of such large tail is not clear; though the extreme hypertrophy suggests a function associated with mating (Cann & Legler 1994). This species displays atypical chelid sexual dimorphism, with adult males being

larger than the adult females (Figure 1.5). The maximum straight carapace length (SCL) recorded in this study for an adult male was 436 mm, and for an adult female 364 mm (SCL), an increase of 17 mm and 16 mm respectively from previous studies (Flakus 2002; Limpus 2008; Micheli-Campbell et al. 2017). This makes adult male *E. macrurus* amongst the largest chelids in Australia (Limpus 2008), and one of the heaviest recorded for an Australia chelid (mass = 8.08 kg, Cann & Sadlier 2017). The home range of *E. macrurus* was previously shown to be ~5 km with no evidence of significant migration (Micheli-Campbell et al. 2017).

Figure 1.5: Sexual size dimorphism evident in *E. macrurus* (Mary River turtle) – adult female (left) and adult male (right).

Like other Australian freshwater turtles, *E. macrurus* has low-fecundity, delayed maturity, and a long reproductive cycle. Close relatives often lay two or more clutches within their respective breeding seasons (Georges 1983; Legler 1985; Legler

& Cann 1980), but laparoscopic studies suggest that *E. macrurus* lays only one clutch per year (Flakus 2002; Limpus 2008). Individuals aggregate near nesting banks during the nesting season and have been shown to revisit those same banks through the years (Cann 1998; Flakus 2002; Micheli-Campbell et al. 2013a). Breeding females lay their eggs into alluvial deposits of sand, with nests located up to 12.2 m above water level and up to 44 m from the water's edge (Espinoza et al. 2018; Micheli-Campbell et al. 2013a). Though these alluvial banks are reworked with each significant flooding event (Limpus 2008), the location of the major nesting banks appears stable. The present-day nesting distribution down-stream from Gympie (Figure 1.8a) is thought to resemble the nesting distribution described from the period of commercial egg harvest in the 1960s (Connell & Wedlock 2006; Flakus 2002; Micheli-Campbell et al. 2013a). There are no historical nesting records for up-stream of Gympie.

E. macrurus was subject to intense egg harvest in the lower reaches of the Mary River to supply hatchling turtles for the pet trade from 1962 to 1974 (Cann & Legler 1994). Between 1,200–1,500 *E. macrurus* eggs were harvested annually from the most productive banks, with 100–200 eggs collected from the less productive (Flakus 2002). By 2002, it was estimated that since 1974, the annual nesting population had declined by 95% in the reach that previously supported egg collection (Flakus 2002). Intense predation of turtle eggs by feral animals (European red fox, *Vulpes vulpes* and wild dogs, *Canis familiaris*), as well as by native species (monitor lizards, *Varanus* spp. and water-rats, *Hydromys chrysogaster*) has prevented the recovery of this species since the egg collection period (Flakus 2002; Limpus 2008). Another limiting factor in the recovery of *E. macrurus* population is its restricted geographic distribution to a single catchment in southeast QLD, Australia.

Emydura macquarii krefftii

Krefft's short-neck turtle (*E. m. krefftii*: Figure 1.6) is found in all the major eastern-flowing rivers of Queensland from north of the Brisbane River to Charlotte Bay in Cape York. It can be found in almost all freshwater habits including natural and man-made permanent and semi-permanent stream and pool habitats (Cann & Sadlier 2017; Limpus et al. 2011). The population of *E. m. krefftii* reaches high densities in impoundments (Haman et al. 2007; Trembath 2005).

Figure 1.6: Adult Krefft's short-necked turtle, *E. m. krefftii*.

Muchelys latisternum

The saw-shelled turtle (*M. latisternum*; Figure 1.7) has a wide distribution down the east coast of Australia from the Cape York Peninsula to the Richmond drainage in New South Wales (Freeman & Cann 2014). A population also occurs in the Northern Territory. This species inhabits deep to shallow pools and lagoons on

permanently flowing waterways particularly in the upper reaches and side channels of larger rivers with numbers often reduced in large rivers (Freeman & Cann 2014).

Figure 1.7: Adult saw-shelled turtle, *M. latisternum.*

Study location

The Mary River catchment covers an area of 9,595 km^2 in the sub-tropical southeast portion of QLD, Australia (Figure 1.8). The headwaters of the river originate in the Conondale Ranges and flows north for 307 km before emptying into the Ramsar-listed wetlands of the Great Sandy Strait, west of Fraser Island. The major tributaries of the Mary River include Obi Obi Creek, Yabba Creek, Six Mile Creek, Munna Creek, Wide Bay Creek, Tinana Creek and the Susan River. The main river channel remains largely unregulated, aside from a 2.9 m high tidal barrage that was constructed in 1982 at 59.3 km Adopted Middle Thread Distance (AMTD) from the river's mouth. This structure converted over 30 km of tidal brackish waters into a freshwater, lentic zone. The impounded waters from this structure extend to the approximate same point as the

head of the tide (Johnson et al. 1982). However, this zone has been excluded from this study due to differences in habitat features within lentic and lotic zones (Clark et al. 2009; Micheli-Campbell et al. 2017).

The Mary River supports a diversity of threatened aquatic species including the endemic Mary River Cod, *Maccullochella peelii*, the Mary River turtle, *Elusor macrurus* and two species that are endemic to this and neighbouring rivers, the Queensland lungfish, *Neoceratodus forsteri* and the white-faced snapping turtle, *E. albagula*. The Mary has one of the highest diversities of freshwater turtles within Australia (Limpus 2008).

Modifications have occurred to the Mary River and the riverine ecosystem since European settlement (Brizga 2004). Hydrological modification has occurred due to water resource development. The installation of the tidal Mary River barrage primarily to supply water for the sugar cane industry, converted flowing brackish water to impounded freshwater. Sand and gravel extraction have changed the bars from pre-European conditions. A range of grasses and legumes have been introduced which have decreased the mobility of bar sediments (Brizga et al. 2004). These modifications will have favoured some species over others (Tucker et al. 2012).

Since the 1970s, Queensland governments have considered a dam on the Mary River a viable option to augment the water supply for Brisbane. In 2006, during the worst drought in 100 years in southeast Queensland, the Queensland government proposed a dam be constructed at Traveston Crossing upstream of Gympie (Wasimi 2010). An enormous level of socio-political forces came together to oppose its construction. An independent expert noted that the dam would adversely affect habitat critical to the survival of *E. macrurus* (Kuchling 2009). The proposal was rejected in

2009 by the Federal Environment Minister, Peter Garrett, who determined that the impacts of the dam on threatened species, including the Mary River turtle, would be too great (Department Environment & Energy 2016).

Figure 1.8: a) The geographical location of the Mary River catchment (QLD, Australia) showing the four study reaches, Lower, Mid-low, Middle and Upper. b) Lower reach of the Mary River. c) Upper reach of the Mary River.

Community-driven conservation program

The Mary River turtle (MRT) conservation program was instigated in 2001 with TDLG as the key partner (Connell & Wedlock 2006). TDLG is one of several thousand individual groups operating around Australia under the Landcare banner. The Australian Landcare movement was formed in 1989 by the Australian Conservation

Foundation and the National Farmers Federation (Sobels et al. 2001). This was primarily in response to the scale of land degradation within Australia. People join Landcare on a voluntary basis with groups formed around a common interest, which is frequently an aspect(s) of natural resource management (Wilson 2004). TDLG membership is comprised of farmers, life-style rural landholders, and business people, who primarily live in the Tiaro district within a 30 km radius from the Mary River. The group was formed in 1998 by landholders who had common concerns, in this instance, the possible environmental impacts resulting from a government proposal to raise the height of the existing tidal barrage (Tiaro & District Landcare Group 1998). On learning of the plight of the Mary River turtle in the late 1990s, members initiated the MRT conservation program as they considered it their responsibility to care for a threatened species that occurred 'at their doorstep'. A multi-faceted program was then developed which incorporated direct and indirect conservation actions such as nest protection, fund-raising through the production and sale of chocolate turtles, awareness raising activities and supporting research (Flakus & Connell 2008). Throughout this period, TDLG have formed formal and informal coalitions with universities, catchment groups, state and local government, not-for profit organisations, landholders, and members of the wider community to maximise human capital in the conservation of *E. macrurus*. A key objective of the program has been to increase turtle recruitment through various predator management actions, which have aided thousands of hatchlings to reach the river. However, the fate of the hatchlings once they enter the river is unknown. Management decisions made by TDLG are likely to deeply impact upon the long-term survival of this species due to its limited geographic range of a single river.

Aims of research

The objective of the present research was twofold: 1) to investigate assemblages of freshwater turtles within the Mary River (QLD, Australia), and 2) investigate the population structure of an endangered turtle species, *E. macrurus*, to determine the effectiveness of a conservation program in aiding the recovery of its population.

First, a standardised sampling protocol was developed that was effective for sampling multiple turtle species within a lotic environment and was sufficiently robust to allow for statistical comparison of assemblages and identify spatiotemporal differences (Chapter 2). Sampling for freshwater turtles has in the past, frequently occurred in an opportunistic, unsystematic manner, with a focus on maximising the capture rate. Consequently, in those instances, the number of turtles captured is a snapshot measure of capture efficiency rather than a measure of true abundance appropriate for a population trend-monitoring program over space and time.

Second, an assessment was made of the impact of a long-term conservation program on the population of *E. macrurus* (Chapter 3). Increasing recruitment is a goal of sea and freshwater turtle conservation projects. For the most part, similar conservation techniques are used world-wide, yet there are no standard criteria nor method for determining success (Burke 215). To evaluate the success of the conservation program, I hypothesised that the population structure in the river reach where the nest protection program operated would have a higher frequency of immature turtles than where implementation had not occurred.

The third aim was to use the study findings to provide direction for management and conservation strategies for *E. macrurus* (Chapter 4).

Structure of thesis

This thesis is composed of two experimental chapters. The first experimental chapter deals with the development of a standardised sampling methodology and its effectiveness in identifying differences in turtle assemblages arrayed along the main river channel. The second experimental chapter estimates the population of *E. macrurus* and compares the population structure at each of the four sampling reaches. Each chapter is a complete work, containing an abstract, introduction, materials and methods, results and discussion sections. The final chapter of this thesis concludes by summarising the findings of the two experimental chapters and provides insights for management and conservation of the endangered *E. macrurus* and presents considerations for future research.

Chapter 2

Identifying discrete assemblages of river turtles using a passive and systematic capture technique

"Next morning, we were back on the Mary. John rowed while I dived, my depth restricted to 1.5m, at which level, visibility, looking up, was only about 80cm. Still, I caught Elseya sp. aff. dentata and E. krefftii. Still he was sure I would succeed (in catching a Mary River turtle) if I stayed another couple of weeks!"

(Cann 1998).

Abstract

Understanding the significance of a river reach to a particular species is critical for informing riverine restoration and management. Generally, the relative significance of a river reach for freshwater turtles is based upon species richness or species counts. However, species counts can be greatly influenced by the capture method(s) employed, species behaviour, localised in-stream conditions and the operator's knowledge and skill. Here I used a protocol that standardised the sampling effort at 20 study sites along the Mary River (QLD, Australia). A large funnel trap with 30 m wing span was deployed at each site and turtles captured over a 4-day period. The location and method of net deployment was repeated identically every 6 months over a two-year period. The turtle species assemblages significantly differed between the upper, mid, and lower catchment (Multi-Variate Analysis of Variance, $P < 0.05$), suggesting species preference for the broad geomorphological and ecological features of each reach. The observed spatial variance in species assemblage was consistent over time and unaffected by the season, illustrating the robustness of the sampling technique. The use of a passive turtle capture method, which can be unattended for prolonged periods, repeated identically and numerously across space and time, provide a robust technique for sampling turtle assemblages. The technique is cheaper and easier to implement than abundance counts and ensures that capture biases remain constant under different conditions and operators. Standardisation of protocols and methods within and amongst studies ensure biases remain constant and thus trends in turtle assemblages can be detected over space and time. These trends may be used to alert natural resource managers to shifts in conditions and ecological health of the river of stretches of the river.

Introduction

Species richness may be similar across an ecosystem; however, discrete patterns of species abundance may characterise assemblages within the same ecosystem (Bluett et al. 2013; DonnerWright et al. 1999; Moll & Moll 2004). Ecological demands of specific taxa and processes operating at different scales can influence the dynamics within an assemblage (Bluett et al. 2011; Habel et al. 2016). At the broader scale, the extent to which biodiversity change in local assemblages contributes to global biodiversity loss is poorly understood (Dornelas et al. 2014). Environmental modification is one the processes that is driving changes to the composition and relative abundance of species within assemblages (Habel et al. 2016; Smith et al. 2006). This process may cause shifts in species abundance resulting in either a positive and/or negative effect upon single or multiple species (Browne & Hecnar 2007; Moll & Moll 2004). Thus, comparative studies of the assemblages' composition will allow for an evaluation of potential responses of taxa and assemblages to environmental changes over time. Implementation of conservation and management decisions are typically at the scale of local or regional ecosystems, thus knowledge of change within assemblages is essential to inform policy as well as to evaluate the efficacy of conservation strategies (Habel et al. 2016).

Freshwater turtles are present in many freshwater systems throughout the tropics, subtropics, and temperate zones, where they occur in assemblages of up to 17 species (Moll & Moll 2004). Like other wildlife (Habel et al. 2016; Wilson 2008), turtles can be grouped according to their ecological tolerance and degree of specialisation (Moll & Moll 2004). The effects of environmental disturbance on a species can be influenced by the level of its specialisation (Wilson et al. 2008). Generalists are adaptable, extremely resilient and may thrive in human-altered

environments often more than they do under natural conditions relative to specialists (Eskew et al. 2010; Roe et al. 2011). For example, the population and biomass of *Phrynops geoffroanus* (Geoffroy's side-necked turtle) was found to be elevated in a polluted river is south-eastern Brazil (Souza & Abe 1999). Conversely, ecological specialists have specific ecological demands and thus are assumed to be more prone to extirpation from anthropogenic factors (Habel et al. 2016; Kennett & Tory 1996; Tucker et al. 2012). For example, the loss of lotic habitat in impoundments has favoured a generalist species, *Emydura macquarii krefftii,* which had a greater relative abundance than the more specialised *Elseya albagula* and *Muchelys latisternum* (Hamann et al. 2007; Tucker et al. 2012). Thus, alteration of habitat can have the effect of shifting the relative abundance of generalists and specialists within a Chelonian assemblage (Browne & Hecnar 2007; Moll & Moll 2004). For this reason, abundance alone may not indicate an ecologically healthy ecosystem, as habitat alteration may not affect all turtle species equally (Moll & Moll 2000).

Time series monitoring, a tenet of adaptive management, is critical for understanding trends in riverine turtle assemblages, as the effects of environmental changes may take years to detect and overcome. Thus, given the length of time required for a change to become evident, it is critical that capture protocols are repeatable across time to detect temporal changes in species diversity and assemblage composition (Gibbons et al. 1997; Habel et al. 2016; Wallace et al. 2007). It is critical for management of species and their habitat, that trends in relative abundance of species within assemblages through space and time are identified. Assessing seasonal, temporal, and spatial variation in assemblages of cryptic and elusive species, such as freshwater turtles, can be challenging due to variable patterns in seasonal behaviour

and environmental conditions. These variables will determine selection of appropriate capture technique.

Multiple techniques used to capture freshwater turtles. The biases inherent in each technique may lead to a misrepresentation of certain species relative to their actual abundance (Breen & Ruetz 2006; Hubert et al. 2012). For example, local environmental conditions can affect each method and thus vary in effectiveness based on the biology of available species (Cagle & Chaney 1950; Frazer et al. 1990; Gamble 2006; Wallace et al. 2007). Factors such as species biology, operator proficiency, and environmental conditions make it difficult to compare results across the various trapping methods because the variation in the probability of capture among species (Cagle & Chaney 1950). However, when sampling protocols and methods are standardised within and among studies, these biases remain constant and trends in turtle assemblages can be detected through space and time (Hill 2005).

Often turtle surveys are carried out in an opportunistic, unsystematic manner with a focus on maximising the capture rate (Sterrett 2010). Consequently, in those instances that the number of turtles captured is a snapshot measure of capture efficiency which may be appropriate for presence/absence studies, but not for comparative studies. When dissimilar capture techniques are employed, the results can be skewed in comparative population studies (Gibbons et al. 1997). Many studies have focused on ways to improve the accuracy of turtle surveys by either: (a) investigating the efficiency and bias of trapping methods (Gamble 2006; Ream & Ream 1966; Sterrett et al. 2010; Stone et al. 1993; Vogt 1980; Weber & Layzer 2011), or (b) making improvements to trapping protocols (Bluett et al. 2011; Frazer et al. 1990; Larocque et al. 2012b; Mali 2012).

In the Mary River in Queensland, there exists six species of freshwater turtles (*Elusor macrurus*, *Elseya albagula*, *Emydura macquarii krefftii*, *Myuchelys latisternum*, *Chelodina expansa*, and *Chelodina longicollis*). It is not known if the turtle species assemblage significantly varies throughout the river as a result of local environmental conditions.

Like many other aquatic organisms, I predict that turtle assemblages (based on relative species abundances) will shift along the river continuum and that Krefft's turtle, *E. m. krefftii* be most abundant given its generalist nature. Because I utilized a standardized protocol and most Australian riverine turtles are not migratory, I predict that turtle assemblages will not vary among and between seasons and years.

Materials and Methods

Study site

The study area encompassed approximately 180 km of the Mary River (QLD, Australia; Figure 1.8). This subtropical river flows in a northerly direction for approximately 307 km. Most stream flow occurs throughout the Austral summer and early autumn months of January to April. While the occurrence and intensity of rainfall is irregular, the flow during the dry season period of July to October is relatively stable (Figure 2.1; Pusey et al. 2004; Queensland Department of Natural Resources and Mines 2017). The low level of flow regulation and instream infrastructures (a barrage downstream of the study area) within the main channel pose minimal obstruction to movement (Figure 1.8; Brizga et al. 2004).

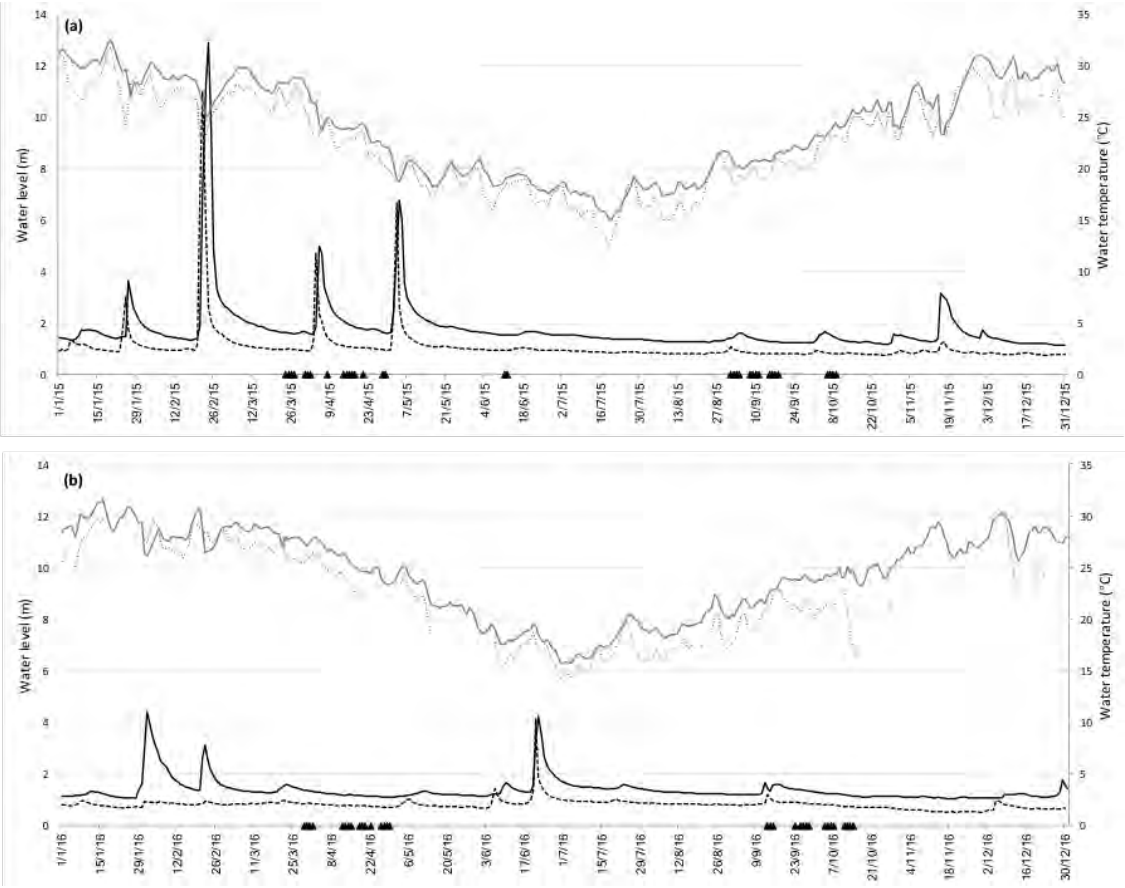

Figure 2.1: Hydrograph of Mary River heights and water temperatures at (a) Bellbird Creek and (b) Home Park Gauging Stations during 2015 and 2016 trapping episodes. Source: Department Natural Resources & Mines.

