

**Population ecology, ecophysiology, phylogenetics and taxonomy of the  
threatened western sawshelled turtle, *Myuchelys bellii*, from the  
Murray-Darling Basin of Australia.**

By

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## **Certification**

I certify that this thesis is my original work and has not been submitted, in whole or in part, for a degree at this or any other university. Nor does it contain, to the best of my knowledge and belief, any material published or written by any other person, except as acknowledged in the footnotes, quotations or references. I certify that any help in preparing this thesis and all sources used have been acknowledged in this thesis.

A handwritten signature in black ink, appearing to read "D.P. Fielder", with a long horizontal flourish extending to the right.

Darren Peter Fielder

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## Abstract

Resolving the evolutionary relationships among species remains an important focus of biodiversity conservation, and is key for assessing and reversing global trends in biodiversity decline. International and national systems of biodiversity protection rely on good taxonomic knowledge at the species level and good information about their biology and life history traits, knowledge that is often incomplete and therefore an impediment to effective conservation action. Over half of the world's turtle species are considered threatened, yet the taxonomy of many turtles species is not adequately resolved, and much work needs to be done to understand relationships between closely related taxa, and for uncovering cryptic species and genera. The *Myuchelys* genus is a little known group of Australian freshwater turtles. The focal species, the vulnerable western sawshelled turtle *Myuchelys bellii* located in the Murray-Darling Basin in eastern Australia, is both taxonomically confused and poorly studied.

I surveyed populations of *Myuchelys* species from within their natural ranges using a combination of baited traps or by hand using a snorkel. Most specimens were live captured and released. For genetic analysis, small skin tissue samples were taken from *M. bellii* individuals while previously collected blood and liver samples (snap frozen at -80°C) were used for other study species. One-off surveys of populations of *M. latisternum* in the Albert River and Lockyer Creek (Brisbane River catchment) were conducted in 2007-2008, while *M. georgesi* and *M. purvisi* were sampled in 2007 from the Bellinger and Manning Rivers located in northern coastal New South Wales (NSW). The study sites of *M. bellii* were located in the New England Tablelands Bioregion (NET) in the Murray-Darling Basin which experiences a temperate climate with cold winters and warm summers. It is not unusual for upland streams in the NET to freeze over and periodic winter snow falls across years to occur. Capture-mark-recapture surveys of *M. bellii* populations were conducted from 2002-2010 in Bald Rock Creek (Border Rivers) from southern Queensland and from 2005-2007 in the Namoi and Gwydir Rivers in NSW.

The phylogeny for the *Myuchelys* genus using 960 bp of the mitochondrial control and ND4 regions revealed a shallow genetic structure of 0.1-0.3% divergence across *M. bellii*'s range in the Namoi, Gwydir and Border Rivers. The phylogeographic patterns

observed for *M. bellii* indicates a recent range contraction to the headwater streams of the Darling River resulting in the three relict populations of today. Similarly, no diagnostic morphological differences between *M. bellii* populations were found but the holotype specimen was confirmed to be an *M. bellii*. Consequently, the genetic and morphological data were concordant in finding no support for the taxonomic distinction of the Queensland population of *M. bellii* to its southern congeners, thus rejecting earlier assertions of a cryptic species in *M. bellii*.

Many turtle species, including those native to Australia, are poorly understood in terms of the life history, ecology, and physiological tolerances. In particular, an understanding of the aquatic respiration abilities in freshwater turtles is essential as a result of habitat modification and loss through worldwide increases in water resource development. My results showed *M. bellii* to have delayed age at first breeding, low reproductive effort (14.3 eggs per adult female) and high survivorship with a predicted lifespan of over 40 years (Type III survivorship). Growth increment data from mark-recapture data provided an estimate of age at maturity with males taking nearly 10 years to mature and females approaching 20 years. Annual breeding rates were found to vary greatly between years with on average 78% of females breeding in any one year. These life history traits mean that *M. bellii* is intrinsically vulnerable to population declines through high levels of nest and hatchling predation (e.g., foxes, exotic fish) and altered habitat.

Not unlike *Rheodytes leukops*, which is considered to be a habitat specialist associated with well oxygenated riffle zones in the Fitzroy River, *M. bellii* is only found above about 700 masl in presumably well oxygenated cold flowing streams. It is restricted to riverine habitats and requires permanent aquatic refugia to persist at the local scale. My study was the first to describe the extraordinary aquatic respiration abilities of *M. bellii* which allow for extended aerobic dives of up to weeks during winter hibernation which I attribute to its cloacal bursae and temperate climate across its range. Despite its moderately specialised bursae morphology in comparison to other Australian chelids, *M. bellii* is second only to *Rheodytes leukops* in having the longest recorded dive for any freely diving freshwater or marine turtle at 15.5 days (maximum). The seasonal diving behaviour of *M. bellii* corresponds closely to those observed for *R. leukops*. In view of that, the intermediate complexity of the bursae structure for *M. bellii* may allow

for an unexpected level of aquatic oxygen uptake, particularly at low temperatures and at depth. Furthermore, the evolutionary forces of a temperate climate have meant that highly specialised bursae like those in *R. leukops* are not required to undertake aquatic hibernation during winter. These findings are important to better understanding the role of the cloacal bursae in pleurodires from temperate zones, and more generally, the lesser studied species within Australia.

In summary, the molecular and morphological evidence of this research do not support the presence of a cryptic species within *M. bellii*. Consequently, the three major populations of *M. bellii* from the Gwydir, Namoi and Border Rivers need to be managed as a single species entity. The relatively small size and extent of each population also means that *M. bellii* is certainly worthy of its national vulnerable status and international endangered status. Of particular concern for *M. bellii*, is the long term conservation of the isolated Queensland population . This thesis provides a clear foundation and insight into the conservation priorities for *Myuchelys* species, and in particular, for the threatened western sawshelled turtle *M. bellii*.

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## Chapter 1. General Introduction



Picture: The study species, the western sawshelled turtle *Myuchelys bellii* in its natural habitat in Bald Rock Creek, Girraween National Park in the Murray-Darling Basin of eastern Australia. Photo by Robert Ashdown.

## Chapter 1. General Introduction

### Conservation priorities for Australian freshwater turtles

Loss and alteration of habitat are major causes of turtle declines around the world while human exploitation has brought many south-east Asian species to the brink of extinction (Klemens 2000; Buhlmann *et al.* 2009). Illegal wildlife trade is also endangering species globally (Alacs 2008; Alacs and Georges 2008). Nearly half of the world's modern turtle fauna (Superorder Chelonia) are threatened or are already extinct in the wild making them the most threatened group of animals (Turtle Taxonomy Working Group 2010). Accordingly, a significant proportion of turtle diversity could be lost over the next century without strategic conservation intervention (Buhlmann *et al.* 2009).

Australia is not immune from these global trends with the coastal region of eastern Australia identified for additional conservation effort (Buhlmann *et al.* 2009). Worldwide, there are currently 333 recognised extant species of turtles, with approximately 10% occurring in the Australasian region (Turtle Taxonomy Working Group 2010; Georges and Thomson 2010). Of these turtles, six species are listed as nationally threatened under the Australian *Environment Protection and Biodiversity Conservation Act 1999*.

The most critically endangered species, the western swamp tortoise *Pseudemydura umbrina* (Siebenrock 1901), has suffered one of the most severe population declines through loss of habitat from agricultural and urban development. There are only about 100 individuals in total with around half of them remaining in their natural habitat of a single swampland area and the rest maintained in a captive breeding program (Burbidge 1981; Kuchling and de Jose 1989; Burbidge and Kuchling 1994). The endangered Mary River turtle *Elusor macurus* was subject to intense egg harvest to produce hatchling turtles for the pet trade (1960-1974) which was thought to have resulted in a 95% reduction in the size of the annual nesting population by 1999 (Flakus 2002). A third endangered species, the Gulf snapping turtle *Elseya lavarackorum*, was first described from fossils only later to be discovered living in northern Australia (White and Archer 1994; Thomson *et al.* 1997). This turtle is endemic to a single drainage system with little known about its ecology or biology.

The vulnerable Fitzroy River turtle *Rheodytes leukops* is known from a single drainage and is believed to be a habitat specialist relying on shallow riffles for its diet of macroinvertebrates (Legler and Cann 1980; Georges *et al.* 1993). In combination, these factors make it vulnerable to threatening processes which are thought to include habitat modification from water infrastructure impoundments and a lack of juvenile recruitment from high levels of nest predation (up to 100% predated, C.J. Limpus unpublished data) by native and feral animals (foxes, pigs, goannas and water rats). The species upon which this thesis is focused, the western sawshelled turtle *Myuchelys bellii* is also listed as vulnerable primarily because of its restricted range in the New England Tablelands and a poor understanding of its biology and ecology (Cann 1998). Recent investigation of the sixth species listed as threatened, the vulnerable Bellinger River Emydura *Emydura macquarii nigra*, has revealed that it is an introduced population of the common, widely distributed, Macquarie River turtle *Emydura macquarii* (Georges *et al.* 2007). Although conservation authorities will likely delist this turtle as a threatened species in time: such a move will probably be unpopular with local communities who have attached an elevated conservation status to this turtle population (NSW National Parks and Wildlife Service 2001; Georges *et al.* 2007).

There is increasing concern worldwide for the significant loss of aquatic ecosystems and associated biodiversity (Georges and Cottingham 2002; Saunders *et al.* 2002; Cullen 2003). Human consumption of water for urban, agricultural and mining purposes continues to be debated across Australia with the Murray-Darling Basin a key focus for government regulation. Not surprisingly, a common and ongoing threatening process affecting most threatened turtle species in Australia is water infrastructure development and possible detrimental long-term effects from changed conditions from flowing streams to stagnant reservoirs (Clark *et al.* 2009).

Detrimental anthropogenic impacts on aquatic ecosystems are particularly important for those turtle species that rely on aquatic respiration as part of their life history because the decreased oxygen levels and increased turbidity associated with impoundment and other forms of river regulation would negatively impact on cloacal ventilation (Clark *et al.* 2009). For example, dive duration was substantially reduced in Mary River hatchling turtles and Fitzroy River turtles in simulated hypoxic conditions which were considered

to potentially affect other aspects of their ecology (Gordos *et al.* 2004; Clark *et al.* 2009). In addition, an important microhabitat for the Fitzroy River turtle is the riffle zone which are becoming lost by virtual drowning from impoundments and water releases downstream of major weirs or dams (Georges *et al.* 1993). Turtles that rely on aquatic respiration are particularly vulnerable to these types of instream changes (Tucker *et al.* 2001). Consequently, an understanding of the ecological costs of further development activities is required for decision makers (Georges *et al.* 2002).