The Mary River catchment supports one of the highest diversities (six species) and endemicity (two species) of freshwater turtles in Australia (Cann, 1994; Limpus 2008). The Mary River turtle (*Elusor macrurus*) and the white-throated snapping turtle (*Elseya albagula*) are river specialists (Flakus 2002; Thomson et al. 2006). Krefft's turtle (*Emydura macquarii krefftii*) - a generalist - does not show any preference and can be found in lentic and lotic environments and man-made pools (Tucker et al. 2012). The saw-shelled turtle (*Myuchelys latisternum*) prefers the upper reaches and side channels of larger rivers (Freeman and Cann 2014). The broad-shelled turtle (*Chelodina expansa*) and the snake-necked turtle (*Chelodina longicollis*) have

preference for shallow, ephemeral wetlands often remote from a river (Kennett et al. 2009).

The study was confined to the freshwater lotic reaches of the river, thus determining the location of the downstream study reach. Four study reaches were selected with each consecutive reach spaced at 60 km intervals (Figure 1.8). The Upper river reach (Adopted Middle Thread Distance [AMTD] 264 km from river mouth) is characterised by a generally narrow valley, low to moderate channel sinuosity, with pools and runs interspersed by long reaches where the transient channel moves across a shallow braided bed (Brizga et al. 2004). The Middle reach (AMTD 208 km) consists of highly sinuous meandering units, composed of longer pools intercepted with substantial lengths of riffle and glide habitat. The Mid-low (AMTD 143 km) and Lower (AMTD 86 km) reaches are typified by reduced stream slope, a highly sinuous channel, and long deep pools with occasional riffles. Because of these distinctive features and the distance from the river mouth, the study sites will be referred to as 'Upper', 'Middle', 'Mid-low', and 'Lower', respectively (Figure 1.8). The stream bed material was comprised of sand, gravel, cobble stones or bed rock and varied between sites. Five sampling sites, approximately 1 km apart, were located within each reach. Accessibility, landholder agreement, and geomorphology dictated the final selection of each sampling site.

Sampling methodology

A passive sampling technique, fyke nets, was used for this study (Figure 2.2). Set net, such as fyke nets are commonly used as a standardised sampling method when monitoring fish and turtle populations (Vogt 1980). Passive sampling involves the

capture of the target species by a device that is not actively moved by humans or machines while the organisms are being captured, thus facilitating standardisation of sampling gear (Hubert et al. 2012). Unlike hoop nets, fyke nets have a single, double, or even triple leaders that assist in guiding the animal into the hoop sections. Fyke nets been successfully used in large rivers, creeks, fast moving water, small ponds and large lakes (Breen & Ruetz 2006; Vogt 1980; Wallace et al. 2007).

The net dimensions, mesh size, size of mouth, and funnel openings were specific to the physical turtle sizes, ranging from hatchlings (35 mm) to large adults (436 mm). The cylindrical section of the net was 4 m in length (from mouth to cod-end) and included four 0.9 m diameter aluminium hoops with two internal funnels, each having a fixed opening of 0.4 m. The entire fyke net was covered with 20 mm (stretched) mesh made from 2 mm braided cord. A polystyrene float was placed in each compartment to provide space for captured turtles to surface for air (Figure 2.2c; Larocque et al. 2012a). The cod-end of the net was secured to a metal star-picket as an additional measure to maintain access to the surface for trapped animals. Two leaders extended from either side of the mouth of the hoop net to form a 'V' (Figure 2.2a). Each leader was 10 m long with a drop of 1.2 m. The ends of each leader were secured with metal star-pickets, set approximately 7-10 m apart, with the end of one leader set on the water's edge. A float line ran along the top of each leader. To ensure the nets sat firmly on the riverbed, 1 m lengths of 8 mm galvanised chain were randomly clipped at random intervals along the mouth and the bottom of both leaders to reduce the possibility of individuals passing beneath the net (Figure 2.2b).

The nets were set facing upstream, parallel to the river bank within the vicinity of a riffle. Here the water flow and depth were conducive to the physical dimensions of the set-nets. Sampling sites were excluded from below riffles due to higher flow

rates, which created an unsafe environment for researchers. To standardise sampling protocols, each net was set in the same location and for the same time-period on subsequent sampling episodes. The nets were not baited and relied on the turtles encountering and entering the net. Each morning, all the nets were checked, the by-catch released, litter removed, and the turtles processed.

Figure 2.2: Trapping technique: a set fyke net showing leaders, float line, mouth, funnel throats and cod-end. (a) Funnel section set parallel to riverbank, facing upstream with double lead-in wings. A float was placed in each chamber and the cod-end was secured to a post. (b) Additional weights added to the bottom of the leaders. (c) Captured turtles within one of the net compartments, and (d) turtles removed from net.

Sampling occurred n two discrete seasons: austral spring (September to October) and autumn (March to April) over a two-year period (21015-2016).

Throughout these seasons, there is a reduced likelihood of flood events thus minimising the risk of a trapping event being compromised by a rise in the river height. Nonetheless, minor flooding occurred in April/May 2015, which delayed the completion of initial autumn sampling until June 2015 (Figure 2.1). Five nets were set over four consecutive nights within each of the four sampling reaches (a total of 320 trap nights, i.e. 20 nets set within each of the four sampling reaches over four sampling periods).

Turtle processing

Species were identified by head and plastron characteristics (Cann 1998). Sex was determined by dimorphic tail sizes in all species (McDiarmid et al. 2012). Although carapace length is not an absolute indicator of reproductive status, assigning a constant straight carapace length (SCL; minimum) allowed for individuals of all species to be assigned an age class. Sex of individuals of all species with a SCL of < 150 mm could not be determined with confidence, thus all were considered juveniles with no discrimination between species (Thomson et al. 2006). Only the SCL of all juveniles of every species was measured. The number of male, female, and juvenile turtles of each species captured within each reach was recorded.

Data analysis

A non-parametric multivariate analysis of variance, PERMANOVA - R (Version 3.4.1) package Vegan - was conducted to assess for the influence of river reach, season, year, as well as the interactions between these variables (Anderson 2001; R Development Core Team 2016). The model was run using 200 random

permutations. Post-hoc pair-wise comparisons were run using an F-test. The Hellinger transformation was used to offset the zeros in the model before applying the Bray-Curtis index. Non-metric multi-dimensional scaling (nMDS) was used to visualise differences (i.e. dispersion or clustering) among data in ordination space (Clarke & Warwick 2001). In both the nMDS and PERMANOVA, dissimilarity matrices were calculated using the Bray–Curtis index on transformed (square root) abundance values ($species\ assemblage = dis.bray \sim stretch * season * year$). Bubble plots of raw species abundance were plotted for each trapping site on the nMDS in PRIMER® to show the dispersal and separation of species and trapping sites. Rarely captured species were removed from the PERMANOVA analysis (Clarke & Gorley 2006).

Results

Distribution and abundance

A total of 782 individuals were captured during the two years of sampling (Appendix 1). *Elusor macrurus* was most frequently trapped followed by *Elseya albagula*, *Emydura macquarii krefftii*, *Myuchelys latisternum*, *Chelodina expansa,* and *Chelodina longicollis* (Table 2.1). Although the number of trapped turtles across each of the four reaches was similar, species relative abundance varied between study locations (Figure 2.3). In the Upper reach, the abundance of *E. albagula* was greatest but their numbers gradually decreased moving upstream. The abundance of *E. m. krefftii* and *E. macrurus* both peaked within the Middle reach, with a reduction in abundance up and down stream of the Middle Reach. These species were at their smallest abundance in the lower reach. The Upper reach had highest number of *M. latisternum*.

Table 2.1: A comparison of three studies that used different survey methods showing relative species abundance. Each study was conducted in the Mary River, QLD.

Species	Survey method		
	Multiple (Limpus 2008)	Snorkelling (Thomas 2007)	Set net (this study)
E. macrurus	26%	29%	38%
E. albagula	31%	42%	31%
E. m. krefftii	40%	27%	26%
M. latisternum	3%	1%	4%
C. expansa	n/a	1%	<1%
C. longicollis	n/a	n/a	<1%
Total number of individuals	554	1 032	782

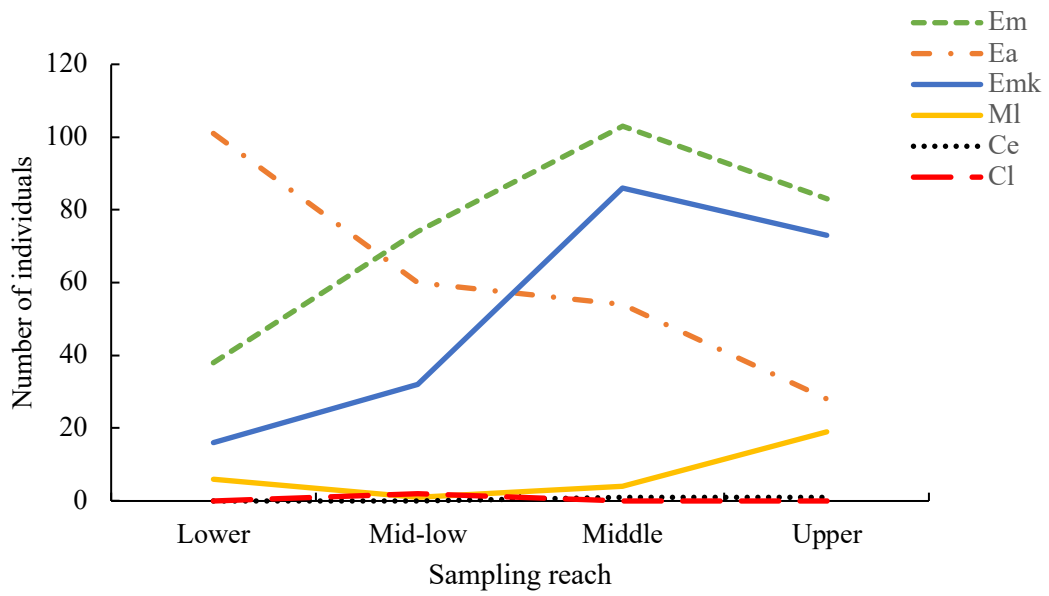

Figure 2.3: Spatial distribution of individuals from each species across the four sampling reaches – Em (*E. macrurus*), Ea (*E. albagula*), Emk (*E. m. krefftii*), Ml (*M. latisternum*), Ce (*C. expansa*) and Cl (*C. longicollis*).

Three significantly discrete turtle assemblages were identified over the four sampled reaches using PERMANOVA (Tables 2.2 and 2.3). The assemblages within the Lower and Mid-low reaches were similar ($F_{3,80}$ = 1.6, P = 0.19), but significantly different from the Middle reach (Lower $F_{3,80}$ = 11.34, P < 0.01; Mid-low $F_{3,80}$ = 4.03, P < 0.01), and the Upper reach was significantly different from the other sampled reaches (Lower $F_{3,80}$ = 9.02, P < 0.01; Mid-low $F_{3,80}$ = 4.73, P < 0.01; Middle $F_{3,80}$ = 3.04, P < 0.05). The assemblages were not significantly altered by either the season or the year of capture (Season $F_{1,80}$ = 1.05, P = 0.38; Year $F_{1,80}$ = 1.77, P = 0.17). Two species, *C. expansa* and *C. longicollis,* were infrequently captured and consequently were removed only from the PERMANOVA analysis.

Table 2.2: Results from PERMANOVA paired-wised comparisons to test for significant differences in turtle assemblages between river reaches.

Paired Comparisons	*F* model	R-squared	*P* Value
Lower vs Mid-low	1.614	0.039	0.190
Lower vs Middle	11.345	0.229	0.003
Lower vs Upper	9.025	0.191	0.003
Mid-low vs Middle	4.031	0.093	0.026
Mid-low vs Upper	4.736	0.108	0.008
Middle vs Upper	3.043	0.074	0.05

Table 2.3: Results from three-way PERMANOVA test showing the influence of co-variables on the turtle assemblage.

	df	Sum of squares	Mean squared	F. Model	R-squared	Pr (>F)
Season	1	0.090	0.090	1.051	0.011	0.363
Year	1	0.158	0.158	1.838	0.019	0.184
Site	3	1.442	0.481	5.597	0.175	0.005
Season:Year	1	0.140	0.140	1.632	0.017	0.224
Season:Site	3	0.134	0.045	0.519	0.016	0.781
Year:Site	3	0.027	0.009	0.106	0.003	0.980
Season:Year:Site	3	0.666	0.222	2.584	0.081	0.010
Residuals	65	5.581	0.086		0.678	
Total	80	8.238			1.000	

Species assemblage differences by reach, were evident in PERMANOVA, but were less obvious in the 2-D nMDS plots (Figure 2.4b). More of the variability in the data was explained by the 3-D nMDS plot (Stress 0.1) compared to the 2-D plot (Stress 0.17). Whilst the 2-D nMDS still provides a useful picture, the stress value obtained for the 3-D plot corresponds to a good level ordination of the information (Clarke & Warwick 1994). In the 3-D nMDS plot (Figure 2.4a) the Upper reach is ordinated in a different plane to the other reaches. The combination of the spatial distribution of species (Figure 2.3), the nMDS plots (Figure 2.4) and the bubble plots (Figure 2.6) enable interpretation of the data and indicate the species most responsible for dispersion of data. Although only a small number of *M. latisternum* were captured (Table 2.1), it is the abundance of this species primarily in the Upper reach that is responsible for the separation of this reach from the others (Figure 2.3). The bubble plots revealed the effect of *M. latisternum* in the Upper reach on the dispersion of the

data and ordination changes between the Upper reach and other reaches (Figure 2.5). *E. albagula* had a similar effect in the Lower reach. Likewise, changes in abundances of *E. m. krefftii* and *E. macrurus* across the reaches also cause separation of the reaches in ordination. The nMDS and bubble plots are consistent with PERMANOVA results and indicate the dissimilarity of turtle assemblages between sites.

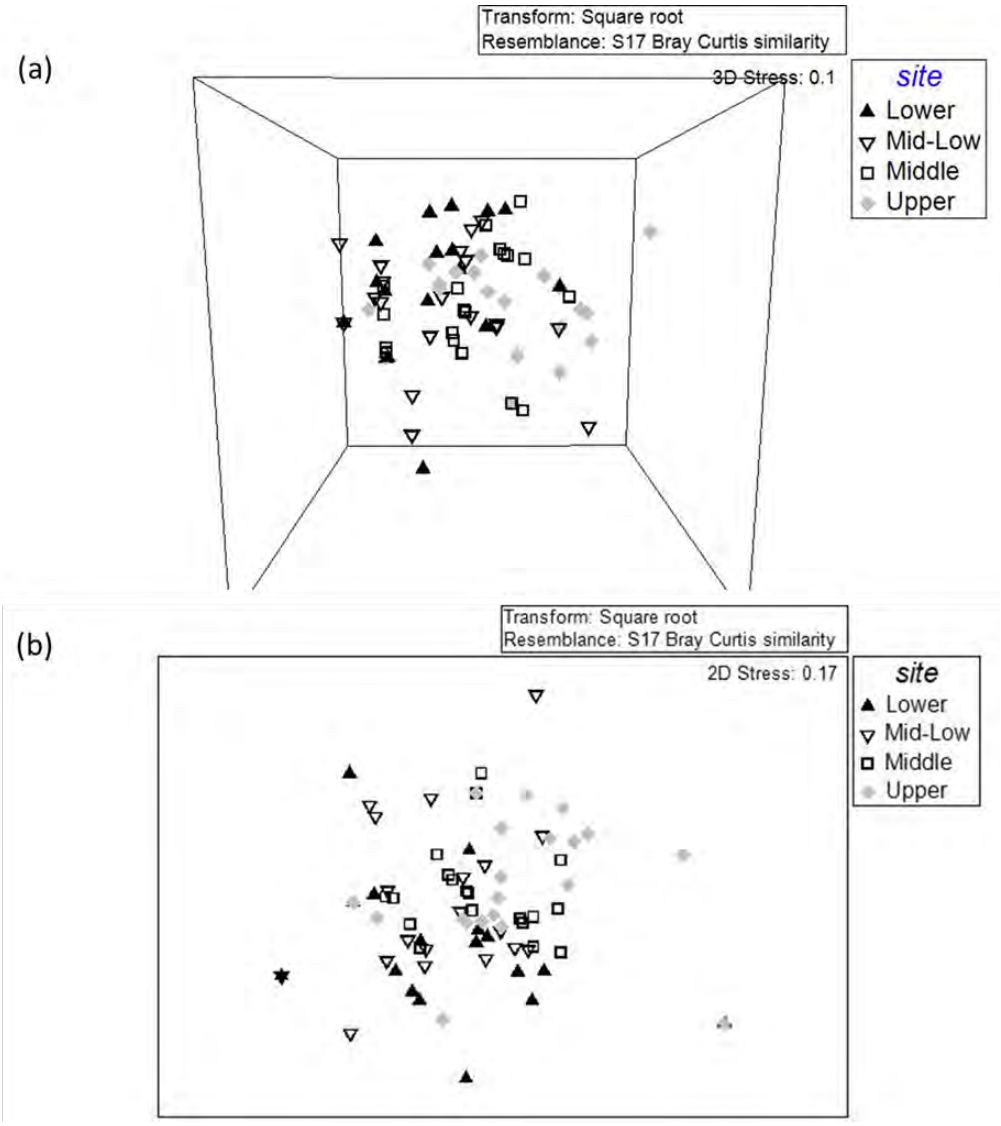

Figure 2.4: Non-metric multidimensional scaling (nMDS) plot of turtle assemblages in the Mary River, (a) 3-D and (b) 2-D highlighting the impact of site. Data points relate to individual trapping events.

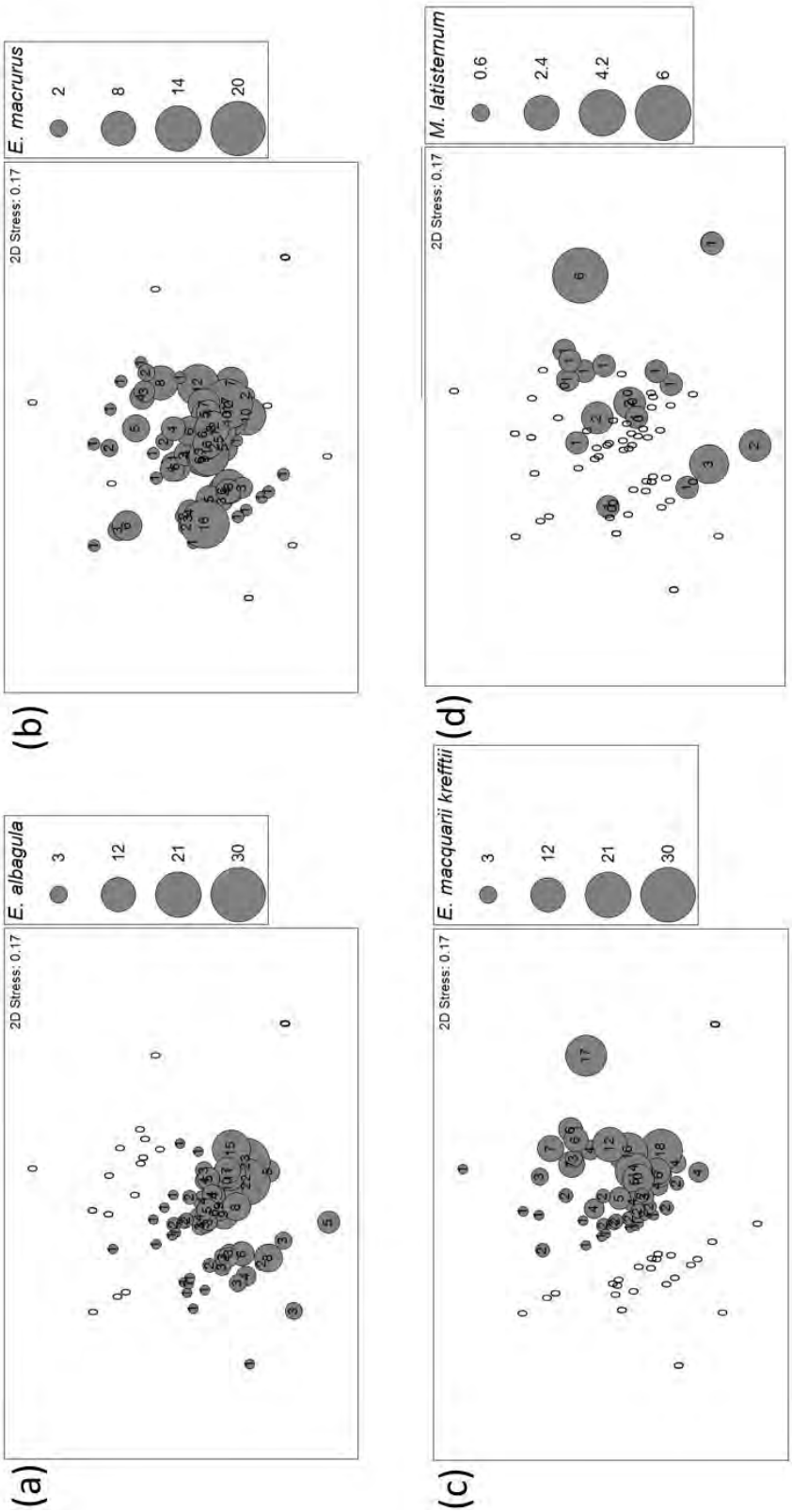

Figure 2.5: Bubble plots of raw species abundance overlaid on the 2D nMDS for (a) *E. albagula*, (b) *E. macrurus*, (c) *E. m. krefftii* and (d) *M. latisternum*. Data points relate to the number of individuals caught per trapping event at each site. Zeros represent locations where no turtles were caught.

Juveniles comprised 6% of the overall number of captured turtles (Figure 2.4). The Upper reach yielded 67% (*n* = 33), while the Lower reach contained only 4% (*n* = 2) of juveniles captured. Four species (*E. macrurus, E. albagula, E. m. krefftii, M. latisternum*) were represented in the Upper reach, three species in the Middle (*E. macrurus, E. m. krefftii, M. latisternum*) and Mid-low reaches (*E. macrurus, E. albagula, E. m. krefftii*), with two species (*E. albagula, M. latisternum*) found in the Lower reach. However, an insufficient total number of juveniles were captured within each reach to undertake statistical analysis of species assemblage differences.

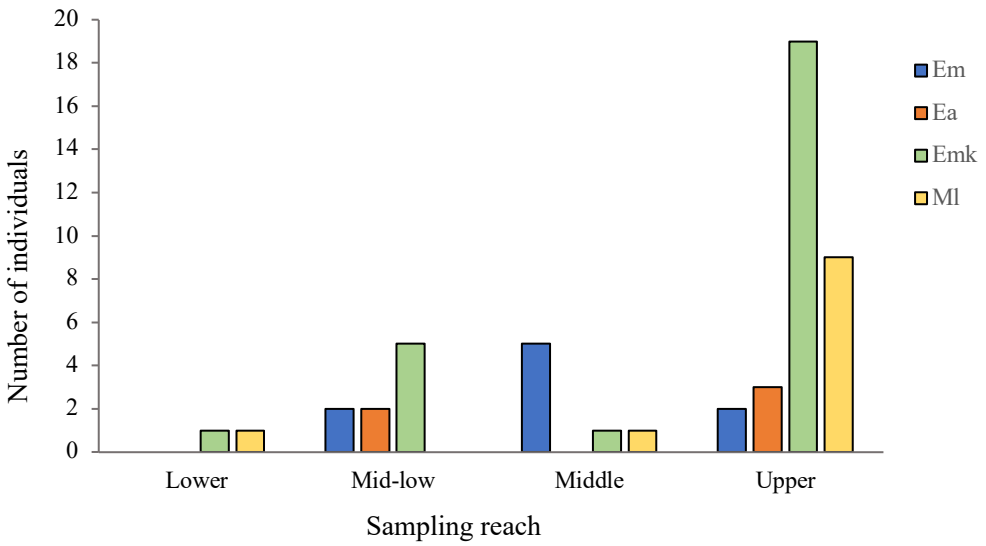

Figure 2.6: Number of juveniles of each species captured per study site – Em (*E. macrurus*), Ea (*E. albagula*), Emk (*E. m. krefftii*), Ml (*M. latisternum*).

Sex ratios

No significant difference in abundance of male turtles was evident between reaches (F <0.9; DF = 3, 80; P <0.05). However, there were three discrete species assemblages for females: 1) the Mid-Low (F = 9.34; DF = 3, 80; P <0.01), 2) the

Middle (Lower F = 8.51; DF = 3, 80; P <0.01), and 3) the Lower and Upper reaches being similar (F = 1.5; DF = 3, 80; P = 0.20). Season and year of capture did not significantly alter the assemblage for either males of females (Season: F = 1.05; DF = 1, 80; P = 0.38; Year: F = 1.77; DF = 1, 80; P = 0.17).

Males were most abundant for *E. macrurus* and *M. latisternum*, whereas only female *C. expansa* and *C. longicollis* were captured (Figure 2.7). The number of males and females captured for *E. albagula* and *E. m. krefftii* were not significantly different.

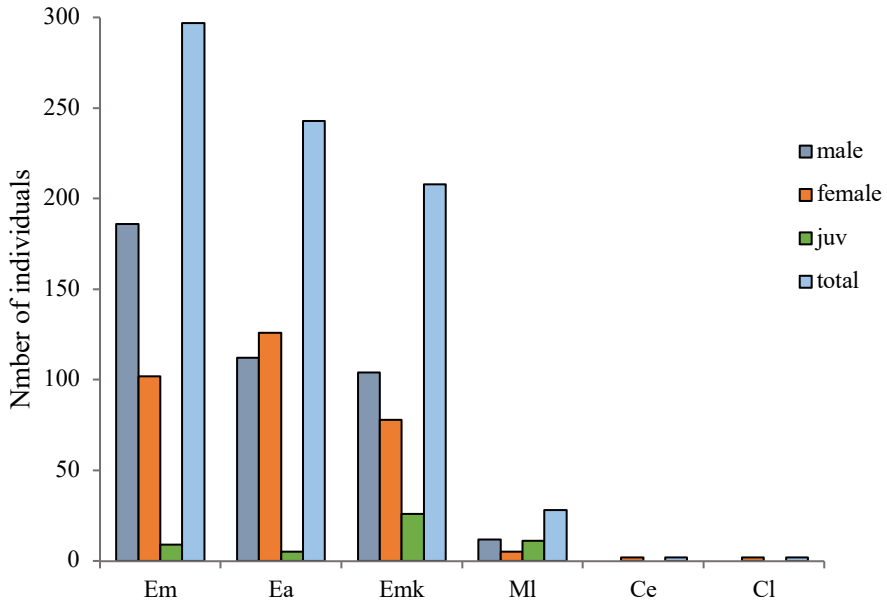

Figure 2.7: Number of males, females, juveniles, and total per species captured in the present study – Em (*E. macrurus*), Ea (E. albagula), Emk (*E. m. krefftii*), Ml (*M. latisternum*), Ce (*C. expansa*), and Cl (*C. longicollis*).

Discussion

This study revealed significant changes in the relative abundance of the six-turtle species along the length of the Mary River, in Queensland Australia. Although

all species were captured throughout the river, different reaches of the river served as hot spots for particular species. The spatial variation in species assemblages was consistent through time, illustrating the robustness of the sampling method for defining freshwater turtle assemblages. Only a few studies have previously investigated riverine turtle assemblages along a river gradient (DonnerWright et al. 1999; Bluett et al. 2013) but I argue that it is a quick and easy method to assess turtle community health. These studies investigate the relationship between environmental gradients (DonnerWright et al. 1999) and stream order (Bluett et al. 2013) on species richness or abundance. Like Bluett et al. (2013), this study showed that turtle assemblages vary along the continuum of the Mary River.

Although it was beyond the scope of the study to identify the ecological processes (for example competition or specific environmental gradients) that are driving the composition of assemblages (like DonnerWright et al. 1999), this study showed an association between variation in the abundance of specific species and broad geomorphological features, such as stream reaches.

The abundance of numerous aquatic organisms from a variety of taxonomic groups and community assemblages have been found to vary predictably along the entire length of river systems (Vannote et al 1980). For example, a longitudinal study of the Meuse River in Europe found a gradual shift from a macroinvertebrate assemblage dominated by insects to a community dominated by crustaceans and molluscs (Usseglio-Polatera 2002). My study also detected variation in species assemblages along the river gradient and the significance of specific reaches for individual species. For example, *Elseya albagula* was most numerous in the Lower reach, suggesting the significance of this reach for this species. Importantly, this spatial difference in assemblage was consistent among seasons and years.

This spatial variation of species abundance varied predictably within the known habitat requirements of individual species. It is indicative of variation in stream morphology, species niche requirements, anthropogenic influences, and exotic competitors (Morrison et al. 2006). *E. albagula* tends to prefer slow moving deep pools, and feeds on filamentous algae and crustaceans foraged from the muddy vegetated shallow margins of deep water pools (Micheli-Campbell et al. 2017). Hence, it was expected that this species would be more abundant in the Lower and Mid-low reaches, where the pools are deeper and longer. In contrast, *Elusor macrurus* has a significantly larger, linear home-range, frequents riffle zones, and prefers different food items, such as bivalves, gastropods, and aquatic insects that are found within rocky riffles (Micheli-Campbell et al. 2017). The abundance of this species in the Middle reach suggests that this reach has the most appropriate ratio of pool-riffle sequences, and therefore more food sources. *Myuchelys latisternum* prefers deep to shallow pools typified in the upper reaches and side channels of rivers, and is chiefly carnivorous, occasionally feeding upon vegetative material (Freeman and Cann 2014). Accordingly, this species was most abundant in the Upper reaches. *Emydura macquarii krefftii* is known to inhabit a wide range of natural and man-made water bodies, and has an omnivorous diet consisting of filamentous algae, sponges, and terrestrial insects (Hamann et al. 2008; Limpus 2008; Wilson and Lawler 2008). This is the only generalist species of the Mary River. The abundance of this species followed a similar distribution pattern to *E macrurus*, and this was unexpected given it has the least habitat specialisation. It was anticipated that given its generalist nature, the relative abundance of *E. m. krefftii* would be consistently high across all reaches. This suggests that the quality of the habitat has not declined to such an extent to select this species over the more specialised ones. The two *Chelodina* species (i.e., *C.*

expansa and *C. longicollis*) prefer lentic habitats, and thus the low capture rates observed were expected (Kennett et al. 2009; Bower and Hodges 2014), though their non-detection within a reach does not imply it was genuinely absent.

Our methodology yielded different relative abundances of individual species when compared to previous studies that employed alternative sampling techniques (i.e. snorkelling, dip netting, and visual surface sightings; Table 1). *E. macrurus* was the most abundant species in our study (0.39 across reaches); however, a snorkelling study within the same reach reported a relative abundance of only 0.29 (Thomas 2007; Table 1), whilst a study utilizing multiple capture methods (snorkelling, dip netting, puddling, and visual surface sightings) reported a relative abundance of only 0.18 (Limpus 2008; Table 2.1). While this comparison may suggest the influence of capture methods on detection probability, the variation in capture methods and sampling protocols precludes rigorous comparisons. The snorkelling study was a single episode methodology across multiple seasons, and presumably capture method was affected by operator, and local river conditions (Thomas 2007). For example, the abundance of a highly mobile species like *E. macrurus* may have underestimated due to their visibility being inhibited by presence of dense beds of macrophytes, turbidity, the size and depth of pools, and difficulty in hand capturing (Thomas 2007). Whereas the abundance of a less mobile species, such as *E. albagula*, may have been underestimated in our study as it is more likely to be captured by snorkelling, but less likely to be captured in a set-net. The habitat generalist, *E. m. krefftii* was most abundant when multiple techniques were used: snorkelling, cathedral traps (telescoping, vertical, cylindrical nets), seine and dip nests, and muddling (Limpus 2008), thus using a single capture method, such as in the present study, may have underestimated its abundance.

Most juveniles were captured in the Upper reach. Other studies have also found the upper reaches to yield the highest capture rate of juvenile turtles (DonnerWright et al. 1999). The proportion of juvenile turtles was low for all species (varied from 0.02 – 0.13), with exception of *M. latisternum* (0.37). Entrapment equipment can affect catch and escape rates and may explain the low juvenile capture rate. While the dimensions of the mesh, 20 mm, would preclude juveniles escaping through the mesh, the dimensions of the net funnel would not preclude them from escaping through the mouth.

Typically, very few studies capture juvenile turtles in the water and low numbers are not infrequent in turtle surveys (Hamann et al. 2008; Pike et al. 2008; Tesche and Hodges 2015). This likely reflects the population dynamic of freshwater turtles in general, but also may be due to young turtles being cryptic and less mobile (Micheli-Campbell et al. 2013; Micheli-Campbell et al. 2017). Previous snorkelling studies in the Mary River captured a greater proportion of juvenile turtles (0.125; Thomas 2007) than in the present study (0.062). Whilst I accept that differences in capture method may be partially responsible for the disparity, the 50% decline is worrisome and worthy of future investigation. These studies were completed ten years prior to the present study and whilst it may be a bias of my capture technique, it is worth investigating further as it may demonstrate an actual decline or change in recruitment processes

Studies that have investigated riverine turtle assemblages are sparse (Bluett et al. 2013; DonnerWright et al. 1999). Those studies explored the influence of environmental gradients or stream order on species richness or abundance, rather than testing the robustness of the method to detect spatial variation over time. While this study suggested an association of abundance for specific species with broad

geomorphological features, it was beyond the scope of the study to identify the processes driving the composition of assemblages. A study of the environmental features and abundance of the two threatened turtle species of the Mary River was undertaken by Collett (2017). That study found the abundance of *E. albagula* was influenced by the presence of pebbles in the riverbed, whereas the abundance of *E. macrurus* was influenced by the presence of algae in the substrate, in-stream condition, and mesophyll to notophyll vine forests fringing the waterway.

Numerous explanations have been offered for sex ratios that differ from 1: 1 (Lovich et al 1990). Overall, males were the dominant sex for *E. macrurus* and *M. latisternum*. Sex ratio at hatching can be an influencing factor. However, unlike many other turtle species, the sex of *E. macrurus* is not determined by temperature, thus influences such as climate change is unlikely to be a factor in the sex ratio (Georges & McInnes 1998). Sampling bias has been indicted as an explanation (Ream & Ream 1966). Thus, my results may be biased as capture probabilities are likely to be higher for male *E. macrurus* and *M. latisternum* if they are more mobile than females as passive sampling methods rely on the individual encountering the net.

Advantages and limitations of the methodology

The capture technique used in this study was simple to use and, although it requires an initial outlay of capital, ongoing costs are minimal. It requires little specialised training to operate and does not need constant observation. Consequently, a high number of locations can be targeted simultaneously, with low manpower and undertaken by individuals with only rudimentary training. In-stream conditions, such as increased turbidity, operator efficiency and lack of subjective decision-making inherent in many other capture processes do not apply to this technique, thus enabling

the sampling of the turtle species assemblages to be replicated identically across space and time (Hubert et al. 2012). This passive technique can also dampen the effects of any diurnal variation in species behaviour as they can be set continuously over the diurnal period. Furthermore, the turtles can move freely, surface and submerge within the net, ensuring the turtles captured are released in good condition. The non-human disturbance and the period of deployment allow the process to be standardised.

Passive methods are limited to species that move, encounter, and enter the net (Hubert et al. 2012), consequently, the abundance of the less mobile species may be underestimated. There is a minor chance of drowning in the net if perchance the turtles are unable to surface. This is a limiting factor during high rainfall events when water height and velocity may increase rapidly. This limitation can be minimised by securing the cod-end at an elevated position, as well as careful observation of the weather forecast and upstream river heights. Nonetheless, large fluctuations in water level will affect every technique. Passive methods provide a less accurately defined unit of effort compared with active techniques because no spatial measure is included.

The disadvantage of using a single sampling method in a multiple species study is that detectability is likely to be different for each species thus the results may be biased and not reflect actual abundances.

The river levels and temperature were similar from year to year within the trapping episodes (Figure 2.1). The trapping methods and locations were consistent throughout the study; thus, biases were constant. Therefore, the consistency in year to year results was expected.

Survey results were consistent between yearly sampling episodes. Life history characteristics of freshwater turtles, such as longevity, minimal migration, and a limited home range would have minimised variation. This consistency of results is

expected as biases remained constant due to repetition of sampling methods, protocols, and locations.

Conclusion

My objective was to assess the relative species abundance over space and time, and thus identify longitudinal variation in turtle assemblages in the Mary River. Whilst I agree that the results of my survey may not reflect the absolute species abundance, significantly it consistently identified discrete turtle species assemblages. My intent was not to conduct an absolute abundance study where the results may lead to inferences about the population status of a particular species. Thus, it was not critical that the most effective trapping method for individual species was employed. The advantage of my technique and protocol is that future studies will be able to identically replicate my sampling methodology, and thus identify trends in abundance. These trends may be used to alert natural resource managers to shifts in conditions and ecological health of the river. The Mary River catchment has been affected by human activities (such as gold mining, vegetation clearing, sand and gravel extraction, water extraction, and introduction of exotic plants and animals) for over 150 years (Brizga et al. 2004). Hence, this current assemblage data set is unlikely to coincide with pre-European settlement assemblages, but rather, reflects individual species responses to habitat modification.

Chapter 3

Is nest protection an effective strategy to increase the population of a threatened river turtle?

"We can get completely carried away by the conviction that our efforts are indeed saving a species yet fail to undertake critical appraisal of our efforts to show whether these techniques are as beneficial as we think. However, taking no action to help save a turtle species is indefensible."

(Pritchard 1980)

Abstract

A common strategy used to conserve threatened species is to increase the recruitment of juveniles into the population. The release of the juveniles is often promoted as a measure of conservation success, but only if these individuals reproduce will these efforts be effective. For long-lived, slow maturing animals, measuring the success of increased recruitment can be challenging and requires monitoring of the population over many years. Chelonians are long-lived and slow maturing animals. Forty-one percent from within the group are threatened, and nest protection is generally used and recommended as a conservation strategy. Here, I test the effectiveness of nest protection as a conservation strategy for the endangered freshwater turtle, *Elusor macrurus*. This species is only present within a single river system and has undergone a decline of about 95% in the lower reaches over the past 50 years. A program to protect the turtle nests from predation and increase the number of hatchlings entering the river has been operating for the past 15 years. This has resulted in more than 2,843 hatchling turtles entering the river. Here, I instigated a mark and recapture program, which estimated the population of *Elusor macrurus* to be 211, 537, and 369, within the mid-low, middle and upper reach, respectively. The size class frequency was heavily weighted towards large adults, with a very low proportion of immature turtles in each study reach. No immature *Elusor macrurus* were captured in the stretch of river where the nest protection program took place. This demonstrates that factors other than nest predation are responsible for juvenile turtles not maturing to reproductive age in this section of the river, and these pressures appear to not be so significant in other areas of the species range. The findings suggest that the current conservation actions are ineffective in the long-term preservation of this

population and should either be changed or moved to other areas where they may be more effective in population preservation.

Introduction

Increasing recruitment is promoted as a global conservation strategy to redress population declines for a wide range of plant and animal species (Burke 2015). This strategy is often the foundation of conservation and management acts because helping individuals to survive, especially during the vulnerable early life stages, seems an obvious way to increase persistence of a population (Burke 2015). Animals with little or no parental care, relatively high fecundity and low juvenile survival rates appear well suited for mass-rearing programs – which is the case for several reptile and amphibian threatened species (Heppell et al. 1996).

Marine and freshwater turtle conservation programs are no exception, with programs frequently focusing on the early life stage to increase recruitment (Burke 2015; Crouse et al. 1987; Dodd & Seigel 1991; Eng-Heng 2013). Commonly, conservation measures target the early life stages using techniques such as head-starting, captive breeding, hatcheries, translocation, reintroductions, predator control and protection of nesting beaches/banks (Buhlmann et al. 2015; Burke 2015; Ratnaswamy et al. 1997). However, given the high mortality of turtles during the first few years, most conservation programs have not been operating long enough nor have the results been adequately monitored to make a definitive conclusion of the effectiveness of each technique (Moll & Moll 2004). Nevertheless, the least-manipulative techniques are advocated as they minimise the possibility of human error and thus allow the turtles to carry out their life history in the fashion dictated through

eons of natural selection (Bjorndal 1982; Meylan & Ehrenfeld 2000; Moll & Moll 2004).

Although turtle recruitment programs operate worldwide, few articles have been published regarding the long-term success of freshwater turtle recruitment projects (Buhlmann et al. 2015; Dutton 2005; Heppell 1996; Paez et al. 2015). This may indicate that head-starting programs are short lived and/or that those involved have little incentive to report their work in peer-reviewed literature (Burke 1991, 2015). Furthermore, the lengthy period between hatching and maturity may exceed the tenure of the manager or researcher, thus exacerbating the lack of published accounts (Buhlmann et al. 2015; Burke 2015; Heppell et al. 1996). In addition, there is no standard definition of success (Burke 1991). Nevertheless, an evaluation of the population's response to conservation actions is critical for making effective, informed management decisions (Crouse et al. 1987; Dodd & Seigel 1991; Heppell et al. 1996; Pritchard 1980).

Here, I undertake an assessment of an Australian freshwater turtle conservation program that has been in operation for 15 years. The depredation of the nests of *E. macrurus* has been identified as a major threat to the population (Flakus 2002; Limpus 2008). Therefore, a key strategy of the conservation program was to minimise egg predation through an annual nest protection program. The conservation measures adopted focused on the least manipulative methods, such as fencing the nesting banks and in-situ protection of individual nests.

During the 1960s and 1970s, thousands of *E. macrurus* eggs were collected from the wild during each year for the pet trade. Hatchlings were sold through commercial aquariums until 1974 when the legal trade of turtles in Australia ceased following the introduction of the Fauna Conservation Act (Cann 1998; Flakus 2002;

Limpus 2008). By the late 1990s, it was estimated that the nesting population of *E. macrurus* was functioning at a 5% capacity when compared to the 1960s anecdotal harvest data, which was obtained from the pet-trade period (Flakus 2002).

In this study, I assessed if the nest protection program has led to an increase in the *E. macrurus* population by comparing the population structure around the nest protection areas to other sites within the river. I hypothesised that the population of immature turtles would be greater in the river reach where the nest protection program operated. Four study reaches were selected, of which one was adjacent to the nest protection areas. Standardised capture methods were used to ensure sampling could be replicated in future studies to detect population trends and consequently inform conservation actions.