In order to better manage and conserve threatened turtles, and those that are widely distributed and abundant, good science must inform conservation directions and be integrated into management and policy decision making (Klemens 2000; Georges and Thomson 2010). Conservation agencies and community groups that are focussed on the stewardship of turtle populations and their habitats must be able to access the most up-to-date information to develop management and recovery strategies. Often though, not enough is known about species and their individual requirements for unqualified assessments of their conservation status and significance (Georges 1993). In particular, alpha taxonomy (describing biological entities at the species level or lower ranking taxa) for freshwater turtles in Australia has typically lagged behind scientific discoveries with some species remaining undescribed for many years (Georges 1993; Georges and Thomson 2010).

### **Alpha taxonomy**

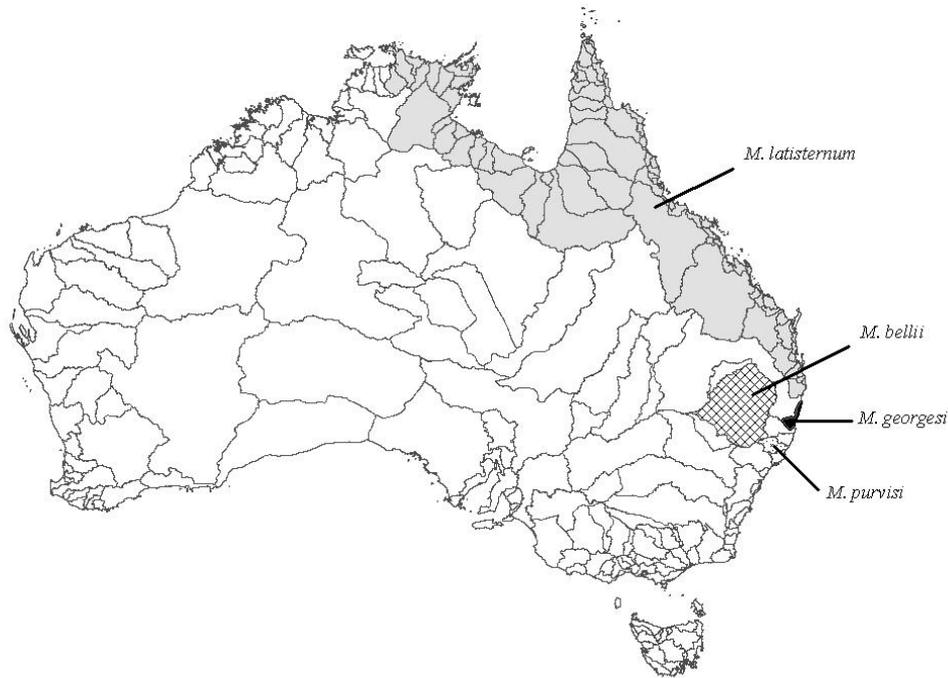
In a vacuum of information on poorly known and undescribed species, and substantial increases in molecular data, proliferation of scientific names can occur in the literature with unnecessary and subjective name changes lacking a strong consensus on their use (Artner 2008; Pauly *et al.* 2009; Georges and Thomson 2010). For example, North American anuran species lists have seen 56 new names out of 100 taxa in the latest edition (Pauly *et al.* 2009). These nomenclatural changes, such as those by Wells and Wellington (1985) for Australian amphibians and reptiles, and non-peer reviewed species descriptions circulating in private articles (Wells 2002; 2007; 2007; 2009) or hobbyist magazines (Cann 1997a; 1997b; 1997c) can lead to confusion and sometimes erroneous descriptions or identification of a biological entity (Georges and Thomson 2010). Georges and Thomson (2010) in their taxonomic review of freshwater turtle fauna in the Australasian region argue that nomenclatural stability in taxonomy is now

more important than ever as nearly half of all chelonian fauna are threatened globally, and without a clear understanding of taxa, conservation outcomes can be stifled. With confused alpha taxonomy, application of conservation criteria such as population declines, population size, area of occupancy and population viability analysis may be misdirected or erroneous.

In another example of nomenclatural confusion, the oblong turtle *Chelodina colliei* (Georges and Thomson 2010) from Western Australia has six different binomial names in use since its original description in 1856 (Gray 1841; Gray 1856). This situation arose partly because of an incorrect holotype specimen (now identified as a northern snake-necked turtle *C. rugosa*) being assigned to former names (*C. oblonga*) (Thomson 2000b). Subsequent attempts to rectify this situation by renaming the species and fixing a neotype specimen (McCord and Ouni 2007) were considered in violation of Articles 75.6 and 82.1 of the 1999 ICZN Code by Kuchling (2010). Consequently, timely revisions of groups of organisms, such as that provided by the recent review of Australasian freshwater turtles by Georges and Thomson (2010), can punctuate the scientific literature providing nomenclature clarification and stability for established biological entities.

### **The study species – *Myuchelys bellii***

Across the Australian continent, there are four recognised species in the short-necked genus of *Myuchelys* (*M. bellii*, *M. georgesi*, *M. purvisi* and *M. latisternum*) with a fifth species from New Guinea (*M. noveaguinea*) tentatively included in *Myuchelys* (Figure 1.1) (Thomson and Georges 2009; Georges and Thomson 2010). The nomenclature of the *Myuchelys latisternum* clade, incorporating the threatened western sawshelled turtle *M. bellii* from the Murray-Darling Basin in eastern Australia, has been a contentious subject with recent reviews by Thomson and Georges (2009) and Georges and Thomson (2010) rejecting proposed genus and species names raised in a series of articles (Wells 2002; 2007; 2009). Georges and Thomson (2010) argue that these documents do not meet the International Commission on Zoological Nomenclature publication guidelines. For the purposes of my thesis, I have adopted the nomenclature of Georges and Thomson (2010), a position that is inline with other authors (see Nowak-Kemp and Fritz 2010). Where it was considered relevant, I have restated this position in the following chapters.



**Figure 1.1** Distribution of the Australian short-necked *Myuchelys* species mapped to the major river drainage basins of Australia.

The genetically distinct taxon, *Myuchelys bellii*, was first described by Dr. John E. Gray in 1844 as *Phrynops bellii* (Gray 1844), and its name later resurrected by Cann (1998) after confirmation from molecular analyses of its species status (Georges and Adams 1992; 1996). Without knowing the location of the *P. bellii* holotype (Oxford University Museum, 8460) or having other related specimens from Australia at the time, Gray associated the species with the South American side-necked genus *Phrynops* (Cann 1998). An ‘undescribed’ species of *Myuchelys* from the Namoi and Gwydir River catchments of the New England Tablelands bioregion in northern New South Wales (NSW) was included in ‘Tortoises of Australia’ (Cann 1978) after its discovery in the 1970’s. Gray’s description of *P. bellii* and its holotype went unnoticed for nearly 150 years; but, on recent examination, populations of *Myuchelys* from the Namoi and Gwydir River drainages were assigned to *M. bellii* (Cann 1998). However, without a known location for the *P. bellii* holotype specimen and inconclusive morphological characteristics (Cann 1998) there remains a question mark on the holotype specimen’s true species identity.

In addition to these nomenclatural issues, a small disjunct population of *Myuchelys* was discovered in southern Queensland in Bald Rock Creek (Border Rivers region) in 1992. This discovery was believed to possibly represent a cryptic species within *M. bellii* based on subtle morphological variations (Cann 1998). However, in the absence of genetic evidence and detailed morphological assessments, it remained unclear if this Queensland population warrants specific or sub-specific status separate from populations of *M. bellii* located in the Namoi and Gwydir Rivers of NSW. Furthermore, a general lack of information about the ecology of *M. bellii*, including its level of reliance on aquatic respiration, has hindered effective conservation management for its long-term survival and recovery.

### **Thesis aims and structure**

An interdisciplinary inquiry into species and their ecology provides for the greatest opportunity to advance their conservation. For example, conservation biology – a multidisciplinary area of science that emerged in the 1980's – in its broadest sense, is primarily concerned with the description, explanation, appreciation, protection, and perpetuation of biological diversity (Meine *et al.* 2006). In particular, the merging of the complimentary fields of conservation biology and genetics has proven invaluable in assisting conservation agencies and organisations on decisions of where limited resources are invested to achieve the greatest conservation outcome (Soule and Simberloff 1986). Conservation biology in its most modern form provides an interface of scientific disciplines in the areas of systematics, genetics, ecology and evolutionary biology (Meine *et al.* 2006), and in its broadest sense, an interface between science and social, political and economic factors. Similarly, the affiliated field of ecophysiology has advanced our understanding of the evolutionary adaptations of turtles' diving abilities and their interactions with a variable environment (see Jackson *et al.* 1984; Ultsch *et al.* 1984).

For these reasons, I draw upon a range of disciplines to address this taxonomically confused and poorly studied species, *M. bellii*. Broadly in my thesis, I aim to:

- (i) Seek taxonomic stability for *M. bellii* through the identification of the holotype specimen and the investigation of supporting genetic and morphological evidence for a potential cryptic species from Bald Rock Creek;

- (ii) Establish baseline data on the reproductive biology and population ecology of *M. bellii* and relate this to its population status and long-term survival; and
- (iii) Investigate the aquatic respiration ability of *M. bellii* and relate this to its behavioural ecology across seasons.

These aims require the merging of several scientific disciplines including conservation genetics, morphology, reproductive biology and ecology and ecophysiology to provide a clear foundation and insight into the conservation priorities for *Myuchelys* species, and in particular, for the threatened western sawshelled turtle *M. bellii*. More specific objectives are introduced in the following outline of thesis structure.