Materials and Methods

Nest protection

A local community group, Tiaro & District Landcare Group (TDLG), instigated the Mary River turtle (MRT) Conservation Program in 2001 (Figure 3.1; Flakus & Connell 2008). The strategic focus of the program was to increase recruitment of *Elusor macrurus* through minimising nest depredation (Connell & Wedlock 2006; Limpus 2008). Nesting behaviour was monitored during a defined period within austral mid-spring to mid-summer (October - December) – *E. macrurus* typical nesting season (Cann & Legler 1994; Flakus 2002; Micheli-Campbell et al. 2013a). Multiple strategies were used to increase hatching success: 1) nesting banks were surrounded by electric fencing to stop the beef cattle from trampling the nests; 2) feral animal threat abatement programs targeted wild domestic dogs (*Canis lupus familiaris*) and the European red fox (*Vulpes vulpes*); and 3), freshly laid nests were

protected (0.9 x 0.9 x .05 m flat plastic mesh, secured by 8 x 300 mm plastic sand pegs) to reduce the predation from feral and native predators, such as European red fox, monitor lizards (*Varanus panoptes* and *Varanus varanus*), and water rats (*Hydromys chrysogaster*; Beukeboom 2015). Every nest was numbered sequentially in order of date laid. The nest number was recorded on the exposed surface of a sand peg. A black 100 mm-long cable tie was attached to the mesh to unobtrusively note the position of the clutch. The nest number and date of oviposition was recorded on a 150 mm length of surveyor's tape, which was then buried adjacent to the clutch, approximately 100 mm below the surface. These measures were vital in locating and confirming the nest data at the end of the season. Following incubation, nests were excavated, and the number of empty eggshells counted as a means of estimating the number of hatchlings that emerged from protected nests. Hatched eggs were clearly distinguishable as there was no evidence of an embryo or egg yolk, and the ribboning effect caused by the egg tooth was evident on the eggshell.

Figure 3.1: MRT nest protection (a) TDLG members building an electric fence around a nest bank. (b) TDLG members after the fencing of a nesting bank. (c) Clutch of hatched eggs. (d) *E. macrurus* hatching emerging from protected nest.

Turtle sampling methods and measurements

A mark-recapture study was conducted at four reaches arrayed along the main channel (Figure 1.8). These stretches of river were labelled 'Upper', 'Middle', 'Mid-low', and 'Lower', respectively (see Chapter 2 for details). This program was conducted every spring and autumn during 2015 and 2016. Turtles were captured in purpose designed set-nets and the sampling protocol replicated identically through space and time (see Chapter 2 for details).

Each captured turtle was sexed, measured, weighed, tagged and photographed. Sex was visually determined based on morphological characteristics: the tail of male *E. macrurus* are substantially larger, both in length and circumference, compared to the females (Cann 1998; Flakus 2002). Males with a SCL of <285 mm and females

with a SCL of <270 mm were classified as immature (for details see Limpus 2008). Morphological measurements were taken (Figure 3.2). Straight-line carapace length (SCL), straight-line carapace width (SCW) and tail length were measured using with Haglof™ 650 mm callipers. Body mass was measured (±10 g) with a digital spring balance (Kathmandu™ compact scale). Each turtle was scanned with a Biomark® Pocket EX reader. If a passive integrated transponder device (PIT tag) was not detected, a single-use Trovan® ISO All-In-One applicator was used to insert an FDX-B PIT tag (1.8 mm x 30 mm) through the muscle layer into the right-anterior inguinal region (Buhlmann & Tuberville 1998; Hamann et al. 2007). Supplementary identification methods were employed in case of failure of the PIT tag. Digital photographs were taken of the carapace and the plastron for each individual and a 2 mm^2 section of tissue was taken from the webbing of the right rear foot.

Figure 3.2: Turtle processing methods: (a) Measuring straight carapace length; (b) scanning for PIT tags; (c) tail measurement; (d) weighing; and (e) inserting PIT tag.

All turtles were released downstream of the set net in which they were captured immediately following processing, to reduce any stress and minimise the chances of immediate recapture.

Population estimates

This study generated a mark-recapture history for each tagged *E. macrurus,* data which was used to generate a population estimate using program MARK (White 1999). The POPAN formulation of the Jolly-Seber model was used to generate population estimates for each study reach (Pollock et al. 1990). POPAN has five main assumptions: 1) equal probability of capturing marked and unmarked individuals; 2) animals retain their marks through the experiment and are read correctly; 3) sampling periods are instantaneous; 4) the probability of survival is the same for marked and unmarked individuals; and 5) the study area is constant (Cooch & White 2004). Assumptions 1, 3, and 5 were met based on the sampling design. It was assumed that there were minimal trap wariness effects as 10 individuals were recaptured in the same trapping episode (same study reach) as their initial capture. Assumption 2 was met by having multiple identification methods. The loss of internally injected PIT tags is considered to be low and is suitable for both juveniles and adults (Buhlmann & Tuberville 1998). If a PIT tag failed to read, an individual could be identified by presence of a tissue scar, photographs and morphological measurements. Assumption 4 was also met as the PIT tags were inserted into the animal's body cavity and therefore did not increase visibility of the marked individual.

The following parameters were estimated: Φ (apparent survival), *p* (recapture probability), *PENT* (probability of entry into the population) and *N* (size of population). A set of candidate models was tested for each reach of all captured turtles

that encompassed sex, age and time variation (sampling occasion). Models were compared by the Akaike Information Criterion (AIC_c) and the most parsimonious model from the set of candidate models was identified. The AIC_c weight of each model was used as an objective means of model selection in combination with knowledge of the species (Burnham & Anderson 2004). Each study reach was classed as a closed population based on: 1) the home range of *E. macrurus* was previously shown to be ~5 km, and 2) each study reach being approximately 60 km apart (Micheli-Campbell et al. 2013b). Based on these two facts, it was considered unlikely that an individual would emigrate from one study reach to another.

An overall estimate of the population size was made for the main river channel – from the upstream end of the most downstream impoundment (AMTD 83.8 km) to the furthest upstream area where an individual *E. macrurus* has been caught (AMTD 289 km). This length of river was divided into four sections, with section 1 reaching from the most downstream point (AMTD 83.8 km) to the midpoint between the Lower and the Mid-low study reaches (AMTD 113.4 km). Section 2 was therefore from that point to the midpoint between the Mid-low and the Middle study reaches (AMTD 175 km), and so on. The population for each section was estimated by dividing the POPAN population estimate for each study reach by the maximum potential home range of *E. macrurus* around the sampling locations. In three of the four reaches this was accepted to be 5 km (the known home range of *E. macrurus* is 4.98 km) and the nets were set within a 5 km range (Table A1.2). In the case of the Upper study reach, the nets were greater than 5 km apart in three cases. The total length used for that reach was 15 km. The POPAN population estimate/km was then multiplied by the length of the section of river (in km).

The sex ratio of *E. macrurus* was compared to a 1:1 ratio using a chi-squared (χ^2) goodness-of-fit test, and a Yates' correction was used for continuity for each trapping episode, season, and study reach (Hasler et al. 2015). The chi-squared heterogeneity test was used to test whether the data could be pooled to perform an overall chi-square analysis for each site (Zar 1984). All sites with pooled samples were then tested for heterogeneity and a final overall chi-square analysis of those pooled sites was conducted. Significance was tested at $\alpha = 0.05$.

A comparison was done of size frequency (SCL) differences between this study and two historical *E. macrurus* studies (Flakus 2002; Limpus 2008). Frequencies of the turtles in each 10 mm SCL length bins were compared between studies. The data was grouped into 50 mm bins for the purposes of display. A two-sample Kolmogorov-Smirnov test was used to compare these studies with the current study (Gordon & Klebanov 2010; Zar 1984).

Results

Nest protection

The program protected 410 *Elusor macrurus* nests over a period of 15 years in the lower reaches of the Mary River (Figure 3.3). Post incubation, 4,428 eggs were recovered from these protected nests, of which at least 2,843 hatched resulting in a 64% hatching success rate. Unfortunately, it was not possible to record the true numbers of hatchlings entering the river as not all nests were located after the incubation period, mostly due erosion of nesting material by sudden increases in river levels. A high level of variability was evident in the number of clutches laid each year, with an above-average number of nests laid and hatched turtles occurring in only 3 of the 15 years (Figure 3.3). Additionally, there were 4 years of very low numbers of

nests and hatchlings (2001, 2003, 2012, and 2013). Extreme climatic conditions were experienced in the Tiaro district during this time, with a very dry January in 2001 and 2003 when <20 mm rain was recorded for the month (mean for January = 153 mm; Australian Government Bureau of Meteorology 2017). In January 2012, a significant flood inundated all the nesting banks. The river rose 12 m within 24 hours and peaked at 23.14 m at Home Park Gauging Station – the classification of a major flood at this Gauging station is 13 m (Queensland Department of Natural Resources and Mines 2017).

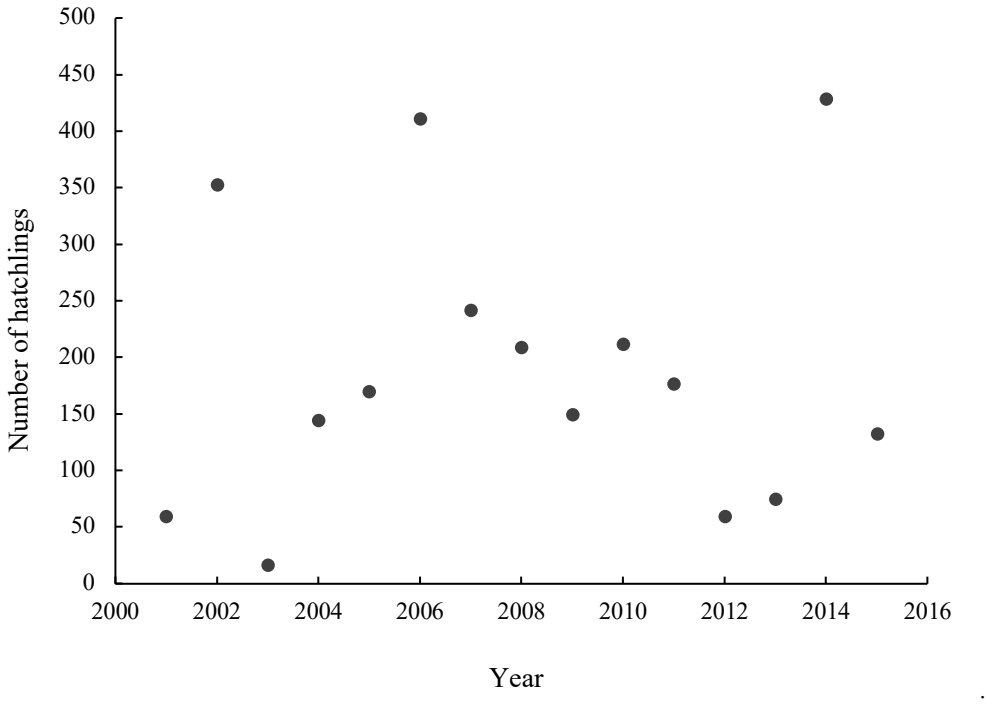

Figure 3.3: The number of known hatchlings *E. macrurus* entering the river as a direct consequence of the nest protection program from 2001 to 2016 in the Lower reach of the Mary River.

Mark-recapture

I captured and marked 268 *E. macrurus* over the two years of sampling (168 males, 78 females, and 22 immature turtles; Table 3.1; Table A1.1). Twenty-nine

turtles were recaptured with one individual recaptured twice (19 males, 10 females, 1 immature). All recaptures occurred within the same study reach as their initial capture. This supports the assumption of a closed population. Five turtles were collected upstream of the net of initial capture, 13 in the same net and nine downstream, which suggests there was minimal effect on capture from the process of releasing the turtles downstream of the net. Ten turtles were recaptured during the same trapping episode, four in the same net as the initial capture, one upstream and five downstream, suggesting little effect of trap wariness. This study also recaptured 14 *E. macrurus* from a tagging exercise undertaken 20 years previous. That study notched the marginal scutes of the carapace and inserted self-piercing monel tags into the webbing of the right hind foot (Flakus 2002).

Table 3.1: Number of *E. macrurus* marked and recaptured during each trapping Episode (TE) within each study reach for each sex.

	N (male, female, immature)			
	Lower	**Mid-low**	**Middle**	**Upper**
Number of individuals marked	38 (31, 7, 0)	63 (42, 17, 4)	94 (59, 22, 13)	73 (36, 32, 5)
Number of recaptures	0	11 (7, 4, 0)	9 (7, 2, 0)	10 (5, 4, 1)
TE1	10 (10, 0, 0)	12 (10, 2, 0)	16 (8, 7, 1)	20 (15, 4, 1)
TE2	4 (3, 1, 0)	32 (21, 9, 2)	19 (12, 5, 2)	23 (7, 14, 2)
TE3	7 (7, 0, 0)	17 (10, 5, 2)	40 (28, 6, 6)	6 (4, 1, 1)
TE4	17 (11, 6, 0)	2 (1, 1, 0)	19 (11, 4, 4)	24 (10, 13, 1)
Total per reach	38 (31, 7, 0)	63 (42, 17, 4)	94 (59, 22, 13)	73 (36, 32, 5)
Total number of marked individuals captured: 268 (168, 78, 22)				

Population estimates

The population estimates, and models used varied among study reaches. Estimates ranged from 304 males and 233 females in the Middle reach to 148 males and 63 females in the Mid-low reach (Table 3.2). No turtles were recaptured within the Lower reach, preventing a population estimate for this reach.

Table 3.2: POPAN population estimates for the three study reaches, male, female, and immature. An estimate was not made for the Lower reach as no turtles were recaptured in that reach.

	Mid-low		**Middle**		**Upper**	
	Number	95% Cl	Number	95% Cl	Number	95% Cl
Male	148	92–269	304	160–653	188	93–444
Female	63	37–124	233	98–605	176	82–446
Immature	n/a		n/a		5	3–32

The density of the estimated *E. macrurus* population peaked at 107.4 turtles/km in the Middle reach, 42.2 turtles/km in the Mid-low reach and 24.4 turtles/km in the Upper reach. The density estimate for the Upper reach is overly conservative and potentially lower due to trap locations being greater than 5 km part requiring the POPAN estimates to be divided by 15 rather than 5 (see Methods). The adult *E. macrurus* population within the main channel of the river is estimated to be 5,991 males and 4,092 females (Table 3.3). This is an underestimate, as it omits Section 1 (no population estimate) and immature turtles as population estimates were unable to be made for either due to a lack of recaptures.

Table 3.3: Population estimates for three sections of the river (males, females, and immatures). Section 1 has not been included, as no recaptures occurred in this section.

	Section 2		**Section 3**		**Section 4**	
	Number	95% Cl	Number	95% Cl	Number	95% Cl
Male	1,823	1,133–3,314	3,450	1,816–7,207	717	355–1,694
Female	776	456–1,527	2,644	1,112–6,866	671	312–1,702
Immature	n/a		n/a		19	11–122

At the Lower study reach a POPAN model could not be conducted due to a lack of recaptures. There is limited evidence that population estimates can be estimated with no recaptures, however this was not progressed (Bell 1974).

The preferred (most parsimonious) POPAN model for the Mid-low reach, Φ(.) p(.) *PENT*(.) and N(g), had a constant (.) survivorship, recapture probability and probability of entry, with a population estimate for each group (g = male and female; Table A2.1). Immature turtles were excluded from the model as none were recaptured. Apparent survivorship (Φ) estimate was high at 0.999 (95% CI: 0.996–0.999 SE: $0.562*10^{-003}$), and probability of recapture (p) was 0.024 (95% CI: 0.013–0.043 SE: 0.007; Table A2.2). The probability of entry (*PENT*) estimate was very low, possibly due to the physical characteristics (such as length of pool) of this area of the river that the sampling net had to cover, given the increased size of habitat in this reach relative to that in the Middle and Upper reaches (Figure 1.8b and c). Estimated population size (N) for male *E. macrurus* was 148 (95% CI: 92–269 SE: 42), and for females 63 (95% CI: 37–124 SE: 20; Table 3.2).

The preferred POPAN model, Φ(g) p(t) PENT(.) and N(g) for the Middle reach estimated survivorship and population for each group with recapture probability varying over time and constant probability of entry (Table A2.3). Immature turtles

were excluded as none were recaptured. Apparent survivorship (Φ) for males was estimated at 0.999 (95% CI: 0.067–1.000 SE: 0.002) and for females 0.995 (95% CI: 0.976–0.999 SE: 0.003). The significant variation between the lower and upper estimates indicates the model had difficulty computing male survival resulting in low confidence in the estimate (Table A2.4). The probability of recapture (*p*) was variable over time and ranged from 0.007–0.057. The variability in recapture probability reflects the uneven probability of capture over time. During autumn 2016 (Trapping Episode 3 - parameters 11–14), an unusually high number of male *E. macrurus* were captured in this reach (Table 3.1; Figure 3.4). Probability of entry (PENT) was estimated as 0.037 (95% CI: 0.010–0.131 SE: 0.025). Estimated population size (N) for male *E. macrurus* was 304 (95% CI: 160–653 SE: 116), and for females 233 (95% CI: 98–605 SE: 117; Table 3.2).

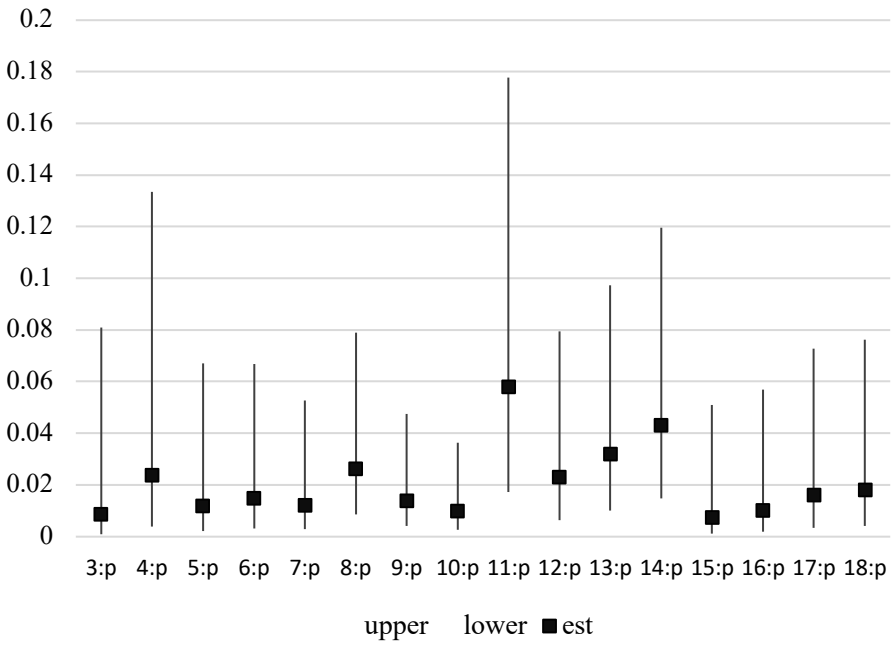

Figure 3.4: Variable recapture probability (p) showing the upper and lower POPAN estimates for each trapping occasion in the Middle reach.

The preferred POPAN model, Φ(.) *p*(g) PENT(.) and *N*(g), for the Upper study reach had constant survivorship and estimated probability of entry, with estimates for recapture probability and population for each group (Table A2.5). Apparent survivorship (Φ) was estimated at 0.998 (95% CI: 0.990–0.999 SE: 0.001), probability of recapture (*p*) for immatures was 0.087 (95% CI: 0.009–0.490 SE: 0.093), for males 0.027 (95% CI: 0.009–0.758 SE: 0.014), and for females 0.025 (95% CI: 0.007–0.082 SE: 0.015) (Table A2.6). Probability of entry (PENT) was 0.035 (95% CI: 0.015–0.079 SE: 0.014). Estimated population size (*N*) for immature *E. macrurus* was 5 (95% CI: 3–32 SE: 5), for males 188 (95% CI: 93–444 SE: 81) and for females 176 (95% CI: 82–446 SE: 83; Table 3.2). The variation in the lower and upper values (95% CI) reflects the low number of captured and recaptured immature turtles.

Several *E. macrurus* were recaptured that were first marked in a movement study conducted between 1998 to 2001 (Flakus 2002). These turtles were recaptured within the same reach as their initial capture. In the current study, the linear movement of recaptured turtles ranged from 0–1.8 km with a single exception of an individual (male turtle) that travelled 12.3 km in the Upper reach, thereby indicating that they occasionally migrate outside their home range over extended periods of time. However, they predominately remained within the 5 km.

Population structure

The sampling yielded a range of size classes (108–436 mm SCL; Figure 3.5). Adult male mean SCL was 376 mm (n = 187, range 292–436 mm) and 320 mm (n = 87, range 275–364 mm; Table 3.4) for adult females. However, the size frequency plot is skewed with few smaller individuals and a high frequency of turtles within the 350–

400 mm range. Age was not determined because the annuli were indistinct in most turtles. Overall, adults dominated the population (92%, n = 246) with 8% immature (n = 22). Laparoscopic studies showed that individuals with a SCL <280 mm were immature though discernible secondary sex characteristics were evident in many individuals (Limpus 2008). There was no evidence of tail elongation in turtles with SCL <150 mm, thus they remained unsexed.

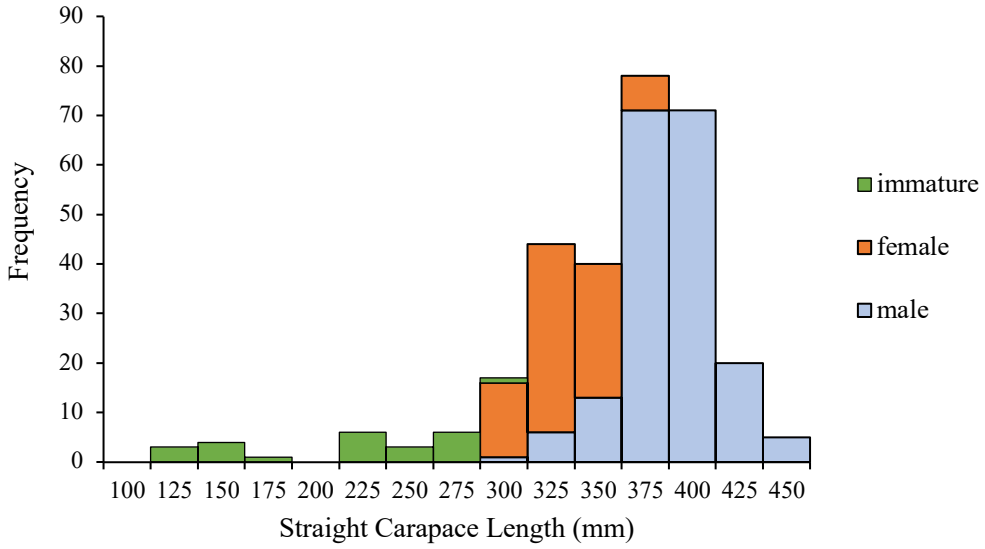

Figure 3.5: Sexual maturity of all *E. macrurus* captured. Graph shows combined data from all four study sites. Males are represented by blue bars, females by orange bars, and immature by green bars.

Table 3.4: Mean and range of sizes (SCL and Mass) for male, female, and immature *E. macrurus* per study reach.

			SCL (mm)		Body mass (kg)	
Age class	Study reach	*N*	mean	Range	mean	range
Adult male	Lower	31	373	344–406	5.76	4.57–7.13
	Mid-low	42	393	307–422	5.63	2.67–7.63
	Middle	59	369	292–424	5.28	2.34–7.37
	Upper	36	385	352–436	5.72	4.23–8.08
Adult female	Lower	7	320	304–334	3.64	3.22–3.91
	Mid-low	14	325	292–348	3.59	2.74–4.61
	Middle	22	310	275–332	3.25	2.43–3.97
	Upper	32	326	283–364	3.75	2.4–5.34
Immature	Lower	0		n/a		n/a
	Mid-low	4	252	237–263	1.89	1.5–2.27
	Middle	13	194	116–282	0.9	0.18–2.22
	Upper	5	172	108–248	0.7	0.23–1.54

Overall, the sex ratio was significantly skewed towards males with 2.81:1 ratio (male: female) and was statistically different from 1:1, (P <0.001; Table 3.5). The heterogeneity test indicated that the results from the Upper reach could not be pooled with the other reaches due to the variability in trapping results, with some trapping events having zero values. The bias towards males was most evident in the Lower reach (4.43:1), whereas in the Upper reach the ratio was closer to 1:1 (1.14:1). Seasonal influence was apparent in the ratio of males to females captured with autumn trapping predominately skewed towards males. In contrast, during spring trapping, only one occasion (trapping episode 2 in Mid-low reach) was the result significantly different to 1:1.

Table 3.5: Number of *E. macrurus* by study site, trapping episode (TE), season, sex, ratio, and significance. Figures in bold denotes significantly different ratios.

Site	Season	Male	Female	Ratio	Significance
Lower					
TE 1	Autumn	10	0	n/a	**0.002**
TE 2	Spring	3	1	3.0:1	0.317
TE 3	Autumn	7	0	n/a	**0.008**
TE 4	Spring	11	6	1.83:1	0.225
Overall		31	7	4.43:1	**<0.001**
Mid-low					
TE 1	Autumn	10	2	5.0:1	**0.021**
TE 2	Spring	25	11	2.27:1	**0.02**
TE 3	Autumn	12	5	2.4:1	0.09
TE 4	Spring	2	3	0.67:1	0.65
Overall		49	21	2.33:1	**0.001**
Middle					
TE 1	Autumn	8	7	1.14:1	0.796
TE 2	Spring	12	5	2.4:1	0.09
TE 3	Autumn	35	7	5.0:1	**<0.001**
TE 4	Spring	11	5	2.20:1	0.134
Overall		66	24	2.75:1	**<0.001**
Upper					
TE 1	Autumn	16	4	4.0:1	**0.007**
TE 2	Spring	8	15	0.53:1	0.144
TE 3	Autumn	4	2	2.0:1	0.414
TE 4	Spring	13	15	0.87:1	0.705
Overall		41	36	1.14:1	n/a
Lower, Middle, Mid-low, Upper		146	52	2.81:1	**<0.001**

A two-sample Kolmogorov-Smirnov (KS) test compared frequency distribution of *E. macrurus* size classes with previous studies (Figure 3.6). Significant differences occurred in the Lower reach between this current study and Flakus (2002; $P = 0.04$, dmax = 0.289). Non-significant differences were found between this current study and Limpus (2008; $P = 0.121$, dmax = 0.244) and between Limpus (2008) and Flakus (2002; $P = 0.921$, dmax = 0.111). No significant difference was found in the Middle reach between Limpus (2008) and this study ($P = 0.156$, dmax = 0.156; Figure

3.6). The two-sample KS test loses power when there are unequal sample sizes, to the point that the increased sample size for one sample weakens the ability of the test to reject the null hypothesis when it is actually false (Gordon & Klebanov 2010).

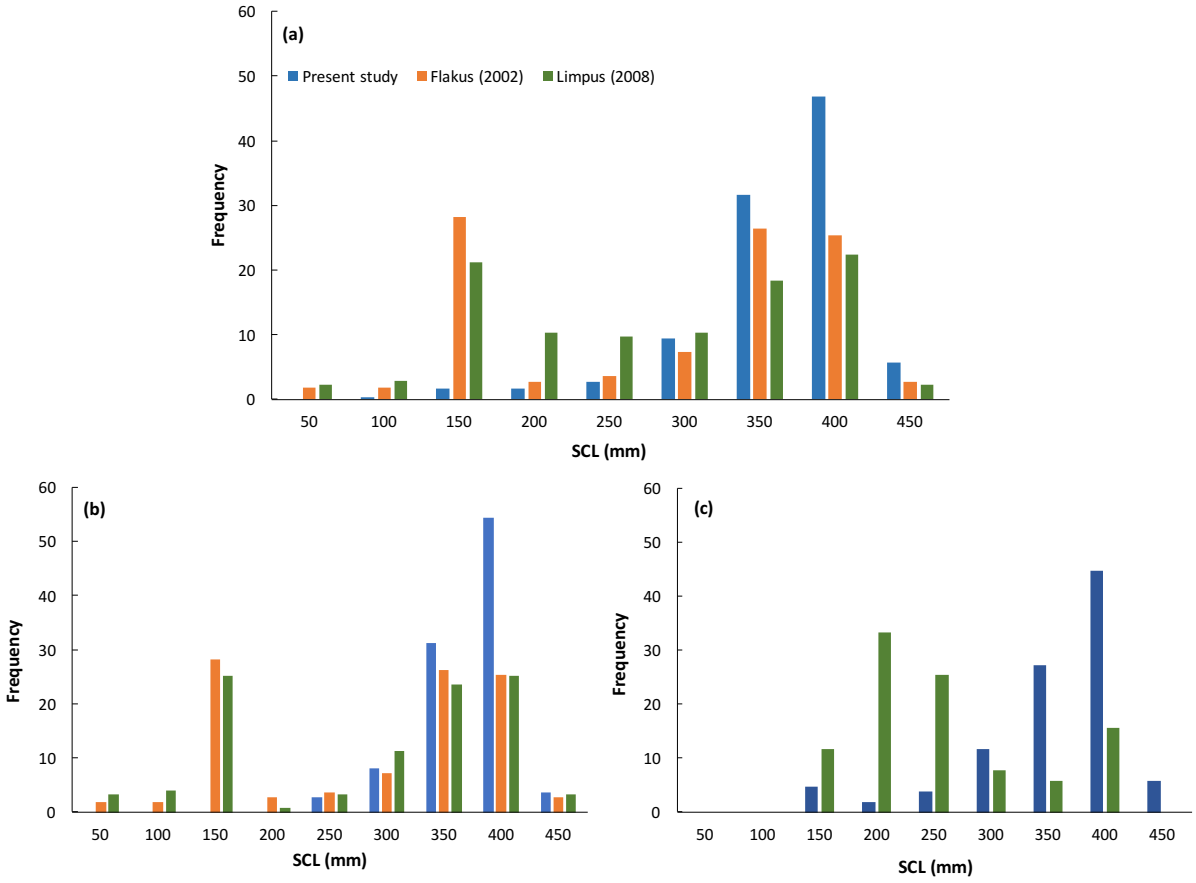

Figure 3.6: Comparative distribution of size class frequencies (SCL mm) of *E. macrurus* of three studies. (a) Overall results from Flakus (2002; orange bars; n = 110), Limpus (2008; green bars; n = 174) and the present study (blue bars; n = 297). (b) Combined results for Mid-low and Lower reaches from Flakus (2002; n = 110), Limpus (2008; n = 123), and the present study (n = 112). (c) Middle reach comparison of Limpus (2008; n = 51) and the present study (n = 52).

Discussion

Nest protection

Nest protection is used world-wide as a conservation action for freshwater and marine turtles. This has been an effective strategy to increase survival of the egg/hatchling phase as, without human intervention, the majority of *E. macrurus* eggs in the mid to lower catchment of the Mary River would be destroyed from depredation or other threats (Connell & Wedlock 2006; Limpus 2008). The nest protection program undertaken by TDLG has been successful in that it has resulted in thousands of hatchling turtles entering the Mary River over the last 15 years. However, it was unclear whether this has led to an increase in the population. Extensive trapping using fyke nets within this stretch of river captured no turtles of a size class that would indicate that they were descendants of the nest protection program. This result reveals that the count of hatchlings was not a good indicator of conservation success for the nest protection program and even calls into question the use of nest protection as a stand-alone measure for this species.

There are four possible explanations for the lack of juvenile turtles captured in the lower reach of the river. First, the turtles were present but were not captured, second, the hatchling turtles emigrated from this stretch of river, third, the population of reproductive females is too low in the lower reach given the survivorship of freshwater turtles and fourth, there is an increased level of juvenile mortality in the lower reach.

The first theory can be disregarded because the capture technique did capture immature *E. macrurus* at other locations, even though they were still at a low abundance (Figure 2.4). Fyke netting may have been biased towards the capture of

adult *E. macrurus* since adults travel greater distances than hatchling and juvenile turtles (Micheli-Campbell et al. 2013b; Micheli-Campbell et al. 2017). Furthermore, other studies have also not found juvenile *E. macrurus* in this reach (Limpus 2008; Thomas 2007).

Previous studies have hypothesised that there is an upstream migration of the juvenile *E. macrurus* from the nesting area. This theory would explain the relative lack of juveniles in certain areas (Kuchling 2009; Limpus 2008). Genetic population analyses support a single genetic population along the length of the Mary River, suggesting genetic mixing (Schmidt et al. 2017). However, a migration from the nesting area to the locations where I did capture juvenile *E. macrurus* would require the immature turtles to have moved 60, 120 or 180 km, respectively. This extent of movement for such a small animal against the water flow seems unlikely and is supported by a biotelemetry study of juvenile *E. macrurus* turtles. This study found that over a 9-month period, the juvenile turtles had travelled no further than 2.5 km and this was downstream (Micheli-Campbell et al. 2013b). Whilst I cannot absolutely discount upstream migration, the paucity of immature turtles found at all sites surveyed suggests that mass emigration was not occurring from the nest protection reach. However, genetic mixing within this population could occur through mixing of individuals over smaller distances over the breeding life of the turtles spanning many generations.

Freshwater turtles are R-strategists and therefore it would be expected for *E. macrurus* hatchlings to have a high rate of mortality. Iverson (1991) completed a review of survival estimates for both freshwater and marine turtles and found an average survivorship of 0.229 for freshwater turtles in the egg to hatching age-class. The MRT conservation program had an annual mean of 405 *E. macrurus* eggs laid,

i.e. 27 clutches with an average 15 eggs per clutch. The number of hatchlings produced was on average 259 suggesting an annual survivorship of 0.64 to hatchling stage. Thus, this program has achieved a substantive increase in the survival of this age-class than compared to the average for freshwater turtle species. In addition, local threats such as depredation are thought to be responsible for severe losses (>75%) based on similar studies in the Fitzroy River for other species (Limpus et al. 2011). Therefore, based on current depredation threats, the nest protection program in the lower reach has been highly successful in rearing hatchlings.

Although the nest protection program has been successful in conserving hatchlings from the limited nesting that has occurred, a proportion of these small number of hatchlings released each year (average 405 individuals) need to survive to adulthood to breed. Iverson (1991) suggested that the average annual survivorship for the age-class from egg to age 1 was 0.185, for juvenile freshwater turtles 0.672 and for sub-adults, 0.837. Actual data on the survival rates of juvenile *E. macrurus* is limited. Micheli-Campbell (2013b) found a 60% loss rate of individual juvenile *E. macrurus* over a 9-month period, this being similar to the juvenile values given by Iverson (1991). An estimated number of surviving turtles can be completed using the known hatchling survival rate (0.64), the first sub-adult males appearing at age 4 (Limpus 2008), a maturity age of 20 years (Limpus 2008), and the average published survival rates by Iverson (1991). Only 23 individuals of the 259 hatchlings would survive to sub-adults and one individual would survive to adulthood. In contrast, zero individuals would survive to adulthood based on the high depredation rate without nest protection. No juveniles were captured in the lower reach suggesting that the levels of mortality of these size classes is significantly higher. Therefore, the number of clutches laid

would have to significantly increase in the lower reach for the program to achieve the stated goal of population recovery.

The present study captured immature *E. macrurus* in all reaches of the river apart from the Lower reach. Those three reaches also had higher relative abundance of adult females, supporting the higher recruitment. This suggests that the adult female population in the Lower reach is inadequate to provide a base level for the population of *E. macrurus* even with nest protection in place.

The quantity of eggs reportedly collected from the most productive banks (10 - 12,000 eggs per annum) between 1962 to 1974, suggests mass nesting occurred in the Lower reach (Flakus 2002). Based on the data from the nest protection program, *E. macrurus* has continued to nest communally in the same well-defined ancestral nesting areas (Beukeboom 2015; Espinoza et al. 2018; Flakus 2002; Micheli-Campbell et al. 2013a). However, the number of eggs laid during the MRT conservation program indicates that the nesting population in the Lower reach is functioning at only 4% of that observed in the 1960s. This suggests that the number of reproductive females has remained depressed for at least the past 20 years.

Thus, the adult female population level and the predation of the hatchling turtles once they had entered the river are the most likely causes of their lack of recruitment into the population. It is possible that the low levels of recruitment are not the result of the egg collection era nor the predation of nests. Other unidentified threatening processes may have caused a decline in the abundance of mature females since that observed in the 1960s resulting in a substantive reduction in nests laid. Thus, the nesting program may never achieve the expected results.

Many have questioned the use of nest protection and headstarting as a stand-alone strategy to arrest a declining turtle population (Brooks et al. 1991; Congdon et al. 1994; Congdon et al. 1993; Crouse et al. 1987; Enneson & Litzgus 2008; Frazer 1992; Heppell et al. 1996; Klemens 2000a; Seigel & Dodd 2000). At Mon Repos beach, a major loggerhead turtle (*Caretta caretta*) rookery on the eastern seaboard of Queensland Australia, it was estimated that even with beach protection efforts that resulted in 90% hatchling emergence, it would not prevent population decline (Heppell et al. 1996). Likewise, the current study found that the reliance on a single conservation measure has had limited impact on recovery of the turtle population, and that a diversity of actions is required to ensure the preservation of *E. macrurus.* For example, protection of females may be required particularly in the Lower reach, as the large adult male sex bias in this reach and the historical mass nesting suggests the possibility substantial female mortality.

Population estimates

A general pattern of spatial variation in abundance occurred for this species, with the density greatest in the centre of the range. This coincided with the mid reach of the river and declined gradually toward the boundaries, that is the upper and lower reaches. A 2007 study of turtles of the Mary River also found *E. macrurus* to be most abundant in the middle reaches (Thomas 2007). Thus, we suggest that the preservation of *E. macrurus* habitat within the middle catchment of the Mary River the most critical for the long-term survival of this species.

The low rate of recaptures of *E. macrurus* resulted in a wide range of the lower and upper estimates for the population (Table 3.3). This is not unusual as low recapture

rates are not uncommon in chelonian studies (Bernardes et al. 2014; Bluett et al. 2011; Burke et al. 1995). The model chosen for the Mid-low reach had a similar Model likelihood value to other reaches (Table A 2.1), although the next three models have a smaller likelihood. An assessment of the next three likely models for this reach either provided similar population estimates or were more restrictive models with only a global population estimate. Hence, the most likely model was chosen.

There were no recaptured turtles in the Lower reach. Therefore, population estimates could not be made for this stretch of river. The relative abundance of *E. macrurus* was the lowest in this section compared to all the other survey reaches and may have contributed to the lack of recaptures. The Lower reach was also where the nest protection occurred and therefore a population estimate for this stretch is important to assessing the long-term success of the nest protection program in sustaining or increasing the population. I was only able to undertake four trapping episodes during this thesis, but further episodes of mark-recapture are planned and will serve to improve the accuracy of the population estimates.

The extrapolated population size estimated for *E. macrurus*, of approximately 10,000 individuals, indicates an abundant species throughout the main trunk of the river.

Population structure

The *E. macrurus* population is characterised by a male-biased adult sex ratio, a low proportion of immatures and a population weighted towards large adults. The male bias was most heavily skewed in the Lower reach, 4.43: 1 (male: female). This number may be the result of a higher rate of terrestrial predation of adult females than

in the other reaches. It is possible that a male bias exists in the population as females are exposed to additional threats during the nesting season (Spencer 2005). Seasonal influences on sex ratios were apparent in this study but were minimised as sampling occurred over multiple seasons. Previous studies of *E. macrurus* that employed multiple survey methods found a sex ratio that was also weighted towards males, 1.4:1 (Limpus 2008), 1.28:1 (Flakus 2002), but to a lesser extent than this study (2.81:1). If the bias towards males in the Lower reach is a true representation of a bias within the population in this reach, then it is unlikely that the population will recover to the level observed during the egg collection period of the 1960s unless there is an increase in the number of females nesting in this reach and an increase in survival rates of immature turtles.

The size class frequency of *E. macrurus* was heavily weighted towards large adults with a very low proportion of immature turtles in each study reach. It is possible that the variability in overall abundance of immature turtles may be partly due to the absolute abundance of hatchlings resulting from variable, annual rates of nesting success (Figure 3.3). Neither the current study nor a study undertaken in 2007 (Thomas 2007) detected immature turtles in the lower reach. The lack of immature *E. macrurus* turtles in the lower reach has been evident for more than a decade (Flakus 2002). This suggests that the lack of immatures found in this study is not symptomatic of a sampling bias as different sampling methods were used. Thus, the 12 years of egg collection (1962 to 1974) that occurred over 50 years ago, can no longer be the cause of the scarcity of immature *E. macrurus*, given the history of a paucity of immature turtles throughout the river.

Understanding drivers of population change

A logical next step in attempting to understand the drivers for *E. macrurus* population change is to develop a simple stage-based population model. Whilst many of the parameters for such a model are available for this species (juvenile mortality, adult survival, fecundity), there is no validated age data for this species. What age information is available is limited and had not been peer-reviewed (Limpus 2008). Whilst this current thesis has outlined a sampling strategy and used mark-recapture to estimate the current population, it is limited in the predictive ability to quantitatively understand the sensitivities of the key life stages of the species.

Conclusion

There are two compounding issues limiting recovery of the species in the lower reach. The first is the low number of reproductive females in the lower reach and thus a low number of hatchlings entering the river. The second is the high rate of predation and because the number of hatchlings is not on mass as it was before the 1960s (in the hundreds rather than thousands), it appears there is not sufficient immature female turtles surviving to reproductive age. It is unknown if this decline in the number of nesting females is peculiar to the lower reach or symptomatic across the population as the historic data is limited to the lower reach (Limpus 2008).

The aim of conservation management is either to maintain the *status quo* or to manipulate the system to achieve some predefined target (Legg & Nagy 2006). The results of this study suggest that though the MRT conservation program increased hatchling recruitment it has not translated to population recovery. Whilst no population estimate could be derived from the mark-recapture study, there was a low abundance

of turtles in the lower reach overall. While the abundance of *E. macrurus* found in this study indicates a resilient species, the demographic structure suggests the population is at a point where the next two decades are crucial in terms of the recovery or demise of this species.

Chapter 4

General Discussion and Conclusion

"Turtles cannot be saved in any one place,

or by controlling any one phase of the life cycle."

Archie Carr, the 'Father' of sea turtle research and conservation

(as cited in Frazer 1992)

Typically, freshwater chelonian studies focus on a single species rather than multiple co-existing species. Conversely, the overall aim of this study was to investigate the freshwater turtles of the Mary River (QLD, Australia). This required the development of a standardised sampling methodology that was effective in capturing all sizes and species of turtles over space and time (Chapter 2). The methodology is simple and easy to use, thus enabling future studies to identically replicate the methodology and identify trends in species abundance over space and time. Changes to abundance of the ecological specialists within these assemblages will provide an alert to managers of changes in the health of the Mary River ecosystem. Individual species occurred in maximum number in specific reaches, highlighting the significance of a river reach for particular species. For example, the population of the critically endangered *Elseya albagula* peaked in the Lower reach, whilst the numbers of the endangered *Elusor macrurus* were highest in the Middle reach and of *Myuchelys latisternum* in the Upper reach. The Upper reach was also more significant for juveniles of all species. This has implications for river management and species conservation.

In Chapter 3, I investigated the efficacy of in-situ nest protection as a technique to increase the population of an endangered freshwater turtle – *E. macrurus*. The comparison of the population structure of the target species at four sites inferred that not enough hatchlings were produced to counteract in-stream threats. The findings presented in this study suggest that while in-situ nest protection is a successful technique at increasing hatchling recruitment, addressing a single threat may not be adequate to achieve the recovery of a population. The results were contradictory to the study hypothesis.