In Chapter 2, I present the results of the phylogeny for the Australian *Myuchelys* species with a primary focus on the taxonomic issues for the three disjoint populations of *M. bellii*. Here, I use mitochondrial DNA to examine the genetic divergence between species (including *Emydura macquarii macquarii*) and within species to test whether there are shallow or deep genetic divergences indicating recent or long-standing genetic separation. The results obtained in Chapter 2 are then used in Chapter 3 as a phylogenetic framework to determine diagnostic morphology between the four *Myuchelys* species and also to investigate the morphology of the three populations of *M. bellii*.

From the analyses presented in Chapter 3, insights into the sequence of species radiation across the Australian continent are inferred and the likely ancestral phenotype of the *Myuchelys* is described. In addition, the combined molecular and morphological results are used to determine if *M. bellii* contains an undescribed cryptic species. I also examine the morphology of the *M. bellii* holotype specimen.

In Chapter 4, I present an analysis of the life history and population ecology of *M. bellii* across its natural range. The specific objectives of this chapter were to (i) assess the population size and distribution in Queensland, and (ii) estimate age at maturity, fecundity and life expectancy, and (iii) identify known and potential threatening processes hindering the recovery of this threatened turtle. I use long-term mark-recapture data and yearly analysis of the reproductive outputs of male and female *M. bellii*. I also describe the health of the populations of *M. bellii* including observations on

diet. These are key population parameters that will be of value to managers and turtle ecologists for long-term conservation and monitoring of the population into the future.

In Chapter 5, I describe the seasonal and diel dive behaviour and ecophysiology of *M. bellii* in Bald Rock Creek in Girraween National Park. The specific objectives of this chapter were to (i) investigate the seasonal and diel diving performance of *M. bellii* and relate this to its level of reliance on aquatic respiration via its cloacal bursae, (ii) describe the morphology of the cloacal bursae, and (iii) relate its diving behaviour to the ecological significance and life history traits identified in Chapter 4.

Chapter 6 presents a synopsis that draws on each of the previous chapters and identifies their contribution to chelonian science, and the conservation and management of Australian turtles.

**Chapter 2. Mitochondrial gene variation among Australian freshwater turtles in the genus *Myuchelys*, with special reference to the endangered *Myuchelys bellii***

This chapter has been submitted for publication to the journal *Endangered Species Research*:

Fielder, D.P., Vernes, K., Alacs, E.A. and Georges, A. (2011) Mitochondrial gene variation among Australian freshwater turtles in the genus *Myuchelys*, with special reference to the endangered *Myuchelys bellii*. *Endangered Species Research*, submitted.



Picture: A female and male *Myuchelys bellii* from Bald Rock Creek, Girraween National Park. Photo by Robert Ashdown.

**Chapter 2. Mitochondrial gene variation among Australian freshwater turtles in the genus *Myuchelys*, with special reference to the endangered *Myuchelys bellii*.**

**Abstract**

Identifying species and the relationships among them remains important for assessing biodiversity trends and is a critical focus for reversing global biodiversity loss. The saw-shelled turtles of Australia, in the genus *Myuchelys*, show cryptic diversity and include species that range from endangered (*M. bellii*), through those that are locally abundant but extremely limited in distribution (*M. georgesi* and *M. purvisi*) to those that are common and widespread (*M. latisternum*). Endangered *Myuchelys bellii* is restricted to three small isolated populations in the headwaters of the Murray-Darling basin, in the Border, Gwydir and Namoi tributaries. There is no evidence of strong divergence among these three populations based on mitochondrial DNA (mtDNA) divergences – there is only shallow genetic structuring ranging from 0.1–0.3% divergence. The mtDNA phylogeny supports previous phylogenies based on multi-locus allozyme electrophoresis, with the exception of the finding that *Emydura macquarii* and *Myuchelys georgesi* are sister taxa inside the *Myuchelys* radiation. Our mtDNA phylogeny corroborates earlier findings of a deep phylogenetic divergence between the morphologically similar *M. purvisi* and *M. georgesi* (13.5% mtDNA) and the sister taxa relationship of *M. latisternum* and *M. bellii*.

## Introduction

Biodiversity loss is of increasing concern to governments nationally and internationally. A substantial array of legislative and regulatory systems are in place to protect native biota, recognising the value the community places on biodiversity for the services it delivers in maintaining the quality of the air we breathe, the water we drink and the food we eat (Cairns 1997; Salzman 1998; Baumgartner 2007; Anderson *et al.* 2009; Doherty *et al.* 2010). While these legislative instruments recognise biodiversity at a range of levels, the central focus of biological conservation is at the species level, so identifying species, and relationships among species, remains a core focus of managers aiming to preserve biodiversity. For example, international systems of biodiversity protection such as the IUCN Red List and national systems such as the United Kingdom Endangered Species Act (ES Act), United States ES Act and the Australian Environment Protection and Biodiversity Conservation Act (EPBC Act) rely on good taxonomic knowledge at the species level. However, because such knowledge is often incomplete, conservation action can be misguided, or misdirected (Roman *et al.* 1999; Engstrom *et al.* 2004).

Recent developments in molecular approaches to describe species genetic makeup have accelerated progress in documenting biodiversity and have revealed an unexpected level of cryptic biodiversity. For example, the Asian leaf turtle *Cyclemys dentata* was regarded as a single widespread polytypic species until molecular and morphological analysis revealed a species complex of several formerly cryptic species (Stuart and Fritz 2008; Fritz *et al.* 2008). The Endangered giant narrow-headed softshell turtle, *Chitra indica*, is also likely to be a species complex, as mitochondrial ND4 sequences reveal three deeply divergent, monophyletic lineages (Engstrom *et al.* 2002). Similarly, the European pond turtle, *Emys obicularis*, contains a cryptic species endemic to Sicily (Fritz *et al.* 2005; Fritz *et al.* 2006). The Sicilian form, *E. trinacris*, is highly distinct genetically while morphologically resembling *E. obicularis galloitalica* (Fritz *et al.* 2005).

Failure to recognise underlying cryptic diversity at or below the level of species can have a great influence on biological conservation outcomes, either through declines in cryptic species that go undetected and so poorly managed (as with *Cyclemys* – *op cit*) or because diversity that is suspected but with poor foundation leads to misallocation of resources (Georges *et al.* 2011). In addition, phylogenetic information has a role to play

in assessing distinctiveness, an attribute that has an influence on conservation priorities (Faith 1992; Moritz 1995).

This study investigates populations of the threatened western sawshelled turtle, *M. bellii* (Thomson and Georges 2009), and expands on the allozyme studies of Georges and Adams (1992 and 1996) by including all known populations from three headwater streams of the Darling River in the Murray-Darling Basin of Australia. *M. bellii* was first described by Gray (Gray 1844), and the name was later re-applied to turtle populations from drainages in New South Wales (Cann 1998). However, a third extant population from Queensland is believed to possibly represent a new cryptic species within *M. bellii* based on scant morphological evidence (Cann 1998). This paper uses a phylogeny from two mitochondrial genes (ND4 and control region) to investigate the taxonomic relationship of populations of the western sawshelled turtle in the Murray-Darling Basin, to see if they are deeply or shallowly structured, as an indication of longstanding or recent divergence. The outcomes of this study will provide future direction for the conservation priorities for *Myuchelys bellii*.

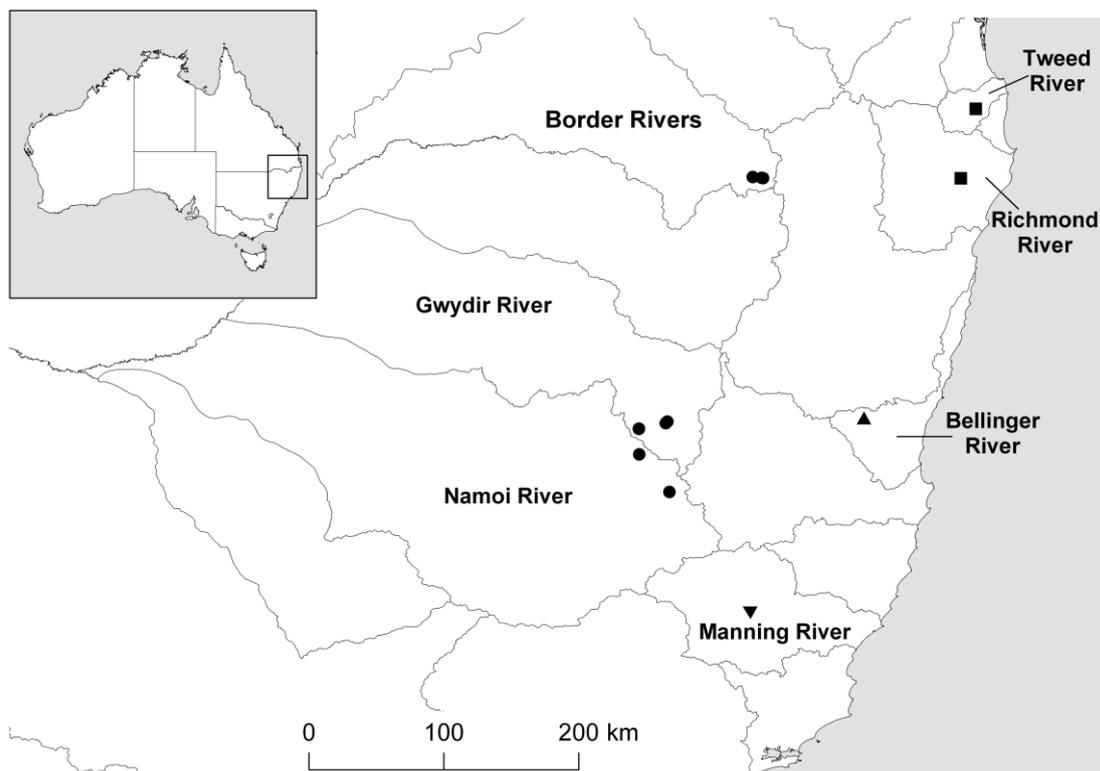
The levels of mitochondrial divergence across the four recognised species in the *Myuchelys* genus (including an *Emydura macquarii* as an in-group member in the analysis) was examined to test the current phylogenetic arrangements among species based on earlier allozyme electrophoresis studies (Georges and Adams 1992; 1996). Since those allozyme studies, a taxonomic revision by Thomson and Georges (2009) rectified the unacceptable paraphyly between the *Myuchelys latisternum* clade (previously *Elseya*) and *Elseya dentata* clade with respect to *Emydura* species. Consequently, I add the independent mitochondrial data set to the current understanding of genetic relationships of Australian freshwater turtles.