Population trajectory

While it is recognised that populations are not static, detecting a long-term trajectory can be informative for species management. Although an accurate population trend could not be assessed for *E. macrurus*, inferences can be made by examining significant historical events, the results of Flakus (2002), and this study. It is unknown how many turtles were captured in each of the two study reaches in the 2002 study. However, a greater number (six) were tagged and tracked in the Mid-low reach than the Lower reach (three). This suggests that a higher proportion of the turtles (110 individuals) captured also occurred in the Mid-low reach. Thus, it is assumed that - like in this study - fewer individuals were captured in the Lower reach in comparison to the Mid-low reach. This suggests that the population in the Lower reach has been low for at least two decades.

A comparison of data from the current and 2002 studies, suggest that the *E. macrurus* population is on a trajectory towards an ageing population, a falling number of reproductive females in addition to the limited recruitment found in this study. A shift in the size frequency to larger individuals was evident when compared with historical surveys for the lower reach (Flakus 2002). The study from 2002 found similar proportions of turtles in the 150, 350, and 400 mm classes (Table 2.1), as well as a sex ratio not significantly different to parity (1:1). In contrast, the present study found fewer turtles < 300 mm SCL, a greater abundance of turtles >350 mm, and a sex ratio heavily skewed to males (Figure 3.6). Assuming reproductive females lay a single nest each year, the number of nests protected indicate that the nesting female population has not returned to that observed in the 1960s and 1970s (Flakus 2002).

It was outside the scope of this study to investigate factors which may have contributed to the variation in abundance of this species throughout the river. The

abundance of *E. macrurus* in the Middle reach suggests this area has the most appropriate ratio of pool-riffle sequences and thus food sources. However, there are likely to be multiple factors influencing abundance such as variation in populations of terrestrial predators, in-stream refugia, aquatic competitors, basking sites, and riparian vegetation condition.

The lack of juvenile recruitment into the turtle populations in eastern Australia is symptomatic of several other Australian freshwater turtle species. It was first highlighted for *Emydura macquarii* in the early 1980s (Thompson 1983). Population studies of freshwater turtles in the adjacent Burnett River found that populations were skewed towards adults for three of the four species sampled, with a similar shortage of turtles in the smaller size classes (Hamann et al. 2008). Similarly, the decline in recruitment is evident for *Rheodytes leukops, Elseya albagula* (Hamann et al. 2008; Hamann et al. 2007; Limpus 2008; Limpus et al. 2011), the Murray River turtle, *E. macquarii*, in the Murray River and Cooper Creek and Krefft's river turtle, *Emydura macquarii krefftii*, in the Ross River in north QLD (Thompson 1983; Trembath 2005). While the causes are yet to be identified, research indicates that populations of many Australian freshwater turtle species are largely comprised of ageing adults and are in effect surviving on borrowed time (Cann & Sadlier 2017).

The extent of habitat alteration and the abundance of *E. macrurus* found in this study appear to be contradictory. The abundance levels found in this study infer a resilient species that has survived several threatening events and significant habitat alteration. However, turtles have long generation times, and consequently may persist at higher abundances despite decreases in reproductive success or increases in mortality at early life stages that could eventually cause population extirpation (Gibbs & Amato 2000). Despite the abundance of *E. macrurus* found in this study, the

population structure suggests that the long-term trajectory for the population is in decline.

Factors contributing to the trajectory of the current population

The present study has revealed the significance of distinct river reaches for *E. macrurus* and has established a baseline population estimate. However, the critical question remains: is the population trajectory increasing or decreasing?

Some modifications are still on-going and may have had a gradual, long-term impact on the population, such as the clearing of riparian vegetation with the subsequent reduction in large log tangles used for refugia (Limpus 2008). Other events have dramatically modified the aquatic and riparian environments of the Mary River, which has disrupted the ecological integrity of the river (Flakus 2002; Limpus 2008; Mary River Catchment Coordinating Committee 2001; Queensland Department of Primary Industries Water Resources 1995), and thus likely to have had significant effects on the *E. macrurus* population. Cann (personal comm. 2016) estimated a male *E. macrurus* captured in 2016 to be approximately 100 years old. Given this assumption, the current population is likely to be a consequence of river management and events that have occurred over the past 100 years (Bodie 2001; Schaffer et al. 2016). Thus, an understanding of the current and near future population trajectory can be informed by an overview of major events that are likely to have had a direct impact on the population of this species.

The Gympie Gold rush began in 1867. This mining operation released up to 20,000 tonnes of tailings, which polluted the Mary River with heavy metals. Several mercury-contaminated sites remain, including a permanent watercourse that is likely

to still carry mercury to the Mary River (Dhindsa et al. 2003). Unnaturally large quantities of sediment choked the river from the gold rush period until 1904 (Queensland Department of Primary Industries Water Resources 1995). Channels had to be excavated within the sediment to permit the river to flow. Historical photos (Figure 4.1) illustrate the extent of the disturbance.

Figure 4.1: Deep Creek Bridge, Gympie (QLD, Australia), showing mining activities within and adjacent to the creek, circa 1890. This bridge is located within 200 m of the Mary River. Photo: State Library Queensland.

Official Australian Museum collectors visited the Mary River in 1870. However, they failed to obtain any *E. macrurus* specimens, whilst successfully collected samples from every other species during their trip. The reason for this remains unknown (Cann & Sadlier 2017).

Commercial timber operations started in 1853, when large areas of native forest were cleared around the Mary River. Much of the sediment generated would have been

introduced into the river during the large floods of the 1890s (Queensland Department of Primary Industries Water Resources 1995). Post 1898, most of the sediment would have remained on the hill slopes and alluvial plains, until the decade of large floods in the 1950s, which included the worst flood of the century in 1955 (Australian Government Bureau of Meteorology 2017; Queensland Department of Primary Industries Water Resources 1995).

Chronic sedimentation and turbidity affect local food webs, the growth, rate of reproduction, and mortality on a variety of freshwater fauna (Wood & Armitage 1997). Bimodal respiring freshwater turtles, such as *E. macrurus*, are likely to be affected by increased concentrations of suspended-sediment, as it affects their ability to aquatically respire and reduces their dive duration, increasing their exposure to predators (Clark 2008b; Schaffer et al. 2016). The frequency and the magnitude of the 1950s and subsequent major flood events (1955, 1968, 1974, 1989, 1992, 1999, 2011 and two in 2013) would have had a compounding effect on aquatic species.

Significant removal of sand and gravel from the floodplain and instream commenced in the early 1970s in the Mary River and has had implications far beyond the original extraction site (Brizga et al. 2004). The rapid growth of the building industry in the Sunshine Coast and Hervey Bay during the 1980s led to a four-fold increase in extraction of coarse sand from the Mary River (Queensland Department of Primary Industries Water Resources 1995). Most of the sand was extracted from five areas: one in the upper reach (between Conondale and Moy Pocket), two areas in the middle reach (Tuchekoi, and between Traveston Crossing and Gympie), one area in the mid-low reach (downstream of Bells Bridge), and one in the tidal reach at Maryborough (Queensland Department of Primary Industries Water Resources 1995).

E. macrurus preferentially nests on sandy banks, thus excavation at any scale is likely to have caused a reduction in recruitment.

Numerous exotic plant and animal species have been introduced into the Mary River and have significant impact on native wildlife (Brizga 2004). When the affected species are predators such as varanid lizards, a subsequent increase in abundance of prey species are likely (Doody et al. 2015; Jolly et al. 2015). The introduction of the cane toad, *Rhinella marina,* into the sugar cane growing districts of Queensland, including the Mary River catchment, occurred in 1935. Extensive research has demonstrated that population-level declines of predatory varanid lizards consistently follow the arrival of *R. marina* due to toad-induced lethal toxic ingestion (Lever 2001; Phillips & Shine 2006). On the Daly River in the Northern Territory, Australia, cane toad invasion killed many yellow spotted monitors and presumably, as a result rates of predation by varanid lizards on the nests of pig-nose turtles, *Carettochelys insculpta*, fell from an average of around 17–23% to zero after the toad invasion (Doody et al. 2006). Varanid lizards, *Varanus panoptes* and *Varanus varius*, are known predators of *E. macrurus* nests, thus it is likely that the rate of recruitment for *E. macrurus* would have increased for a period following the arrival of *R. marina* (Beukeboom 2015; Limpus 2008).

Changes in the populations of exotic and native terrestrial predators of *E. macrurus* nests were noted by an egg collector. In a recorded interview, he noted the differences in predation of eggs between the 1960s and the mid-1990s. Predation by neither foxes or varanids was a big problem during the egg collection era (1960-70s; Flakus 2002). However, when this egg collector revisited one of the most productive nesting banks in the mid-1990s, only one nest was found with the remainder destroyed

by foxes (Flakus 2002). The egg collector's observation was that since the farmers stopped shooting, the fox numbers increased (Flakus 2002).

Changes have occurred in the aquatic hierarchical structure of the aquatic food web with the introduction of predatory species and the demise of the top aquatic predator. The stocking of native and non-native predatory species, such as the sooty grunter (*Hephaestus fuliginosus)*, saratoga (*Scleropages leichardti)*, and golden perch (*Macquaria ambigua*), with the aim of enhancing recreational fishing has been identified as a possible threat to the sympatric Australian Lungfish (*Neoceratodus forsteri*; Lintermans 2004; Pusey et al. 2004). These introduced fish species are likely to be impacting upon the population of *E. macrurus* as well. The Mary River cod (*Maccullochella mariensis*), is one of the top aquatic predators in the Mary River, but they are now very rare or absent in many areas where they were once common (Simpson & Mapleston 2002).

Possible threats specific to the lower reach

The absence of immature *E. macrurus* and low numbers of immature turtles from the other species suggest the existence of an instream threat(s) particular to the lower reach. It was thought that the nest protection program in the lower reach would have negated any variation in the rate of terrestrial predation of eggs or hatchlings between study reaches.

A noticeable difference between the lower and the other reaches is the presence of a barrage. The barrage, constructed in 1982, transformed over 30 km of a flowing tidal reach into a 'lake', drowning pools and riffles (Figure 1.8; Brizga et al. 2004; Tucker et al. 2012), and as a consequence stream flow, habitat features, and the

assemblage of fish species have been modified (Berghuis & Pilz 2005; Bodie 2001; Bunn & Arthington 2002; Hamann et al. 2008; Kuchling 2009; Tucker et al. 2012; Vandewalle & Christiansen 1996). While the Lower reach is not contained within the impoundment, this study reach includes the adjacent upstream pool. All aquatic species can move freely between the Lower reach and the impounded waters (Micheli-Campbell et al. 2013b). Impounded waters are known to favour the native fork-tailed catfish (*Arius graeffei*; Pusey et al. 2004a) recognised natural predator of freshwater turtles in waterholes (Blamires & Spencer 2013). During the 2016 trapping season, 53 of the 57 *A. graeffei,* trapped in the fyke nets, occurred in the Lower reach. *A. graeffei* is a listed as a threatening process for another Australian freshwater turtle, *Wollumbinia georgesi* (Department of the Environment and Energy 2016), thought its direct impact upon *E. macrurus* population is unknown.

In addition, the barrage blocked the passage of diadromous and estuarine fish species, such as barramundi (*Lates calcarifer*), bullsharks (*Carcharhinus leucas*), kingfish (*Polydactylus sheridani*), mangrove jack (*Lutjanus argentimaculatus*), and queenfish (*Scomberoides lysan*) from 1982 until 2001 when the fish-way was upgraded (Berghuis & Pilz 2005; Johnson et al. 1982). Movement of these species into the impoundment is unknown (except for barramundi), as none have yet been detected moving through the upgraded fish-way (Berghuis & Pilz 2005).

Implications for *E. macrurus* management and conservation

Two of the 'fathers' of turtle conservation and research, Dr Archie Carr and Dr Peter Pritchard, provide insights into turtle conservation that should be applied to the MRT conservation program. In 1984, Dr Carr noted that the protection of turtles is not a parochial problem; they cannot be saved in any one place or by controlling any one

phase of the life cycle (as cited in Frazer 1992). Dr Pritchard suggested that though the results of past and future actions in conserving turtles may be uncertain, taking no action is indefensible (Pritchard 1980).

Over the past 50 years, turtle nest protection programs have been embraced on a global species for a wide range of marine and freshwater turtles (Bonin et al. 2006). An indication that head-starting is accepted as a necessary component of turtle conservation projects is that one of the most prominent turtle conservation organisations, Turtle Survival Alliance, is involved in head-starting at least 11 species (Burke 2015). However, studies that evaluated head-starting as a management tool for threatened turtle species, found that it can augment increasing the population only when adult survival is at high levels (Heppell & Crowder 1996, Paez 2015). One of the benefits of head-starting is cited is the public education and support for conservation that is generated by production of hatchling turtles (Chen 2017, Burke 2015; Penaloza et al. 2015).

Adaptive management has been described as a willingness to experiment, monitor and adapt, realising we may not have the one correct answer because ecological and social systems are complex (Grumbine 1997). The present study demonstrated that the current program be adapted and incorporate new conservation actions. This will require collaboration between the community, landholders, other organisations, researchers, and government agencies.

Threats to other life-stages

The highest priority for the conservation of *E. macrurus* is to identify and manage the threat(s) to immature turtles throughout the river and adult females in the

lower reaches. Given the low capture rate of immature turtles, a careful examination of stage-specific mortality sources of *E. macrurus* is urgently required to guide more effective management strategies appropriate to river reaches. Currently, instream threats may be counteracting the increase in hatchlings entering the river as a direct result of the nest protection program. Until the in-stream threats are known and mitigated, it is unlikely that any effort to increase recruitment will make any significant difference to the population of *E. macrurus*. Protection of adult females, in particular, nesting females in the Lower reaches is paramount given the skewed sex ratio that was detected by the present study and the significant decline of nests laid since that observed in the 1960s. Nesting females are susceptible to predation by terrestrial predators during the nesting season, thus implementation of predator management programs are of critical importance.

Recruitment

A challenge for the future conservation of *E. macrurus* is to find conservation solutions that are neither costly nor difficult to implement. The direct and on-ground components of the MRT conservation program have appeal to the general public and funding bodies. The expansion of the nest protection program into other locations may have a greater impact on the population, given the presence of immature turtles and the higher abundance of females found in other more upstream river reaches.

Since the beginning, the on-ground component of the Mary River turtle conservation program has focused on the Tiaro reach. While TDLG members are interested in the survival of *E. macrurus*, their greatest concern is for the population within the geographic range that aligns with that of their group, i.e. the lower reach. Given the existing community interest and the population status, I therefore

recommend that recovery actions to stop the localised decline in population are in greater need in the lowest reach of my study area. Consequently, it is recommended that a suite of head-starting techniques be implemented specifically in the lower reach. There are at least four options to head-start turtles (Burke 2015). First, turtle nests are protected in-situ with predator excluders, similarly to the methods already implemented within the lower reach. Second, nests can be protected with predator-excluders that do not allow the hatchlings to escape. Instead, the hatchings are collected and raised in captivity until the desired age or size for release. Third, nests can be removed to safer locations in the field. Fourth, eggs are collected, incubated in captivity, and the resulting hatchlings are released either immediately or after a period of husbandry. Activities that incorporate captive rearing of eggs and/or hatchling turtles for release in the wild has long been controversial. Therefore, such actions are generally considered a last resort, because it is unknown if there are any long-term impacts on reproductive behaviour of head-started turtles (Burke 1991; Frazer 1992; Seigel & Dodd 2000).

Spencer (2017) found that for the widespread Australian turtle, *Chelodina longicollis*, periodic increases in recruitment can sustain populations. This can potentially allow populations of a particular region to be managed in a mosaic fashion, which means that not all populations need to be managed each year. Thus, the incorporation of a suite of head-starting techniques, implemented periodically, may successfully increase recruitment. Translocations may be required as a last resort to aid the recovery of the population in the lower reach. Nevertheless, head-starting cannot succeed in any meaningful sense if used as the only conservation strategy (Burke 2015).

The long-term commitment to the recovery of a threatened species, as shown by TDLG, suggests that community engagement is critical for the conservation of threatened species. Innovative and simple measures that engender hands-on experiences may strengthen human attachment to the species and thus increase community engagement. Turtles are relatively easy to raise from eggs to juveniles and hatchlings have tremendous public appeal, often generating significant income from tourists (Burke 2015; Wilson & Tisdell 2001). In Mexico, a few eggs were incubated, with hatchlings released by the children at the Festival of Turtle (Burke et al. 2000). Likewise, the Turtle Conservation Society of Malaysia established Terrapin Independence Day, when community participation is encouraged through releasing a token cohort of head-started juveniles into the river (Chen 2017). My recommendation is that community participation is actively encouraged, and a similar program to the aforementioned is implemented across the catchment where a few head-started turtles are ceremoniously released into the river.

Predator management

Management of predatory native wildlife, such as varanids, is complex and investigation into alternative actions such as the use of scent deterrents and detection dogs are recommended. The broad-scale management of introduced species such as foxes and wild dogs is more achievable. Foxes and wild dogs have an economic impact for landholders who manage livestock, thus providing landholders with an additional incentive to manage these species. The establishment of partnerships between local government authorities, TDLG, and other Natural Resource Management groups to implement a wild dog and fox control program during the nesting season of *E. macrurus,* is recommended. Feral pigs are an emerging threat with sightings along

Tinana Creek and the Mary River (Mary River Catchment Coordinating Committee 2015) and a management program to minimise the impact of these species during *E. macrurus* nesting period is recommended.

Habitat management

The population is likely to decrease with time due to the environmental pressures and habitat disturbances upon both adult and juvenile turtles. Protecting nesting areas and a diversity of habitats along the river associated with different channel characteristics and patterns may be an important factor contributing to the long-term viability of the turtle populations. Though it was beyond the scope of this study to identify specific habitat structure and features imposed at a broader spatial scale, the assemblage patterns observed suggest geomorphological features that influence assemblage composition. Thus, preservation of those features, such as pool/riffle frequency, is critical to maintain the current assemblages.

Habitat restoration is a complex and expensive task. Between AUD$6 - 7 million have already been invested in riparian rehabilitation and instream restoration over the past 22 years by the Mary River Catchment Co-ordinating Committee (Smith & Connell 2018). However, targeted investment is required to protect and rehabilitate habitat features and river reaches critical to the survival of *E. macrurus*. The population of three other aquatic species are also at risk: the Mary River Cod (*Maccullochella mariensis*), the Australian Lungfish (*Neoceratodus forsteri*) and the white-throated snapping turtle (*Elseya albagula*). Riparian and in-stream habitat restoration is thus likely to benefit all four-threatened species. Suggested remedial actions and costings outlined by Brizga et al. (2004) remain relevant.

Implications for Government

Water management decisions may continue to threaten the population of *E. macrurus* and of other aquatic species that inhabit the Mary River (Commonwealth of Australia 2017). Since the 1970s, a dam on the Mary River has been considered as a viable option to augment Brisbane's water supply (Queensland Government 2006). In 2006, the QLD government proposed the construction of the Traveston Crossing Dam. The proposal was however, rejected by the Federal Environment Minister at the time, who determined that the impacts of the proposed dam upon the river's threatened species would be too great (Department of the Environment and Energy 2009). The QLD government has identified an unallocated strategic water reserve of 150,000 ML in the Mary basin (Queensland Government 2006). The if, when, and how that reserve will be used remains unknown. The creation of an additional threat that has the potential to reduce flows in the main river channel would likely have a devastating long-term impact upon the survival of *E. macrurus* – an ecologically specialist species. Likewise, such large dam would impact upon their nesting abilities, fragment the population, and increase adult mortality through death from overtopping flows. Embedding flow requirements for species management is required in the Mary River Water Plan to ensure appropriate flow regimes are provided for all threatened aquatic species.

In addition, river management needs to account for alterations to habitats that may favour fork-tailed catfish, such as higher water temperatures, which may result in greater predation upon hatchling and juvenile turtles.

The development of an Australian Freshwater Turtle Action Plan is urgently required. The model of grouping common species together for the purpose of an Action or Recovery Plan is already established for marine turtles, as well as for other

groupings of wildlife species. This Plan could address the threats and the recovery objectives for all Australian threatened freshwater turtles, including the Bellinger River snapping turtle (*Wollumbinia georgesi*), Bell's turtle (*Wollumbinia belli*), Fitzroy River turtle (*Rheodytes leukops*), Mary River turtle (*E. macrurus*), Western Swamp tortoise (*Pseudemydura umbrina*), and the white-throated snapping turtle (*E. albagula*). Access to grants and other funds can be limited without a government-endorsed Plan.

The status of *E. macrurus* is a motivating factor for the MRT conservation program. *E. macrurus* is currently listed as 'endangered', both nationally (Department of the Environment 2017) and internationally (International Union for Conservation of Nature 2011). This study found: 1) the MRT nest protection program has, to date, failed to reverse the low levels of population recruitment, 2) a low proportion of immature *E. macrurus* throughout the study reaches, 3) a lack of recovery of the adult female population from the levels evident during the 1960-70s, and 4) an ageing population. Therefore, I recommended that the listing of *E. macrurus* is reviewed and assessed under Criterion One A2 (population reduction may not have ceased, given that there is no evidence of population recovery since the 1960s); and Criterion Three C1 (the continuing decline of mature individuals of at least 25% in one generation, given the paucity of immature turtles and declining adult female population in the lower reach). Geographic distribution and local population size combined provide a useful notion of rarity.

Directions for future research

There are several specific areas within this thesis where further investigation would lead to improved study conclusions.

A population estimate for the Lower reach is required. Additional sampling episodes are urgently needed to build on the capture records from this study.

Investigation of accurate ageing techniques for this species upon which the time of maturity and longevity in particular, are dependent. Analysis of historical mark-recapture information from the current Queensland Freshwater Turtle database for this species may provide a growth model to be used in a population model. The construction of a stage-based population model may allow investigation of sensitives of life stages to further refine conservation actions for this species. Investigations are underway to determine if carapace ageing by use of bomb radiocarbon dating may be useful (Van Houtan et al. 2016).