### **Methods and Materials**

Specimens of turtles within the *Myuchelys latisternum* clade (sensu Georges and Adams, 1992; 1996) were collected from within their natural ranges (Figure 2.1) by a combination of baited traps (Legler 1960) or by hand with the aid of mask and fins. In the case of the focal species *M. bellii* (Gray 1844) turtles were collected from the three known localities of the Namoi River and Gwydir Rivers of NSW and the Border Rivers of Queensland. The other species in the clade were *M. purvisi* (Wells and Wellington

1985), *M. georgesi* (Cann 1997a) and *M. latisternum* (Gray 1867). *Chelodina rugosa* (Ogilby 1890), *Elseya dentata* (Gray 1863) and *Emydura macquarii* (Gray 1830) were included as a nested series of outgroup species. Specimens of *Chelodina rugosa* were collected from Arnhem Land by Damien Fordham. Details of the specimens, localities and sample sizes are given below.

Small skin tissue samples (2–5 mm<sup>2</sup>) were taken from the neck or hind leg in the case of *M. bellii* or from the outer web margin on the fifth digit of the hind foot in the case of other species using a sharp sterilised scalpel or surgical scissors. Tissue samples were either preserved in 95% ethanol and 5% distilled water or snap frozen in liquid nitrogen, and transported to the University of Canberra where they were stored at -20°C (ethanol samples) or at -80°C (nitrogen samples) for later extraction of DNA.



**Figure 2.1** A map showing the capture localities of the *Myuchelys* specimens used for mtDNA sequencing. Drainage basin names are those of Australia's River Basins 1997 available from Geoscience Australia. Circles are *M. bellii*, squares are *M. latisternum*, the triangle is *M. georgesi* and the inverted triangle is *M. purvisi*.

Total genomic DNA was extracted using a salting out method as described in Miller *et al.* (1988), modified by FitzSimmons *et al.* (1995). DNA was resuspended in lysis buffer (skin or liver tissue: 40 mmol Tris, 20 mmol EDTA, 100 mmol NaCl, 10% SDS; blood or plasma: 10 mmol Tris, 1 mmol EDTA, 10 mmol NaCl, 0.1% SDS). For each sample I assessed nucleotide sequence variation within a relatively fast-evolving segment of mtDNA: the first half of nicotinamide adenine dinucleotide dehydrogenase subunit 4 (*ND4*) gene (~680 bp DNA fragment). The samples were amplified using polymerase chain reaction (PCR) on Eppendorf Mastercylers using the primers ND4F (5'-CACCTATGACTACCAAAGCTCATGTAGAAGC-3') (Arevalo *et al.* 1994), ND4intR (5'-AGGTGTTCTCGTCTTTG-3') (Alacs 2008), TCR500 (5'-CACCACCCTCCTGAAATACTC-3') (Engstrom *et al.* 2004) and PS15841 (5'-CMTTGGYCTTGTAACC-3') (Engstrom *et al.* 2004). Control Regions of specimens were amplified separately using the combination of EmyThr (5'-CACCACCCTCCTGAAATACTC-3') and PS15841 (5'-CMTTGGYCTTGTAACC-3') (~280 bp DNA fragment). Refinement of the primers may lead to longer mtDNA fragments for analysis in future research.

PCRs were conducted on 25 µl in volumes of extract which contained 50-100 ng template DNA, ddH<sub>2</sub>O, 10x NH<sub>4</sub> reaction buffer (Bioline), 50 mmol MgCl<sub>2</sub> (Bioline), 100 mmol dNTPs, 10 mg/ml purified BSA (NewEngland BioLabs), Betaine, 10 µmol/l solution amplification primers and 1 unit of platinum *Taq*-polymerase. PCRs were optimised for each species and for each set of primers used. PCR amplification of double-stranded product was completed using an Eppendorf Mastercycler. ND4 reactions were run at 94 °C for 3 min for the initial denaturation, then 35 amplification cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, followed by the final extension at 72 °C for 6 min. Problematic ND4 PCRs were rerun using a touchdown procedure of initial denaturation at 94 °C for 3 min, then 10x amplification cycles of 94 °C for 45 s, 55 °C for 45 s (-0.5 °C for consecutive cycles) and 72 °C for 1 min, followed by 25 amplification cycles of 94 °C for 45 s, 50 °C for 45 s and 72 °C for 1 min, with the final extension at 72 °C for 6 min. PCRs were visualised using Cyber Safe stain on 2% agarose gel for confirmation of amplification. Some problematic genomic DNA samples were cleaned using a cleanup-kit and dilutions were also used when necessary.

Control Region PCRs were 25 µl reaction: 20-100 ng template DNA, 0.75 mmol each dNTP, 0.4 mmol each primer, 2.5 mmol MgCl<sub>2</sub>, 2.5 µl of 10 X PCR Buffer, 0.6 M Betaine, 5 µg BSA and 1 unit of Taq polymerase (Bioline Red *Taq*). Control region reactions for *M. georgesi*, *M. purvisi*, *E. dentata* and *Em. macquarii* were run at 94 °C for 3 min for the initial denaturation, then 35 amplification cycles of 94 °C for 45 s, 55 °C for 45 s and 72 °C for 1 min, followed by the final extension at 72 °C for 6 min. Control region reactions for *M. latisternum* were run at 94 °C for 2 min for the initial denaturation, then 35 amplification cycles of 94 °C for 30 s, 51.5 °C for 30 s and 72 °C for 45 s, followed by the final extension at 72 °C for 5 min. Control region reactions for *M. bellii* were run at 94 °C for 2 min, followed by 35 amplification cycles of 94 °C for 30s, 55 °C for 30s, 72 °C for 45s and a final extension of 72 °C for 5 min.

PCR products were purified using a standard PEG protocol and sequenced at the facility of Macrogen in Seoul, Korea. Sequencing was conducted under BigDye™ terminator cycling conditions and the reacted products were purified using ethanol precipitation and run using Automatic Sequencer 3730xl. Sequence data were edited using SEQUENCHER v 4.1.4 (Genes Code Corporation) and aligned using CLUSTAL X (J.D. Thompson *et al.* 1997) in the program Geneious Pro 3.8.2 (Biomatters Ltd), with final alignment by eye in PAUP v 4.0 b10 (Swofford 2002).

All sequences were examined to eliminate the possibility that they might be numts. The chromatograms were clean, lacking ambiguous base signals that might have otherwise indicated presence of both mitochondrial and nuclear paralogues in a single PCR product. The coding section of the ND4 sequences for the seven taxa were checked for the presence of premature stop codons (Coin and Durbin 2004; Hay *et al.* 2004; Lerat and Ochman 2005). None were found. The amplified DNA of all seven taxa were aligned to a known complete ND4 genome from a distantly related tortoise, *Geochelone pardalis* (GenBank accession number: DQ080041) (Parham *et al.* 2006). The nucleotides for each sequence, including for *G. pardalis*, were converted to amino acids prior to alignment. All amplified sequences were confirmed to be ND4 gene segments prior to phylogenetic analysis. While none of these approaches eliminate the possibility of nuclear paralogues of mitochondrial sequences in my data, they at least reduce the probability.

The combined data set of 960 bp was analysed using maximum-parsimony (MP) and maximum-likelihood (ML) as implemented by PAUP v 4.0 b10 (Swofford 2002). For the ML analyses, the HKY85+G (Hasegawa *et al.* 1985) model of sequence evolution was used based on a likelihood analysis using ModelTest (Posada and Crandall 1998). Trees were rooted using *Chelodina rugosa* as an outgroup, chosen on the basis of the previous analyses of Georges and Adams (1992; 1996). Robustness of the resultant trees was determined by bootstrap analyses using 2000 and 1000 replicates for MP ML respectively.

Specimens examined. *Myuchelys bellii* Namoi River (NSW) (30.9401S 151.3248E) – UC AA018919-21, (30.4782S 151.3006E) – UC AA042492, (30.6855S 151.1211E) – UC AA018922; Gwydir River (30.4680S 151.3111E) – UC AA018917-8, (30.5085S 151.1195E) – UC AA018915, AA018922; Border Rivers (28.8319S 151.9486) – UC Q19132, (28.8252S, 151.8796E) – UC Q19136-7, (28.8295S 151.9399E) – UC Q19138, Q19146; *M. georgesi* Bellinger River (NSW) (30.4445S 152.6220E) – UC 131 [AM R120965], UC 147 [AM R123043] UC 152 [AM R123044], UC 154 [AM R123045], UC 159 [AM R123046]; *M. purvisi* Manning River (NSW) (31.7333S 151.8500E) UC 129 [AM R120966], UC 130 [AM R120967], UC 138 [AM R123041], UC 140 [AM R123040], UC 153 [AM R123042]; *M. latisternum* Richmond River (NSW) (28.8331S, 153.2668E) – UC 181 [AM R123037]; Tweed River (Qld) (28.3667S 153.3667E) – UC 127 [AM R120997]; *Emydura macquarii* Murray-Riverina (Vic) (36.0930S 146.9484E) – UC 120 [R120954]; *Elseya dentata* Roper River (NT) (14.7139S 134.5044E) – UC AA10883; *Chelodina rugosa*; Nicholson River (17.9603S 139.7522E) – UC AA20211; Mitchell River (Qld) (15.4742S 141.7936E) – UC AA20246. Abbreviations: AM, Australian Museum; UC, University of Canberra Wildlife Tissue Collection (<http://iae.canberra.edu.au/locations.cgi>); Qld, Queensland; NSW, New South Wales; Vic, Victoria; NT, Northern Territory. Drainage basin names are those of Australia's River Basins 1997 available from Geoscience Australia. Species names follow those of Georges and Thomson (2010).