The identification of the cause of immature mortality is also a high priority. One possibility is the predation from the fork-tailed catfish – the extent of the predation rate is unknown. Investigations into this fish's dietary patterns is required, given it is known to be a threat upon the population of *Muchelys georgesi* in the Bellinger River (Blamires & Spencer 2013).

Furthermore, the identification of the cause for the significant decline of adult females in the lower reach since the 1970s is required. If the cause of the downward trend in the population cannot be identified and remedied, the population in this reach is likely to become extinct.

A habitat assessment is needed to identify the key features necessary for continued persistence of *E. macrurus*. This species was most abundant in the Middle reaches with juveniles most abundant in the Upper reaches. A study of the biotic and abiotic factors within those study reaches may indicate those that are necessary for a viable population. These features can then be confidently included in water management and riverine restoration plans.

Identification of the population of *E. macrurus* in the major tributaries is also required as little is known about the utilisation of such habitats by this turtle. Monitoring of these areas is required to determine presence/absence of *E. macrurus*, the structure of the population and the composition of turtle assemblages within the tributaries.

Finally, I recommended that comparative studies, using the same methodology adopted in this study, are conducted within the next 10-20 years. This would allow for the detection of population trends, changes in assemblage composition and thus inform targeted riverine and species management actions.

Funds

A major obstacle to implementing these recommendations is the sourcing of adequate funds. Opportunities for funding are limited, as many funding bodies prefer on-ground actions that, by design, achieve short-term, tangible results. Examples of such actions are: habitat improvement through tree planting, weed control, and number of eggs protected. This obstacle may be lessened if sufficient donations could be attracted to the Mary River turtle conservation Public Fund, where the allocation of resources is not subject to the such restrictions. However, funds from government and other organisations are needed to supplement the one generated by the community, due to the high cost of habitat management and the scale of the work required.

While this research assessed the impacts of the MRT conservation program on the population of *E. macrurus*, the program's impact has not been limited to the ecological sphere. In 2004, Tiaro and District Landcare established the Mary River Turtle Support Scholarship for postgraduate researchers to address knowledge gaps

about the turtle's population, biology and ecology. The funding of this scholarship is in part due to the proceeds from the sale of chocolate turtles, a highly successful, innovative, fund-raising initiative of TDLG. Resultant thesis and peer reviewed papers have provided vital information on the physiology, biology and ecology of *E. macrurus* and contributed to more informed management decisions (Beukeboom 2015; Clark 2008; Clark et al. 2008a; 2008b; 2009; Collett 2017; Espinoza et al. 2018; Micheli-Campbell et al. 2011; 2012; 2013a; 2013b; 2017; Micheli-Campbell 2012; Schmidt et al. 2016; 2017). This support scholarship has contributed enormously to the current knowledge of *E. macrurus,* and I highly recommend that it continues.

Conclusion

Globally, we know far more about how turtles reached their state of decline than how we can reverse this trend. Thus, it is critical that studies such as this one investigate the effectiveness of conservation programs and thus reduce the allocation of finite time and resources to less effective actions.

This thesis used field-based methods to investigate freshwater turtle assemblages and more specifically, the population of an endangered species of freshwater turtle, *E. macrurus*. The primary goal of this study was to generate scientifically sound information that would: 1) establish a base-line data on freshwater turtle assemblages in the Mary River by using a standardised methodology, and 2) assess if the results of a nest protection program had translated into the population recruitment. I believe that the findings of this research will have significant impact on the conservation and on-going management of *E. macrurus* and its habitat – the Mary River and guide the direction of future research endeavours. I anticipate that the

methodologies used in this research will have application for assessing freshwater turtle assemblages in other river systems and for assessing the outcomes of other turtle nest protection programs. Such information is urgently required for the conservation and recovery of declining turtle populations worldwide.

In summary, the ultimate goal of every conservation program, including the MRT program, is a population flourishing within their natural environment (Frazer 1992). The three key levels of actions recommended to achieve this can be summarised as: (1) all age groups must be given adequate protection, (2) critical feeding, nesting, and migration routes must be protected and maintained, and (3) the river system requires protection from damming, draining, erosion, and pollution (Moll & Moll 2000).

What is known about the biology of *E. macrurus* is insignificant compared to what is not known, nonetheless the wisest conservation measures will still involve the least manipulation of the turtle's life history and environment (Meylan & Ehrenfeld 2000; Moll & Moll 2000).

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Appendix 1

Raw data from turtle capture

Table A1.1: *Elusor macrurus* capture records.

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
24-Mar-15		4	Ob1	294	217	60	F	2.54	Y	S8	157505570	N	
24-Mar-15		4	Ob5	376	283	170	M	5	Y	S18	157505573	N	
24-Mar-15		4	Ob1	376	265	180	M	5.37	Y	S11	157505560	N	
24-Mar-15		4	Ob1	378	271	200	M	5.29	Y	S20	157505557	N	
24-Mar-15		4	Ob1	385	287	208	M	6.19	Y	S13	157505558	N	
24-Mar-15		4	Ob5	327	243	92	F	3.86	Y	S3	157505559	N	
24-Mar-15		4	Ob5	389	269	150	M	5.62	Y	S19	157505569	N	
24-Mar-15		4	Ob1	390	284	170	M	6.19	Y	S12	157505561	N	
24-Mar-15		4	Ob5	353	257	80	F	4.21	Y	S5	157505568	N	
24-Mar-15		4	Ob1	364	258	100	F	5.2	Y	S17	157505571	N	
25-Mar-15		4	Ob3	370	269	180	M	4.99	Y	S14	157505497	N	
25-Mar-15		4	Ob1	374	270	180	M	5.32	Y	S16	157505556	N	30702
25-Mar-15		4	Ob1	375	272	170	M	5.44	Y	S4	157505496	N	
25-Mar-15		4	Ob3	387	276	204	M	5.67	Y	S7	157505498	N	
25-Mar-15		4	Ob1	398	290	190	M	6.79	Y	S9	157505495	N	
26-Mar-15		4	Ob2	378	267	180	M	5.39	Y	S1	157505499	N	
26-Mar-15		4	Ob3	209	171	360	I	1.03	Y	S15	12340	N	
27-Mar-15		4	Ob4	364	254	170	M	5.37	Y	S6	157505500	N	
27-Mar-15		4	Ob3	371	274	170	M	4.89	Y	S22	157505502	N	
27-Mar-15	24-Mar-15	4	Ob5	389			M		N	S19	157505569	Y	
27-Mar-15		4	Ob3	436	306	215	M	8.08	Y	S2	157505501	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
31-Mar-15		2	Sp2	323	248	71	F	3.88	Y	S21	956000005361418	N	
31-Mar-15		2	Sp2	398	287	200	M	6.36	Y	S24	956000005352626	N	
01-Apr-15		2	Sp4	314	240	100	M	2.67	N		956000005340031	N	
01-Apr-15		2	Sp5	362	272	180	M	4.8	Y	S39	956000005375276	N	
01-Apr-15		2	Sp2	392	283	200	M	6.25	Y	S32	956000005371869	N	
02-Apr-15		2	Sp5	390	288	210	M	5.63	Y	S37	956000005345798	N	
08-Apr-15		1	Ti3	381	264	195	M	5.65	Y	S27	956000005350656	N	
14-Apr-15		2	Sp5	300	232	50	F	2.74	Y	S29	956000005352024	N	
14-Apr-15		2	Sp5	378	280	180	M	6.89	Y	S33	956000005344412	N	
14-Apr-15		2	Sp5	422	294	200	M	6.6	Y	S28	956000005374768	N	
15-Apr-15		1	Ti5	364	254	190	M	5.47	Y	S26	956000005347812	N	
15-Apr-15		1	Ti5	373	277	175	M	5.99	Y	S34	956000005350550	N	
15-Apr-15		1	Ti5	374	272	210	M	5.95	Y	S36	956000005371937	N	
15-Apr-15		1	Ti5	380	277	200	M	6.39	Y	S31	956000005349454	N	
15-Apr-15		1	Ti5	396	300	180	M	6.64	Y	S23	956000005343279	N	
16-Apr-15		1	Ti5	368	279	200	M	5.86	Y	S35	956000005356060	N	
16-Apr-15		2	Sp4	382	280	200	M	5.89	Y	S30	956000005351569	N	
16-Apr-15		2	Sp4	386	266	190	M	5.73	Y	S25	956000005342482	N	
16-Apr-15		2	Sp5	410	290	210	M	6.18	Y	S38	956000005343369	N	
17-Apr-15		1	Ti4	344	254	210	M	4.57	Y	S40	956000005348558	N	
18-Apr-15		1	Ti4	368	258	200	M	5.27	Y	S41	956000005372224	N	
21-Apr-15		1	Ti2	355	277	190	M	6.03	Y	S44	956000005349711	N	
28-Apr-15		3	Ka4	350	266	170	M	4.68	Y	S47	956000005365391	N	
28-Apr-15		3	Ka4	409	285	200	M	6.71	Y	S43	956000005346239	N	
29-Apr-15		3	Ka1	312	224	70	F	3.05	Y	S46	956000005363019	N	
29-Apr-15		3	Ka3	360	272	170	M	4.83	Y	S51	956000005341178	N	
29-Apr-15		3	Ka4	311	242	60	F	3.42	Y	S49	956000005367059	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
29-Apr-15		3	Ka1	366	274	170	M	5.11	Y	S42	956000005372619	N	
29-Apr-15		3	Ka1	385	279	190	M	5.53	Y	S45	956000005342838	N	
29-Apr-15		3	Ka3	212	175	40	I	0.91	Y	S52	956000005346625	N	
29-Apr-15		3	Ka4	391	283	180	M	6.05	Y	S48	956000005351223	N	
11-Jun-15		3	Ka1	289	224	80	F	2.76	Y	S54	956000005367311	N	
11-Jun-15		3	Ka3	363	273	195	M	5.3	Y	S50	956000005344407	N	
11-Jun-15		3	Ka1	331	242	90	F	3.89	Y	S55	956000005369535	N	
12-Jun-15		3	Ka2	313	232	70	F	3.28	Y	S53	956000005366050	N	
12-Jun-15		3	Ka5	323	242	72	F	3.47	Y	S60	956000005360803	N	
12-Jun-15		3	Ka2	392	292	172	M	6.28	Y	S56	956000005365436	N	
12-Jun-15		3	Ka1	332	247	75	F	3.97	Y	S57	956000005364405	N	
02-Sep-15		1	Ti2	359	248	170	M	5.27	Y	S59	956000005368915	N	
02-Sep-15		1	Ti4	359	257	180	M	5.63	Y	S58	956000005346303	N	
02-Sep-15		1	Ti1	315	244	60	F	3.22	Y	S61	956000005372985	N	
02-Sep-15		1	Ti1	384	273	200	M	6.1	Y	S63	956000005375553	N	
08-Sep-15		3	Ka5	126	114	25	I	0.27	N	-	12341	N	
08-Sep-15		3	Ka5	325	276	150	M	5.11	Y	S62	956000005374469	N	
08-Sep-15		3	Ka3	369	273	185	M	5.39	Y	S81	956000005371432	N	
08-Sep-15		3	Ka5	372	272	190	M	5.44	Y	S60a	956000005363206	N	
09-Sep-15		3	Ka1	345	266	170	M	4.52	Y	S76	956000005353222	N	
09-Sep-15		3	Ka2	301	232	70	F	3.26	Y	S66	956000005369519	N	
09-Sep-15		3	Ka3	379	268	180	M	5.73	Y	S68	956000005370743	N	
09-Sep-15		3	Ka5	324	252	52	F	3.7	Y	S67	956000005351351	N	
09-Sep-15		3	Ka3	382	281	190	M	6.4	Y	S64	956000005343614	N	
09-Sep-15		3	Ka1	405	280	200	M	6.59	Y	S73	956000005347431	N	
09-Sep-15		3	Ka3	282	228	120	I	2.22	Y	S69	956000005345473	N	
09-Sep-15		3	Ka1	420	300	200	M	7.22	Y	S65	956000005360864	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
10-Sep-15		3	Ka2	289	211	63	F	2.71	Y	S71	956000005364004	N	
10-Sep-15		3	Ka3	389	286	200	M	5.8	Y	S70	956000005368668	N	
10-Sep-15		3	Ka5	399	286	185	M	6.08	Y	S75	956000005375343	N	
10-Sep-15		3	Ka2	328	245	74	F	3.7	Y	S74	956000005362116 956000005341549	N	
11-Sep-15		3	Ka3	292	228	70	F	2.74	Y	S77	956000005370777	N	
11-Sep-15		3	Ka4	384	280	180	M	6.05	Y	S82	956000005363738	N	
11-Sep-15		3	Ka3	383	273	190	M	6.12	Y	S79	956000005352546	N	
15-Sep-15		2	Sp2	307	227	130	M	2.69	Y	S83	956000005344595	N	
15-Sep-15		2	Sp5	299	239	64	F	3.06	Y	S88	956000005362008	N	
15-Sep-15		2	Sp5	311	238	60	F	3.09	Y	S87	956000005338488	N	
15-Sep-15	16-Apr-15	2	Sp2	385	271	20.5	M	5.95	N	S25	956000005342482	Y	
15-Sep-15		2	Sp2	380	286	187	M	6.52	Y	S86	956000005337137	N	
15-Sep-15		2	Sp5	385	278	180	M	5.97	Y	S80	956000005373449	N	
15-Sep-15	14-Apr-15	2	Sp5	379	280	180	M	6.29	N	S33	956000005344412	Y	
15-Sep-15		2	Sp5	319	248	90	F	4.14	Y	S85	956000005348042	N	
15-Sep-15		2	Sp4	398	284	210	M	7.31	Y	S78	956000005371296	N	18433
15-Sep-15		2	Sp5	406	296	190	M	6.77	Y	S89	956000005340603	N	
15-Sep-15		2	Sp2	416	293	225	M	7.04	Y	S84	956000005358039	N	10735
16-Sep-15		2	Sp3	369	278	180	M	5.49	Y	S92	956000005351125	N	
16-Sep-15		2	Sp2	369	280	175	M	6.04	Y	S91	956000005363401	N	
16-Sep-15	14-Apr-15	2	Sp2	299	224	60	F	3.21	N	S29	956000005352024	Y	
16-Sep-15		2	Sp4	375	275	195	M	6.63	Y	S105	956000005357962	N	18367
16-Sep-15		2	Sp2	319	241	60	F	3.54	Y	S93	956000005343553	N	10734
16-Sep-15		2	Sp5	385	286	200	M	6.08	Y	S95	956000005342338	N	
16-Sep-15		2	Sp5	388	290	190	M	6.28	Y	S98	956000005363640	N	
16-Sep-15		2	Sp1	388	294	195	M	7.63	Y	S96	956000005353486	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
16-Sep-15		2	Sp5	411	303	200	M	6.76	Y	S90	956000005344867	N	
16-Sep-15		2	Sp5	332	242	65	F	4.51	Y	S94	956000005346562	N	10739
17-Sep-15		2	Sp5	364	271	200	M	5.24	Y	S97	956000005357741	N	
17-Sep-15		2	Sp5	374	261	210	M	5.85	Y	S99	956000005358770	N	
17-Sep-15		2	Sp2	323	237	95	F	3.85	Y	S101	956000005360034	N	
17-Sep-15	15-Sep-15	2	Sp3	394	286		M		N		956000005371296	Y	18433
18-Sep-15		2	Sp3	321	244	130	M	2.7	Y	S107	956000005362707	N	
18-Sep-15		2	Sp1	343	255	175	M	4.2	Y	S100	956000003603010	N	
18-Sep-15		2	Sp4	298	222	70	F	2.8	Y	S109	956000005372249	N	
18-Sep-15		2	Sp3	344	260	170	M	4.47	Y	S102	956000005353709	N	
18-Sep-15		2	Sp4	364	265	170	M	5.2	Y	S106	956000003612729	N	
18-Sep-15		2	Sp4	372	272	180	M	5.4	Y	S103	956000003599993	N	
18-Sep-15		2	Sp1	237	194	70	I	1.5	Y	S111	956000003609028	N	
18-Sep-15		2	Sp4	312	242	70	F	3.7	Y	S110	956000005358426	N	
18-Sep-15		2	Sp1	256	207	48	I	1.9	Y	S112	956000003608742	N	
18-Sep-15		2	Sp3	398	288	205	M	7.3	Y	S108	956000005361692	N	
18-Sep-15		2	Sp2	326	240	85	F	3.8	Y	S104	956000003612101	N	18051
18-Sep-15	14 Apr 15; 15 Sept 15	2	Sp3	378	277		M		N	S33	956000005344412	Y	
18-Sep-15	16-Sep-15	2	Sp3	332			F		N	S94	956000005346562	Y	
06-Oct-15		4	Ob1	352	256	180	M	4.82	Y	S116	956000005342832	N	
06-Oct-15	24-Mar-15	4	Ob1	386	284	210	M	6.19	Y	S115	157505558	Y	
06-Oct-15		4	Ob1	394	292	185	M	6.31	Y	S113	956000005363659	N	
06-Oct-15		4	Ob2	336	242	93	F	3.97	Y	S122	956000005347163	N	
06-Oct-15		4	Ob1	430	300	220	M	7.67	Y	S114	956000005350846	N	
07-Oct-15		4	Ob3	334	256	90	F	3.98	Y	S117	956000005359543	N	
08-Oct-15		4	Ob3	358	259	190	M	4.62	Y	S118	956000005363613	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
08-Oct-15		4	Ob1	304	237	60	F	2.96	Y	S121	956000005372133	N	
08-Oct-15		4	Ob1	306	226	70	F	3.02	Y	S123	956000005364481	N	
08-Oct-15		4	Ob1	315	249	80	F	3.48	Y	S120	956000005344627	N	
08-Oct-15		4	Ob1	341	250	90	F	4.83	Y	S124	956000005346276	N	
08-Oct-15		4	Ob2	353	261	80	F	4.62	Y	S119	956000005366599	N	
08-Oct-15		4	Ob3	248	199	90	I	1.54	Y	S125	956000005373489	N	
09-Oct-15		4	Ob3	294	220	60	F	2.5	Y	S146	956000005365430	N	
09-Oct-15		4	Ob3	362	278	170	M	4.55	Y	S136	956000005341816	N	
09-Oct-15		4	Ob2	108	100	-	I	0.23	Y	S128	12342	N	
09-Oct-15		4	Ob3	378	272	180	M	5.62	Y	S138	956000005369606	N	
09-Oct-15		4	Ob4	313	228	75	F	2.83	Y	S134	956000005373463	N	
09-Oct-15		4	Ob3	383	280	190	M	5.48	Y	T2 S137	956000005350801	N	
09-Oct-15		4	Ob2	320	241	80	F	3.77	Y	S126	956000005369339	N	
09-Oct-15		4	Ob1	323	246	80	F	3.5	Y	S129	956000005348252	N	
09-Oct-15		4	Ob3	324	231	80	F	3.27	Y	S135	956000005363025	N	
09-Oct-15	26-Mar-15	4	Ob3	207	170	40	I	-	N		956000005371413	Y	
09-Oct-15	24-Mar-15	4	Ob1	359	260	90	F	-	N	S17	157505571	Y	
09-Oct-15		4	Ob2	327	248	80	F	3.93	Y	S127	956000005371561	N	
09-Oct-15		4	Ob1	354	263	100	F	4.6	Y	S130	956000005362239	N	
29-Mar-16		1	Ti4	366	255	185	M	5.2	Y	S143	956000005357960	N	
29-Mar-16		1	Ti4	373	258	200	M	5.32	Y	S141	956000005364489	N	
29-Mar-16		1	Ti4	380	276	190	M	6.54	Y	S132	956000005347037	N	
31-Mar-16		1	Ti4	362	265	190	M	4.75	Y	S139	956000005357269	N	
01-Apr-16		1	Ti5	369	271	190	M	5.26	Y	S140	956000005368968	N	
01-Apr-16		1	Ti5	375	267	200	M	6.56	Y	S142	956000005356827	N	
01-Apr-16		1	Ti4	391	274	205	M	6.55	Y	S149	956000005362067	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
12-Apr-16		3	Ka1	331	260	140	M	3.94	Y	S131	956000005338430	N	
12-Apr-16		3	Ka5	354	263	175	M	4.5	Y	S151	956000005358176	N	
12-Apr-16		3	Ka5	353	252	180	M	4.82	Y	S152	956000005352519	N	
12-Apr-16		3	Ka5	317	225	60	F	3.26	Y	S153	956000005349174	N	
12-Apr-16		3	Ka5	359	266	170	M	5.05	Y	S154	956000005366078	N	
12-Apr-16		3	Ka1	382	277	195	M	5.2	Y	S147	956000005350779	N	
12-Apr-16	11-Jun-15	3	Ka4	364	275	180	M	5.41	N	S50	956000005344407	Y	
12-Apr-16		3	Ka3	375	283	185	M	5.57	Y	S148	956000005359721	N	
12-Apr-16		3	Ka3	379	277	185	M	5.75	Y	S150	956000005372552	N	
12-Apr-16		3	Ka1	380	266	190	M	5.78	Y	S145	956000005362998	N	
12-Apr-16		3	Ka1	372	274	200	M	6.01	Y	S144	956000005341854	N	
12-Apr-16	29-Apr-15	3	Ka5	392	284	190	M	6.12	N	S48	956000005351223	Y	
12-Apr-16		3	Ka1	412	290	20	M	6.96	Y	S133	956000005344722	N	
12-Apr-16		3	Ka4	411	291	190	M	7.37	Y	S159	956000005347340	N	
13-Apr-16		3	Ka5	287	225	60	F	2.5	Y	S156	956000005345285	N	
13-Apr-16		3	Ka5	369	276	180	M	4.84	Y	S158	956000005352576	N	
13-Apr-16	29-Apr-15	3	Ka2	369	279	185	M	5.33	N	S42	956000005372619	Y	
13-Apr-16		3	Ka5	374	257	185	M	5.34	Y	S157	956000005367321	N	
13-Apr-16		3	Ka4	380	289	180	M	5.52	Y	S161	956000005342411	N	
13-Apr-16		3	Ka2	388	288	190	M	6.17	y	S160	956000005366180	N	
14-Apr-16		3	Ka1	312	239	120	M	2.92	Y	S165	956000005362244	N	
14-Apr-16		3	Ka2	116	109	20	I	0.18	Y	S171	12344	N	
14-Apr-16		3	Ka3	336	262	140	M	3.59	Y	S167	956000005374916	N	
14-Apr-16		3	Ka2	337	246	190	M	4.3	Y	S163	956000005365590	N	
14-Apr-16		3	Ka2	122	110	23	I	0.18	Y	S170	12343	N	
14-Apr-16		3	Ka1	364	267	190	M	4.52	Y	S169	956000005349854	N	
14-Apr-16		3	Ka1	370	267	190	M	5.16	Y	S164	956000005374107	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
14-Apr-16		3	Ka3	365	272	190	M	5.17	Y	S168	956000005371367	N	
14-Apr-16		3	Ka3	378	267	175	M	5.19	Y	S162	956000005360101	N	
14-Apr-16		3	Ka2	134	122	30	I	0.27	Y	S166	956000005370506	N	
14-Apr-16	12-Apr-16	3	Ka3	411	304		M		N	S159	956000005347340	Y	
14-Apr-16	12-Apr-16	3	Ka1	412			M		N	S133	956000005344722	Y	
15-Apr-16		3	Ka2	288	220	70	F	2.43	Y	S173	956000005368840	N	
15-Apr-16		3	Ka3	302	240	70	F	3.04	Y	S184	956000005352418	N	
15-Apr-16		3	Ka2	349	253	185	M	4.34	Y	S182	956000005374001	N	
15-Apr-16		3	Ka2	310	233	65	F	3.26	Y	S179	956000005371883	N	
15-Apr-16		3	Ka2	362	276	180	M	4.94	Y	S175	956000005351598	N	
15-Apr-16		3	Ka2	370	278	180	M	4.96	Y	S178	956000005356155	N	
15-Apr-16		3	Ka1	365	234	18	M	5.13	Y	S172	956000005363429	N	
15-Apr-16		3	Ka2	371	274	190	M	5.33	Y	S181	956000005356863	N	
15-Apr-16		3	Ka2	321	230	8.5	F	3.67	Y	S176	956000005375740	N	
15-Apr-16	10-Sep-15	3	Ka2	327	247	85	F	3.63	Y	S177	956000005362116 956000005341549	Y	
15-Apr-16		3	Ka4	393	290	180	M	6.26	Y	S185	956000005359682	N	
15-Apr-16		3	Ka2	238	199	80	I	1.27	Y	S174	956000005344839	N	
15-Apr-16		3	Ka2	255	199	55	I	1.59	Y	S180	956000005368914	N	
15-Apr-16		3	Ka1	268	209	60	I	1.93	Y	S183	956000005367223	N	
15-Apr-16	12-Apr-16	3	Ka2	380	270		M		N	S145	956000005362998	Y	
15-Apr-16	09-Sep-15	3	Ka1	424	301		M		N	S65	956000005360864	Y	
18-Apr-16		2	Sp5	292	225	55	F	2.79	Y	S192	956000005357415	N	
18-Apr-16		2	Sp1	344	260	170	M	4.2	Y	S188	956000005360879	N	
18-Apr-16		2	Sp5	351	262	190	M	4.94	Y	S199	956000005358388	N	
18-Apr-16		2	Sp5	363	262	180	M	5	Y	S187	956000005363355	N	
18-Apr-16		2	Sp5	385	278	200	M	5.56	Y	S190	956000005359761	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
18-Apr-16		2	Sp5	255	202	30	I	1.9	Y	S197	956000005347355	N	
18-Apr-16		2	Sp5	391	282	200	M	5.91	Y	S200	956000005371928	N	
18-Apr-16		2	Sp5	326	240	70	F	3.84	Y	S189	956000005348809	N	
18-Apr-16		2	Sp5	348	258	90	F	4.61	Y	S205	956000005348986	N	
19-Apr-16		2	Sp5	348	267	170	M	4.21	Y	S209	956000005374967	N	
19-Apr-16		2	Sp3	311	247	75	F	3.73	Y	S210	956000005347663	N	
19-Apr-16		2	Sp1	382	269	195	M	5.17	Y	S208	956000005358580	N	
20-Apr-16		2	Sp5	360	264	180	M	5.35	Y	S202	956000005358291	N	
20-Apr-16		2	Sp5	377	280	190	M	6.2	Y	S207	956000005344816	N	
20-Apr-16	19-Apr-16	2	Sp1	348	267		M		N	S209	956000005374967	Y	
20-Apr-16	18-Sep-15	2	Sp3	350	260	-	M		N	S102	956000005353709	Y	
22-Apr-16		2	Sp1	362	262	190	M	5.96	Y	S191	956000005363252	N	
22-Apr-16		2	Sp4	263	211	70	I	2.27	Y	S186	956000005348142	N	
22-Apr-16		2	Sp1	328	237	80	F	4.18	Y	S194	956000005345913	N	18356
26-Apr-16		4	Ob3	373	274	180	M	5.16	Y	S215	956000005346476	N	
27-Apr-16		4	Ob5	134	120	-	I	0.25	Y	S219	12345	N	
27-Apr-16		4	Ob1	426	308	190	M	7.12	Y	S198	956000005370545	N	
27-Apr-16		4	Ob1	427	304	200	M	7.15	Y	S214	956000005371894	N	
27-Apr-16		4	Ob2	337	238	80	F	4.27	Y	S206	956000005372863	N	
28-Apr-16	08-Oct-15	4	Ob1	342	252	90	F	4.79	N	S124	956000005346276	Y	
29-Apr-16		4	Ob1	418	309	210	M	6.34	Y	S205	956000005347459 956000005340405	N	
13-Sep-16		3	Ka1	363	267	180	M	5.06	Y	S220	956000005371342	N	
13-Sep-16		3	Ka3	376	268	185	M	5.81	Y	S221	956000005375844	N	
14-Sep-16		3	Ka3	375	268	180	M	5.31	Y	S226	956000005373629	N	
14-Sep-16		3	Ka3	149	129	30	I	0.26	Y	S228	956000005341815	N	
14-Sep-16		3	Ka2	203	167	55	I	0.81	Y	S223	956000005341708	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
14-Sep-16		3	Ka2	207	171	60	I	0.83	Y	S224	956000005362130	N	
14-Sep-16		3	Ka2	220	180	45	I	1.03	Y	S222	956000005373719	N	
14-Sep-16		3	Ka4	329	242	70	F	3.5	Y	S229	956000005367619	N	
14-Sep-16		3	Ka3	325	238	65	F	3.65	Y	S227	956000005357686	N	
15-Sep-16		3	Ka4	353	257	145	M	4.23	Y	S231	956000005372722	N	
15-Sep-16		3	Ka2	275	223	55	F	2.6	Y	S230	956000005364838	N	
15-Sep-16		3	Ka4	364	270	160	M	4.86	Y	S232	956000005363251	N	
15-Sep-16		3	Ka2	364	276	180	M	5.16	Y	S225	956000005365222	N	
15-Sep-16	15-Apr-16	3	Ka1	289	220	70	F	2.49	N	S173	956000005368840	Y	
23-Sep-16		3	Ka1	292	230	95	M	2.34	Y	S234	956000006069933	N	
23-Sep-16		3	Ka1	316	253	110	M	2.95	Y	S239	956000006292971	N	
23-Sep-16		3	Ka1	360	272	200	M	4.55	Y	S235	956000005361119	N	
23-Sep-16		3	Ka1	367	276	180	M	5.43	Y	S233	956000005363362	N	
23-Sep-16		3	Ka5	324	246	95	F	3.85	Y	S238	956000006305590	N	
23-Sep-16		3	Ka4	406	304	200	M	6.75	Y	S252	956000005374054	N	
25-Sep-16		1	Ti5	363	272	200	M	5.93	Y	S250	956000005357324	N	
25-Sep-16		1	Ti1	376	267	195	M	5.38	Y	S240	956000005341111	N	
25-Sep-16		1	Ti5	304	230	80	F	3.39	Y	S251	956000005360633	N	
25-Sep-16		1	Ti1	326	236	70	F	3.77	Y	S236	956000005361524	N	
25-Sep-16		1	Ti5	323	228	90	F	3.91	Y	S254	956000005343565	N	
25-Sep-16		1	Ti5	406	287	215	M	7.13	Y	S256	956000005372777	N	
26-Sep-16		1	Ti3	354	265	190	M	4.98	Y	S258	956000005342252	N	
26-Sep-16		1	Ti5	360	258	185	M	4.8	Y	S249	956000005370583	N	
26-Sep-16		1	Ti1	360	265	180	M	5.14	Y	S253	956000005364952	N	
26-Sep-16		1	Ti1	382	271	195	M	5.78	Y	S259	956000005375162	N	
27-Sep-16		1	Ti1	390	283	195	M	6.67	Y	S261	956000005929574	N	
27-Sep-16		1	Ti1	329	241	80	F	3.84	Y	S260	956000005345896	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
27-Sep-16		1	Ti1	334	241	90	F	3.84	Y	S262	956000006062456	N	
27-Sep-16		1	Ti1	393	286	205	M	6.17	Y	S263	956000006300876	N	
27-Sep-16		1	Ti1	393	277	185	M	6.18	Y	S264	956000006062895	N	
28-Sep-16		1	Ti1	315	237	80	F	3.56	Y	S245	956000005931644	N	
28-Sep-16		1	Ti4	387	277	185	M	5.7	Y	S266	956000005932258	N	
04-Oct-16		2	Sp1	302	217	65	F	2.84	Y	S269	956000005932521	N	
04-Oct-16	18-Sep-15	2	Sp1	328	239	85	F	3.91	N	S104	956000003612101	Y	18051
06-Oct-16		2	Sp3	372	285	150	M	5.4	Y	S243	956000005929679	N	
06-Oct-16	19-Apr-16	2	Sp3	317	243	75	F	3.81	N	S210	956000005347663	Y	
06-Oct-16	16-Apr-15	2	Sp3	405	295	190	M	6.49	N	S38	956000005343369	Y	
11-Oct-16		4	Ob1	357	273	150	M	4.23	Y	S272	956000005339520	N	
11-Oct-16		4	Ob1	359	269	180	M	4.79	Y	S274	956000005364191	N	
11-Oct-16		4	Ob1	368	272	170	M	4.42	Y	S275	956000005338146	N	
11-Oct-16		4	Ob1	303	226	60	F	2.9	Y	S255	956000005339891	N	
11-Oct-16		4	Ob1	386	293	180	M	5.96	Y	S247	956000005338439	N	
11-Oct-16		4	Ob1	388	285	185	M	5.57	Y	S271	956000005338754	N	
11-Oct-16		4	Ob1	391	293	175	M	5.92	Y	S273	956000005340853	N	
11-Oct-16		4	Ob1	402	283	190	M	6.25	Y	S267	956000005932726	N	
11-Oct-16	24-Mar-15	4	Ob1	386	283	180	M	5.84	N	S11	956000005338593	Y	
11-Oct-16		4	Ob1	405	291	190	M	7.02	Y	S270	956000005337993	N	
11-Oct-16	29-Apr-16	4	Ob3	415	299	200	M	7.12	N	S205	956000005347459 956000005340405	Y	
11-Oct-16	27-Apr-16	4	Ob1	428	309	180	M	7.14	N	S198	956000005370545	Y	
12-Oct-16		4	Ob3	374	276	200	M	5.22	Y	S237	956000005930329	N	
12-Oct-16		4	Ob1	311	247	85	F	3.2	Y	S244	956000005373543	N	
12-Oct-16		4	Ob1	324	248	75	F	3.6	Y	S241	956000005340200	N	
12-Oct-16		4	Ob2	343	249	90	F	4.22	Y	S265	956000005337224	N	