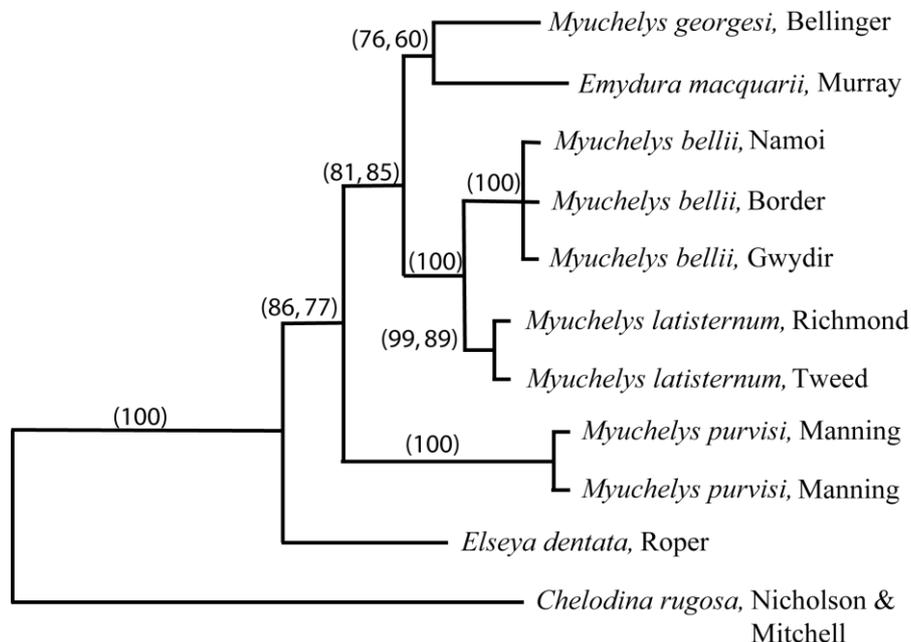
## Results

Of the 960 aligned nucleotides, 286 are parsimony-informative with 12 individual haplotypes identified (Table 2.1). The combined ND4 and control region mtDNA phylogeny (Figure 2.2) reveals that the three populations of *M. bellii* group

unambiguously to exclusion of all other species (100 bootstrap support). Populations from the Border, Namoi and Gwydir Rivers each have one unique haplotype differing by only 1 – 3 bp or 0.1 – 0.3% (Table 2.2). This shallow genetic structure contrasts with other recognised species in the phylogeny such as *C. rugosa* which has 11 base pair differences between catchments or 1.1% divergence (Table 2.2).

The mtDNA phylogeny revealed a paraphyletic arrangement in the *Myuchelys* taxa with respect to *Emydura macquarii*, which is most closely related to *M. georgesi* (8.5% divergence, Table 2.2). This phylogenetic position is well supported, but not unambiguously so (81 MP and 85 ML bootstrap support, Figure 2.2), with differences ranging from 82 bp between *E. macquarii* and *M. georgesi*, and 137 bp between *E. macquarii* and *M. purvisi* (Table 2.2).

The mtDNA analysis has also identified a deep genetic divergence between *M. purvisi* and *M. georgesi* (13.4%, Table 2.2). In addition, *M. latisternum* was revealed as the sister species to *M. bellii* with 100 bootstrap support (Figure 2.2) and a 3.3-3.5% divergence (Table 2.2).



**Figure 2.2** Maximum-likelihood tree obtained for ND4 and Control Region mtDNA sequences. Branch lengths drawn proportional to maximum likelihood estimates of genetic divergence. Where different, bootstraps for MP and ML shown in parenthesis. Nodes with bootstrap value below 50% were forced to collapse and yield polytomies.

**Table 2.1** Observed haplotypes of the ND4 and Control Region mtDNA combined 960 bp for the seven species and populations examined. Total specimens included in parenthesis.

Species	Haplotypes
<i>M. bellii</i>	3 (13)
<i>M. georgesi</i>	1 (30)
<i>M. purvisi</i>	2 (5)
<i>M. latisternum</i>	2 (2)
<i>Em. macquarii</i>	1 (1)
<i>E. dentata</i>	1 (1)
<i>C. rugosa</i>	2 (2)

**Table 2.2** Haplotype divergence matrix (uncorrected) for the ND4 and Control Region mtDNA sequences among 11 populations of seven species of Australian chelid turtles. The lower matrix is percent divergence and the upper matrix is number of base pair differences. Species abbreviations: MBB, *M. bellii* (Border R.); MBG, *M. bellii* (Gwydir R.); MBN, *M. bellii* (Namoi); MLR, *M. latisternum* (Richmond); MLT, *M. latisternum* (Tweed); MG, *M. georgesi* (Bellinger); MP, *M. purvisi* (Manning); EMM, *Em. macquarii* (Murray); ED, *E. dentata* (Roper); CRN, *C. rugosa* (Nicholson); CRM, *C. rugosa* (Mitchell).

Species	MBB	MBG	MBN	MLR	MLT	MG	MP	EMM	ED	CRN	CRM
<i>MBB</i>	-	2	3	33	34	90	125	94	127	223	223
<i>MBG</i>	0.2	-	1	32	33	90	126	94	126	223	223
<i>MBN</i>	0.3	0.1	-	33	34	91	127	95	125	224	224
<i>MLR</i>	3.4	3.3	3.4	-	4	85	128	90	127	201	198
<i>MLT</i>	3.5	3.4	3.5	0.4	-	86	125	88	125	201	198
<i>MG</i>	9.4	9.4	9.5	8.9	9.0	-	129	82	142	217	213
<i>MP</i>	13.0	13.1	13.2	13.3	13.0	13.4	-	137	152	237	237
<i>EMM</i>	9.8	9.8	9.9	9.4	9.2	8.5	14.3	-	127	229	228
<i>ED</i>	13.2	13.1	13.0	13.2	13.0	14.8	15.8	13.2	-	225	222
<i>CRN</i>	23.2	23.2	23.3	20.9	20.9	22.6	24.7	23.9	23.4	-	11
<i>CRM</i>	23.2	23.2	23.3	20.6	20.6	22.2	24.7	23.8	23.1	1.1	-

## Discussion

Multi-locus allozyme data on the delineation of Australian chelid species boundaries (sensu Georges and Adams, 1992; 1996) revealed populations of *Myuchelys bellii* from the Namoi and Gwydir Rivers to be diagnosable and a distinct species, but they did not have samples from the Border Rivers. Since that allozyme study, the Bald Rock Creek

populations of Queensland have been identified as distinct morphologically from those of the Namoi and Gwydir Rivers of New South Wales, and separate species status has been proposed (Cann 1998; Wells 2002). Such distinction failed to be sustained under more rigorous analysis (Chapter 3) and the exceptionally low levels of genetic divergence between the three isolated populations of the endangered *M. bellii* (equivalent to divergence between populations of *M. latisternum* from adjacent drainages – Table 2.2) suggest that it should be viewed as a single taxon. Support for the proposition that the population in Girraween National Park of Queensland is a separate species or subspecies from those in the Gwydir and Namoi Rivers draws no support from my data. Turtles from the three populations occupy a single drainage basin, and although isolated to some degree, it is likely that divergence is impeded by low frequency genetic exchange between these populations. There is at least one anecdotal report of *M. bellii* from the Macquarie Marshes (100 km downstream of its established range and below the confluence of the waters of the Namoi and Gwydir Rivers) which gives credence to the proposition that such low-level exchange is ongoing. Alternatively, the extant populations of *M. bellii* might represent the outcome of recent range contraction.

Consistent with earlier studies (Georges and Adams 1992; 1996), my mtDNA phylogeny establishes *Myuchelys bellii* west of the Great Dividing Range and *M. latisternum* east of the Great Dividing Range and across the north of Australia as sister taxa. There are associated morphological similarities that are interpreted as synapomorphies (Chapter 3) including moderately to deeply serrated shell margins, dorso-ventrally depressed carapace, broad head with furrowed head shield in aged adults, and pronounced neck tubercles. Also consistent with earlier studies is the separation in the phylogeny of cryptic species *M. purvisi* and *M. georgesi* which share external morphological characters (Cann 1998), yet are not sister species. Indeed a 13.5% divergence in the present mtDNA phylogeny corroborates earlier molecular studies of a 20% divergence in fixed allelic differences (Georges and Adams 1996). Their common ancestor has all four species of *Myuchelys* among its descendents. This is either a remarkable case of character convergence in allopatry between *M. georgesi* of the Bellinger River and *M. purvisi* of the Manning River, or these two species have preserved a shared suite of plesiomorphic (retained) characters over many millions of years. This latter interpretation seems more likely, and if correct opens a unique window

on the morphology of the ancestor of the saw-shelled turtle radiation. This study demonstrates that genetic and morphological divergence can be uncoupled, leading to potentially conflicting interpretations of relationships among taxa if based solely on morphological data.

The observed placement of *Emydura macquarii* in the mtDNA phylogeny is a major departure from the currently accepted phylogeny and associated taxonomy (Georges and Thomson 2010). *Em. macquarii* would be expected to be sister to *Elseya dentata* (McDowell 1983; Georges and Adams 1996), and outside the *Myuchelys* radiation (Georges and Adams 1992). This group has had a long and confused history. The genus *Elseya* was originally erected for *E. dentata* (the type species) and *M. latisternum* (formerly *E. latisternum*) (Gray 1867; 1872). Boulenger (1889) redefined the genus on the basis of a longitudinal shearing ridge on the maxillary triturating surface, the alveolar ridge, present only in *E. dentata*. *Elseya latisternum* and *E. novaeguineae* were placed in the genus *Emydura*. Furthermore, Gaffney (1977) was unable to distinguish the genera *Elseya* and *Emydura* using cranial features (though *E. latisternum* was not available to him) and later (Gaffney 1979) used the two in synonymy. McDowell (1983) interpreted a wider range of morphological characters to indicate that the closest relative of *E. dentata* is *Em. australis* (including *Em. krefftii* and *Em. subglobosa*) and not *E. latisternum*. He concluded that 'the generic recognition of *Elseya* seems unwarranted' and synonymised *Elseya* and *Emydura*. In contrast, Legler and Cann (1980) considered that morphological similarities between species in the two genera are sufficient to indicate a common ancestry, but not to warrant lumping the genera. The matter was resolved when Georges and Adams (1992) demonstrated, using allozyme electrophoresis, that the genus *Elseya* was paraphyletic, with *Emydura* coming between clades referred to today as *Elseya* and *Myuchelys* (Thomson and Georges 2009). Unlike Gaffney (1979) and McDowell (1983), Georges and Adams resolved the the paraphyly by recommending a new genus for *E. latisternum* and its relatives (now *Myuchelys*). At the time, it was noted that the nearest living relative to *E. dentata* was *Emydura 'australis'* (including *Em. krefftii* and *Em. subglobosa*) (McDowell 1983) and not *E. latisternum*, a result supported by the allozyme data (Georges and Adams 1992; 1996).

Clearly, the outcome of the mtDNA studies further complicates the matter. In terms of the mtDNA sequence I examined, *Emydura macquarii* and *Myuchelys georgesi* are

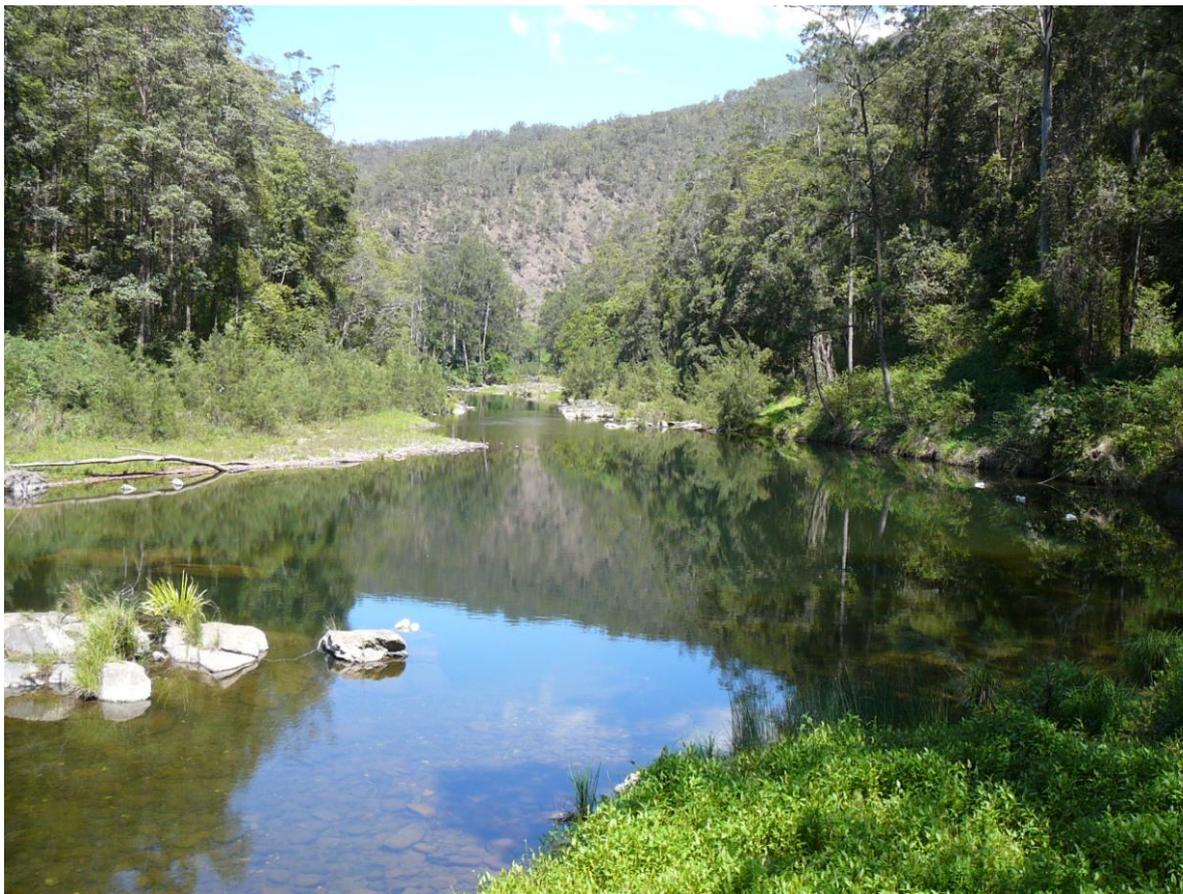
closer to each other than either is to *Elseya dentata*, and this is reflected by a sister relationship between the former two in the mtDNA phylogeny. If verified, this finding would require the generic names and definitions to be revisited, either by synonymising genera, erecting yet another monotypic genus, this time for *M. purvisi*, or recognising and accepting the paraphyly and retaining the existing nomenclature. I do not propose to take this action here because my data are mitochondrial, and potentially subject to the errors that can affect any studies based on a single locus. Hybridization between Australian chelid turtle species is widespread and there is at least one instance of hybridization between *M. latisternum* and *Em. macquarii* (Col Limpus pers. comm., UC AA987). The possibility of historical horizontal transfer of mitochondrial genomes requires that this finding be presented as a hypothesis for testing in future with multilocus data, molecular or morphological.

Notwithstanding the taxonomic uncertainties, *Myuchelys* is a phylogenetically divergent group of chelids emerging as a priority for conservation management. Three out of the four species have extremely restricted distributions, being restricted to headwaters of single drainages which makes them particularly vulnerable to ongoing threatening processes within their catchments. *M. bellii* is of particular concern. Its three major populations are distributed across two jurisdictions (Queensland and New South Wales) and primarily along stretches of river in private hands. The small Queensland population from Bald Rock Creek (<10 km reach) has experienced significant habitat modification through mechanical removal of in-stream boulders and riparian vegetation and loss through impoundment of a major permanent natural waterhole. A significant proportion of the Namoi and Gwydir turtle populations are suffering serious eye damage and blindness from eye cataracts from unknown causes (Cann 1998). Our study has shown little evidence of divergence of the three populations of *M. bellii*, and as such, they should be managed as a single operational taxonomic unit. This will involve additional attention both off and on reserve to protecting the remaining refugial habitat in the headwaters of the Namoi, Gwydir and Border Rivers of the Murray-Darling catchment, as well as attention to the corridors that enable free and effective passage between these when conditions allow. The persistence and quality of wetlands like the Macquarie Marshes may prove pivotal in the long term persistence of the three “isolated” populations of the Endangered *M. bellii*.

**Chapter 3. Ancient phenotypes revealed through present day species – a morphological analysis of Australia’s sawshelled turtles including the threatened *Myuchelys bellii* (Testudines: Chelidae)**

This chapter has been submitted for publication to the journal of Chelonian Conservation and Biology:

Fielder, D.P. (2011) Ancient phenotypes revealed through present day species – a morphological analysis of Australia’s saw-shelled turtles including the threatened *Myuchelys bellii* (Testudines: Chelidae). Chelonian Conservation and Biology, submitted.



Picture: The Bellinger River located in coastal New South Wales – habitat for the Georges helmeted turtle *Myuchelys georgesi*. Photo by Darren Fielder.

**Chapter 3. Ancient phenotypes revealed through present day species – a morphological analysis of Australia’s sawshelled turtles including the threatened *Myuchelys bellii* (Testudines: Chelidae)**

**Abstract**

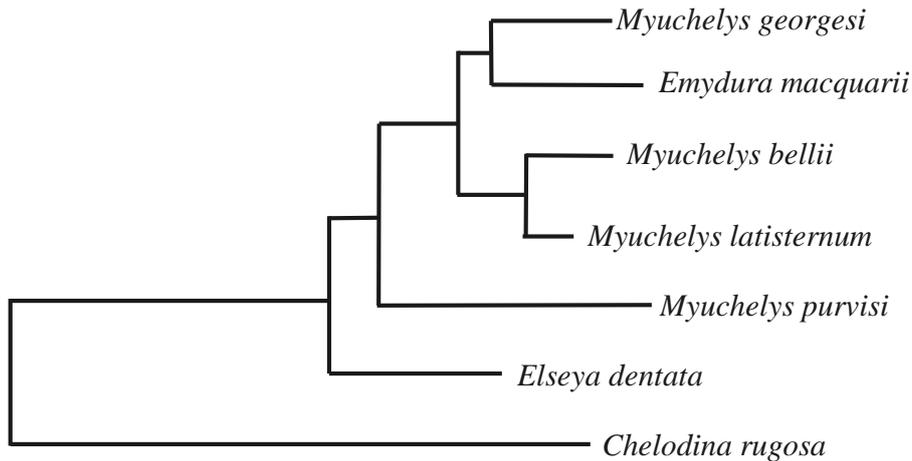
An understanding of the evolutionary relationships and speciation events that lead to the present day phylogeography of species can be gleaned from fossils. However, the phenotypes of ancestral lineages for Australian freshwater turtles are often obscured by gaps in the fossil record or because there is only scant examination of plesiomorphic (retained) or synapomorphic (derived) characters of extant taxa. In this study, the morphology of living *Myuchelys* species was examined to provide insights into the phenotype of their common ancestor and to elucidate taxonomic issues for *M. bellii* populations. The ancestral phenotype of *Myuchelys* was revealed to resemble the present day species of *M. georgesi* and *M. purvisi* by having a cervical scute, a lack of prominent neck tubercles, a relatively small head to body size, minor or nil serrations on marginal scutes, an oval carapace and smooth head shield. These shared traits are considered to be plesiomorphies. In contrast, a broader carapace, larger head size, furrowed head shield, deep serrated shell margins and compressed shell profile of *M. bellii* and *M. latisternum* are considered to be synapomorphies. These diagnostic characters were derived prior to their radiation across eastern and northern coastal Australia, and inland to the Murray-Darling Basin. Subtle, yet diagnostic, characteristics relating to the head, tail and shell morphology separate the superficially similar *M. georgesi* and *M. purvisi*. This study found no support for the taxonomic distinction of the Queensland population of *M. bellii* from populations in northern New South Wales, but was able to conclusively identify the *M. bellii* holotype specimen. A dichotomous key to the Australian *Myuchelys* species is provided. The results of this study have revealed a unique window into the phenotype of the common ancestor, and for the first time, the biogeography of extant *Myuchelys* taxa and the evolution and dispersal events that led to its current distribution across a large portion of the Australian continent.

## Introduction

The discovery of fossils and the information gleaned from them is often fundamental in verifying ancestral lineages and identifying evolutionary relationships (Thomson and Mackness 1999; Thomson 2000a), and in understanding biogeography of contemporary taxa (Noonan 2000; Lieberman 2003). Morphological characters identified from fossils or from living species can connect ancient lineages with their living descendants (Rougier *et al.* 1995). In some cases, species have been described from the fossil record only to be found some time later to be living (White and Archer 1994; Thomson *et al.* 1997). Where gaps exist in the fossil record, it is often left to hypothesise the ancestral phenotypes of particular groups of fauna, and consequently their evolutionary relationships.