Capture date	Recapture date	River reach	Net ID code	Carapace (mm)		Tail (mm)	Sex M F I	Weight (kg)	Genetic sample taken	MRT genetic sample no.	PIT tag No.	Re-capture	Monel tag
				SCL	SCW								
12-Oct-16		4	Ob5	343	258	95	F	4.27	Y	S257	956000005929638	N	
13-Oct-16		4	Ob4	283	215	60	F	2.4	Y	S242	956000005340600	N	
13-Oct-16		4	Ob4	315	230	85	F	3.43	Y	S246	956000005339683	N	
13-Oct-16		4	Ob5	321	246	80	F	3.57	Y	S276	956000005338211	N	
13-Oct-16		4	Ob1	326	242	70	F	3.86	Y	S248	956000005340262	N	
13-Oct-16	12-Oct-16	4	Ob1	310	241		F	3.27	N	S244	956000005373543	Y	
13-Oct-16	12-Oct-16	4	Ob2	341	247	85	F	4.34	N	S265	956000005337224	Y	
13-Oct-16		4	Ob1	358	250	105	F	4.98	Y	S268	956000005340817	N	
13-Oct-16		4	Ob3	361	267	90	F	5.34	Y	S278	956000005933810	N	
14-Oct-16		4	Ob3	293	231	60	F	2.68	Y	S280	956000005340022	N	
14-Oct-16		4	Ob3	163	142	35	I	0.57	Y	S285	956000005338906	N	
14-Oct-16		4	Ob3	400	280	200	M	6.24	Y	S284	956000005340467	N	
14-Oct-16		4	Ob2	342	253	90	F	4.44	Y	S277	956000005338116	N	

Table A1.2: Sampling locations, Mary River, QLD.

Reach	Net Number	Location
Lower	Ti1	25˚43.920'S 152˚31.620'E
	Ti2	25˚44.023'S 152˚31.506'E
	Ti3	25˚44.265'S 152˚31.586'E
	Ti4	26˚45.707'S 152˚31.782'E
	Ti5	26˚45.688'S 152˚31.786'E
Mid-low	Sp1	26˚2.235'S 152˚30.309'E
	Sp2	26˚2.032'S 152˚30.598'E
	Sp3	26˚02.011'S 152˚30.789'E
	Sp4	26˚1.983'S 152˚30.781'E
	Sp5	26˚1.899'S 152˚30.916'E
Middle	Ka1	26˚20.066'S 152˚42.453'E
	Ka2	26˚20.097'S 152˚42.541'E
	Ka3	26˚21.224'S 152˚42.750'E
	Ka4	26˚21.545'S 152˚42.837'E
	Ka5	26˚21.793'S 152˚43.282'E
Upper	Ob1	26˚32.841'S 152˚45.023'E
	Ob2	26˚35.669'S 152˚43.976'E
	Ob3	26˚35.733'S 152˚44.520'E
	Ob4	26˚36.822'S 152˚43.669'E
	Ob5	26˚38.761'S 152˚41.171'E

Appendix 2

POPAN models and outputs per study reach

Comparing: Survivorship (Φ), Recapture Probability (*p*), Probability of Entry (PENT), and Population (*N*), group (g), time (t), no variance by time or group (.) of *E. macrurus*. Only the models with lower AIC_c values are shown.

Table A2.1: Candidate models for Mid-low study reach.

Model	AIC_c	Delta AIC_c	AIC_c Weight	Model Likelihood	Parameters	Deviance
Φ(.) *p*(.) PENT(.) *N*(g)	235.0549	0.00	0.42210	1.000	5	-86.4481
Φ(g) *p*(.) PENT(.) *N*(g)	236.4860	1.4311	0.20638	0.488	6	-87.44063
Φ(.) *p*(g) PENT(.) *N*(.)	236.9111	1.8562	0.16686	0.395	5	-84.5919
Φ(.) *p*(g) PENT(.) *N*(g)	237.2792	2.2243	0.13881	0.328	6	-86.6132

Table A2.2: Output of preferred model for Mid-low reach: Φ(.) p(.) PENT(.) N(g).

			95% Confidence Interval	
Parameter	Estimate	Standard Error	Lower	Upper
Φ	0.9990186	$0.5621518*10^{-003}$	0.9969868	0.9996808
p	0.0248202	0.0073149	0.0138803	0.0439978
PENT	$0.8881784*10^{-015}$	$0.8623376*10^{-008}$	$-0.1690182*10^{-007}$	$0.1690182*10^{-007}$
N_{male}	148.94489	42.719041	92.308998	269.33923
N_{female}	63.546544	20.708321	37.474673	124.52234

Table A2.3: Candidate models for Middle study reach.

Model	AIC_c	Delta AIC_c	AIC_c Weight	Model Likelihood	Parameters	Deviance
Φ(g) *p*(t) PENT(.) *N*(g)	234.248	0.0000	0.99042	1.00	21	-264.1128
Φ(t) *p*(.) PENT(.) *N*(g)	244.945	10.6975	0.00471	0.004	19	-246.6842
Φ(t) *p*(g) PENT(.) *N*(.)	245.322	11.0742	0.00390	0.003	19	-246.3075
Φ(t) *p*(g) PENT(.) *N*(g)	248.126	13.8786	0.00096	0.001	20	-246.8199

Table A2.4: Output preferred model for Middle reach: Φ(g) p(t) PENT(.) N(g).

Parameter	Estimate	Standard Error	95% Confidence Interval	
			Lower	Upper
Φ_{male}	0.9995814	0.0022196	0.0679506	1.0000000
Φ_{female}	0.9951014	0.0039652	0.9763286	0.9990015
p	0.0085668	0.0100553	0.8480585E-003	0.0808530
p	0.0236953	0.0218104	0.0038098	0.1334690
p	0.0119835	0.0107537	0.0020408	0.0671084
p	0.0148092	0.0116221	0.0031447	0.0668400
p	0.0121582	0.0092411	0.0027167	0.0526779
p	0.0262617	0.0150877	0.0084137	0.0789559
p	0.0139723	0.0088275	0.0040200	0.0473914
p	0.0098065	0.0066347	0.0025886	0.0364158
p	0.0579114	0.0350089	0.0171765	0.1777772
p	0.0229296	0.0148900	0.0063383	0.0794767
p	0.0319633	0.0186663	0.0100200	0.0972410
p	0.0430941	0.0232414	0.0147016	0.1196603
p	0.0073462	0.0073640	0.0010215	0.0508385
p	0.0102551	0.0091204	0.0017773	0.0568677
p	0.0159982	0.0126401	0.0033585	0.0727371
p	0.0180466	0.0135846	0.0040738	0.0762743
PENT	0.0377362	0.0250949	0.0100190	0.1319143
N_{male}	304.61252	116.68636	160.44804	653.64440
N_{female}	233.36842	117.33556	98.535674	605.73576

Table A2.5: Candidate models for Upper study reach.

Model	AIC_c	Delta AIC_c	AICc Weight	Model Likelihood	Parameters	Deviance
Φ(.) *p*(g) PENT(.) *N*(g)	273.8564	0.0000	0.99996	1.00	8	-80.0800
Φ(t) *p*(.) PENT(.) *N*(g)	294.0415	20.1851	0.00004	0.00	19	-92.3539
Φ(t) *p*(.) PENT(.) *N*(.)	314.7391	40.8827	0.00000	0.00	18	-68.2295
Φ(t) *p*(g) PENT(.) *N*(g)	328.8891	55.0327	0.00000	0.00	21	-64.7082

Table A2.6: Output preferred model for Upper reach: Φ(.) p(g) PENT(.) N(g).

Parameter	Estimate	Standard Error	95% Confidence Interval	
			Lower	Upper
Φ	0.9982823	0.0014880	0.9906610	0.9996860
p	0.0875800	0.0939504	0.0094904	0.4902106
p	0.0273393	0.0145391	0.0095337	0.0758527
p	0.0258092	0.0156805	0.0077427	0.0825250
PENT	0.0351154	0.0148788	0.0151563	0.0792437
$N_{immature}$	5.8909695	5.0090417	3.2874908	32.071209
N_{male}	188.85409	81.720456	93.210379	444.39395
N_{female}	176.50725	83.586430	82.418893	446.17700

Appendix 3

Contributing author to other relevant publications during candidature

Espinoza, T, **Connell, M,** Marshall S, Beukeboom R & McDougall A 2018, 'Nesting behaviour of the endangered Mary River turtle: Monitoring and modelling to inform e-flow strategies', *Australian Journal of Zoology*. *https://doi.org/10.1071/ZO17044.*

Micheli-Campbell, MA, **Connell, MJ**, Dwyer, RG, Franklin, CE, Fry, B, Kennard, MJ, Tao, J & Campbell, HA 2017, 'Identifying critical habitat for freshwater turtles: integrating long-term monitoring tools to enhance conservation and management'. *Biodiversity Conservation,* vol. 26, no. 7, pp. 1675-1688.

Schmidt, DJ, Brockett, B, Espinoza, T, **Connell, M** & Hughes, JM 2016, 'Complete mitochondrial genome of the endangered Mary River turtle (*Elusor macrurus*) and low mtDNA variation across the species' range', *Australian Journal of Zoology,* vol. 64, no. 2, pp. 117-121.

Schmidt, DJ, Espinoza, T, **Connell, M** & Hughes, JM 2017, 'Conservation genetics of the Mary River turtle (*Elusor macrurus*) in natural and captive populations', *Aquatic Conservation: Marine and Freshwater Ecosystems*, 2017; pp. 1-9.