The Australian fossil record has revealed five families of turtles: Meiolaniidae, Trionychidae, Carettochelyidae, Chelonioidea (superfamily) and Chelidae (Legler and Georges 1993a). Of these families, the chelids dominate the Australian freshwater turtle fauna from the Miocene to the present (Legler and Georges 1993a; Georges and Thomson 2005). However, the chelid fossil fauna of Australia is poorly known with only five species described from the fossil record: the extinct forms of *Rheodytes devisi*, *Chelodina insculpta*, *Elseya uberima* and *E. nadibajagu* (deVis 1897; Gaffney 1981; Thomson 2000a; Thomson and Mackness 1999) and the living fossil *Elseya lavarackorum* (Thomson *et al.* 1997). No chelid fossils to date have been affiliated with the *Myuchelys latisternum* clade (Thomson and Georges 2009).

In contrast to the *Myuchelys* fossil record, the phylogenetic relationships of extant species has been revealed through allozyme electrophoresis (Georges and Adams 1992; 1996; Georges *et al.* 1998) and the mitochondrial phylogeny presented in Chapter 2 (Figure 3.1, modified from Chapter 2). Despite a deep genetic divergence of 13.4% mtDNA (Chapter 2) and 20% loci difference (Georges and Adams 1992; 1996), *M. georgesi* and *M. purvisi* were once regarded to have been the same species having only minor distinguishing features (Cann 1997a; Thomson and Georges 1996). Of note, nested between them in the phylogeny are *M. bellii* (Gray 1844; Cann 1998) and *M. latisternum* (Gray 1867) with a 3.3 – 3.5% mtDNA divergence between them (Chapter 2). The *Myuchelys* common ancestor contains all four species among its descendants.



**Figure 3.1** The current phylogeny for the *Myuchelys latisternum* group using mtDNA (modified from Chapter 2).

In contrast to the molecular relationships of *Myuchelys*, the morphological differences among species have received little attention (Wells and Wellington 1985; Thomson and Georges 1996; Cann 1997a; Cann 1998). In particular, ambiguity based on subtle morphological traits remains for *M. bellii* populations which are suspected to contain a cryptic species (Cann 1998; Wells 2002; 2007). There is also doubt on the origins of the *M. bellii* holotype specimen, *Phrynops bellii* (Gray 1844), because its type locality was never recorded (Cann 1998).

This study is the first comparative morphological assessment of extant *Myuchelys* species providing diagnostic characteristics to separate taxa. These characteristics are also discussed in the context of the phylogenetic framework of *Myuchelys* which offers a unique window into the phenotype of the *Myuchelys* common ancestor. In addition, insights into the sequence of dispersal events of *Myuchelys* across eastern and northern Australia is revealed through a greater understanding of plesiomorphic (retained) and synapomorphic (derived) traits. The morphological variability among *M. bellii* populations is also examined along with an assessment of the holotype specimen. Lastly, a dichotomous key to the Australian *Myuchelys* species is provided.

## Methods and Materials

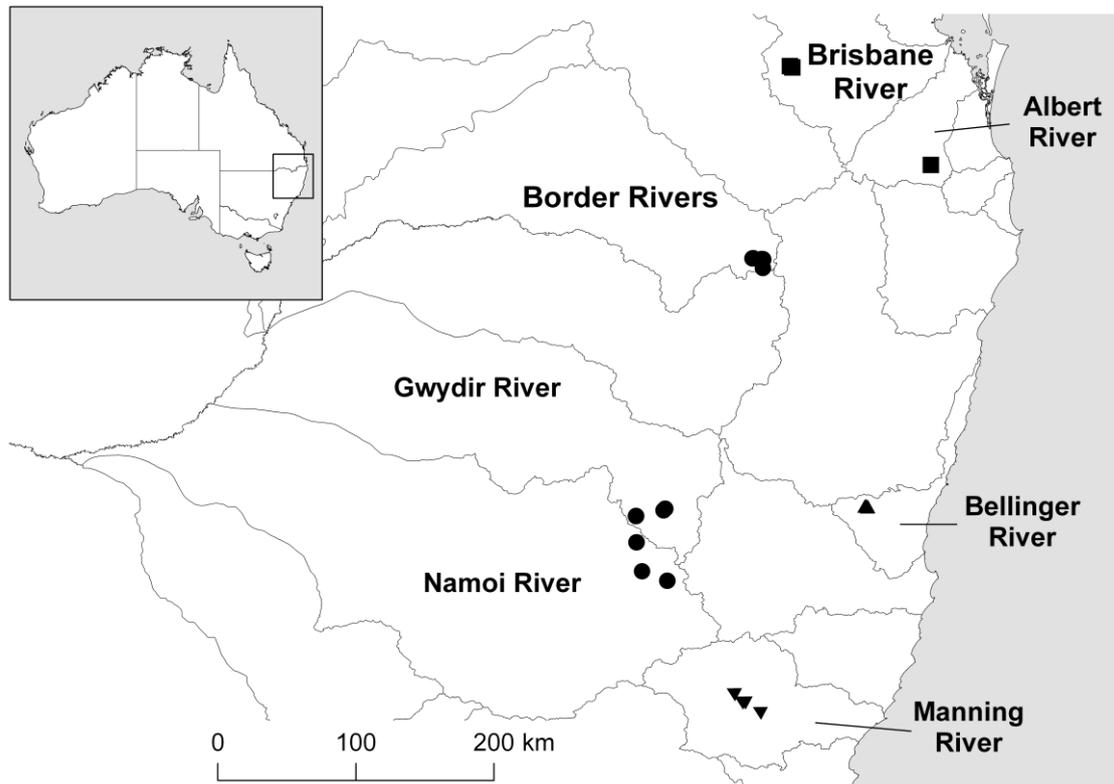
### *Taxonomic issues for Myuchelys*

The *Myuchelys* group of turtles has a chequered taxonomic past. Recent reviews by Thomson and Georges (2009) and Georges and Thomson (2010) have rejected alternative names occurring in a series of articles (Wells 2002; Wells 2007; Wells 2009). They argue these articles do not meet the guidelines for publication by the International Code of Zoological Nomenclature. Consequently in this chapter, the internationally accepted genus name *Myuchelys* is used to describe the *latisternum* species group.

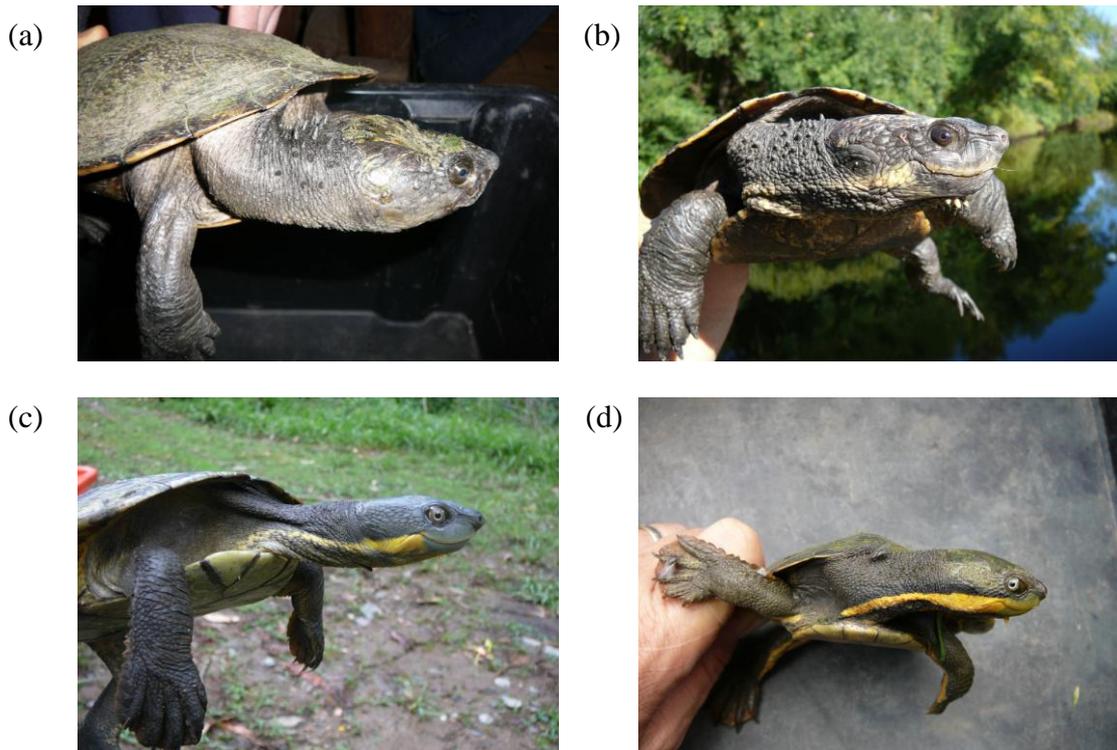
### *Capture and handling*

Most specimens in this study were live captured and released, either having been trapped using modified cathedral traps, or caught by hand and snorkel (Hamann *et al.* 2007). Turtles were generally processed and returned to the place of capture within 24 hours of being caught, or otherwise held in polyethylene tubs of water to prevent desiccation until their release (< 72 hours or infrequently isolated individuals were held for longer periods).

Populations of *M. bellii* were sampled from the Namoi River, Gwydir River and Bald Rock Creek (tributary of the Border Rivers); *M. georgesi* populations from the Bellinger River; *M. latisternum* populations from the Brisbane and Albert River catchments; *M. purvisi* populations from the Barnard River (tributary of the Manning R.) (Figure 3.2). Several specimens representing *M. bellii*, *M. georgesi* and *M. latisternum* (across several river catchments) were examined from the Queensland Museum (QM). Additional datasets of *M. latisternum* from the Albert River and *M. georgesi* from the Bellinger River were supplied for morphometric analysis by C.J. Limpus and D.J. Limpus, and M. Welsh respectively. Measurements and the carapace diagram for the *M. bellii* holotype specimen were taken from Cann (p210, 1998). The Papua New Guinea form *M. novaeguineae* (Thomson and Georges 2009) was not examined. Photographs of the four focal species of *Myuchelys* are presented in Figure 3.3.



**Figure 3.2** A map showing the capture localities of the *Myuchelys* specimens used for morphological analysis (excluding Queensland Museum specimen locations). Drainage basin names are those of Australia's River Basins 1997 available from Geoscience Australia. Circles are *M. bellii*, squares are *M. latisternum*, the triangle is *M. georgesi* and the inverted triangle is *M. purvisi*.



**Figure 3.3** Photographs of the extant Australian *Myuchelys* species: (a) *M. latisternum*, Brisbane R.; (b) *M. bellii*, Namoi R.; (c) *M. georgesi*, Bellinger R.; and (d) *M. purvisi*, Manning R.

A standard set of measurements was recorded for all turtles examined in the study. All shell, head and tail measurements were recorded to the nearest 0.1 mm using vernier callipers and weight recorded to the nearest 2 g using digital scales. Larger turtles (> 2.5 kg) were weighed to the nearest 25 g using a 3 or 6 kg spring balance. All measurements are in mm except weight (g). Shell terminology follows that of Zangerl (1969). Morphometric measurements used in this study are: **CL** (Carapace Length), minimum straight midline length of carapace from the cervical scute or juncture of the first marginals to the junction of the two most posterior marginal scutes; **CW** (Carapace Width), maximum straight distance between the marginal scutes of the carapace perpendicular to the carapace length's axis; **PL** (Plastron Length), minimum midline distance between the intergular shield (anterior margin) and the anal shields groove (posterior margin); **PW** (Plastron Width Anterior), width immediately at the axillary notch (pectoral shields pits) of the plastron bridge anterior; **PWP** (Plastron Width Posterior), width immediately at the inguinal notch (abdominal shields pits) of the plastron bridge posterior; **HW** (Head Width), maximum straight width of skull at tympanum; **HL** (Head Length), maximum straight length between the premaxillae and supraoccipital; **NL** (Neck Length), maximum straight length of extended neck from the

nose to the anterior carapace margin; **TP** (Tail to Plastron), straight length of extended tail from tip to plastron; **TC** (Tail to Carapace), straight length of extended tail from tip to carapace; **TV** (Tail to Vent), straight length of extended tail from tip to anterior edge of the vent; **SD** (Shell Depth), maximum straight midline of the shell from dorsal to ventral surfaces and **WT** (Weight).

#### *Sexual maturity*

Sex and sexual maturity was determined either by laparoscope examination of gonads (Limpus *et al.* 2002), evidence of secondary tail elongation (Georges 1983) or observation of penis extension. Sexually mature *M. bellii* males were identified using a combination of laparoscopic examination ( $n = 26$ ), tail elongation in mature males (tail to carapace length  $>45$  mm) and sometimes observed penis extension (refer to Chapter 4). The smallest mature male *M. latisternum* from laparoscope examination ( $n = 119$ ) had a carapace length of 125 mm, and the largest immature male had a carapace length of 135 mm. In the absence of laparoscopic examination of gonads, a male *M. latisternum* was categorised as sexually mature if its carapace length was greater than 130 mm. Based on the characteristic tail elongation of mature males, male *M. georgesi* and *M. purvisi* were considered to be sexually mature with a carapace length greater than 140 mm.

The smallest mature female *M. bellii* from laparoscope examination ( $n = 27$ ) had a carapace length of 222 mm, and the largest immature female had a carapace length of 212 mm. For statistical analysis, female *M. bellii* that had a carapace length greater than 215 mm were considered sexually mature (refer to Chapter 4). The smallest mature female *M. latisternum* from laparoscope examination ( $n = 100$ ) had a carapace length of 182 mm, and the largest immature female had a carapace length of 189 mm. Consequently in the absence of laparoscopic examination, female *M. latisternum* were considered sexually mature with a carapace length  $>185$  mm. Similarly, sexually mature female *M. georgesi* and *M. purvisi* were identified without laparoscopic confirmation as the point when males and females could be easily distinguished from one another based on tail length. This equated to females  $>140$  mm being identified as adult for the purposes of the statistical analysis.

*Museum specimens*

Specimens examined, including Queensland Museum (QM) voucher numbers, included here. *Myuchelys bellii*: QM 57909-10, QM 60649, Bald Rock Creek (28° 49' S, 151° 53' E) and 80571 (28°50' S, 151°56' E); QM 85206, McDonald River (30° 41' S, 151° 7' E) and 81940 (30°56' S, 151°19' E) n = 6. *Myuchelys georgesi*: paratypes QM 59425, 59430 Bellinger River (30°25' S, 152°46' E) n = 2. *Myuchelys latisternum*: QM 9519, Browns Ck, tributary of Pascoe River (12°51' S, 142°57' E); QM 43823, 43826-27, 43827-33, Lynd River (17°46' S, 144°23' E); QM 74070, Pinjara Hills (27°31' S, 152°54' E); QM 79456, Ross River (19°18' S, 146°45' E) n = 13.

*Statistical analysis*

Morphometric variables of linear distance measurements were converted to ratios in the statistical analyses to reduce the allometry to a minimum. Ratios of morphometric variables have been successfully used in other morphological studies including for freshwater turtles (McCord and Thomson 2002; Zuffi *et al.* 2006), primate fossils (Shapiro *et al.* 2005), and invertebrates (Kartavtsev *et al.* 2006). Weight was log-transformed to normalise its variability prior to inclusion in ratios.

Principal components analyses (PCAs) were carried out on variance-covariance matrixes to summarise the data and reveal the morphometric variables that are best able to distinguish among taxa. For each PCA, variables most highly correlated with the first two principal components were examined in greater detail, since these two components accounted for most of the variation among taxa. An analysis of similarities (ANOSIM) between the samples was conducted on a resemblance matrix for each PCA performed. Multivariate statistical analyses were carried out using PRIMER (Version 6, Plymouth Marine Laboratories) and univariate analyses, including linear regression and one-way analysis of variance (ANOVA), were carried out using Statistix (Version 7, Analytical Software). Bonferroni corrections were applied to all pairwise multiple comparisons following a significant result in the ANOVA. A result was considered significant at the 0.05 level of significance following Bonferroni correction where appropriate.

Only specimens that had a full set of morphometric variables available were included in PCAs. Specimens excluded from PCAs included those with known shell or body

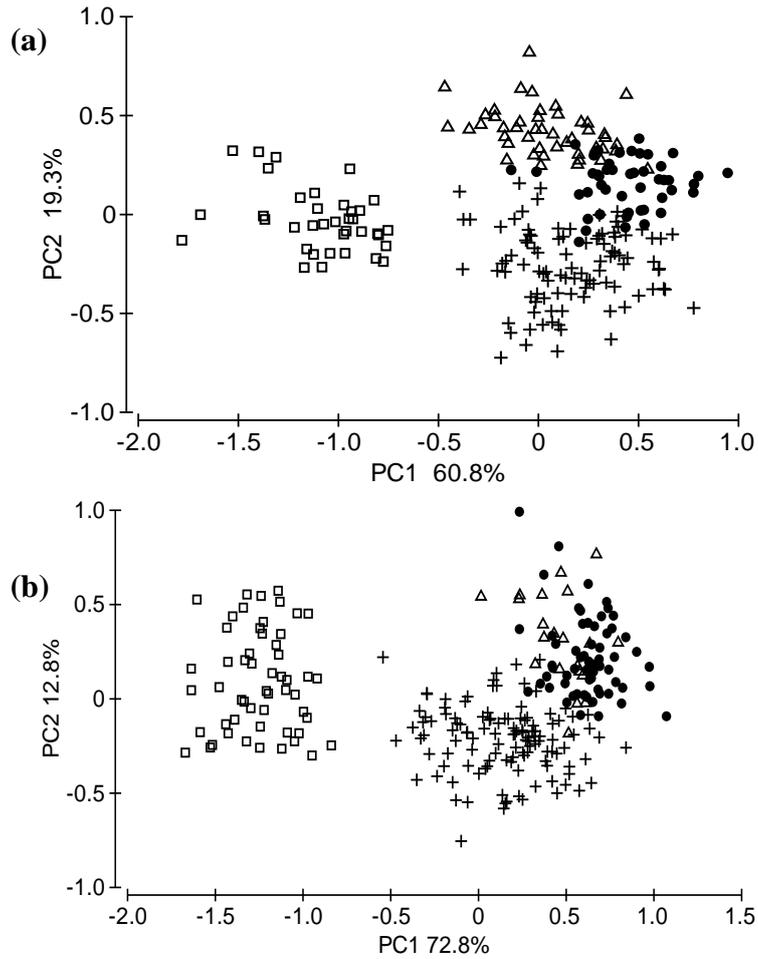
deformities that compromised recorded morphometrics. All available measurements from specimens (whether or not it was previously excluded from PCAs) were incorporated in non-parametric analyses. This allowed for the greatest number of specimens to be analysed. Hatchling turtles up to 70 mm CL were excluded from all analyses.

## Results

### *Morphometric analyses of Myuchelys species*

Principal components analysis (PCA) undertaken separately for grouped males and unsexed (Figure 3.4a) and females (Figure 3.4b) reveals *M. latisternum* to be the most morphologically distinct species. Correlations of the morphometrics for the first two principal components are given in Table 3.1. PC 1 accounts for among species variation of 60.2% for males and unsexed (Figure 3.4a) and 70.1% for females (Figure 3.4b). Along this axis, *M. latisternum* is discriminated from its congeners, with variables most correlated with PC1 being CL/HW (males,  $r = 0.81$ ; females,  $r = -0.72$ ), CW/HW (males,  $r = 0.52$ ; females,  $r = -0.66$ ), PWP/HW (males,  $r = 0.24$ ; females,  $r = -0.25$ ) and CL/HL (females,  $r = -0.36$ ). PC 2 accounts for 19.3% for males and 12.8% for females among species variation along the y axis. The variables most correlated with this axis were CL/TP (females,  $r = -0.90$ ), CL/SD (males,  $r = -0.51$ ; females,  $r = 0.31$ ), CL/PWP (males,  $r = 0.35$ ), CW/HW (males,  $r = -0.23$ ; females,  $r = 0.16$ ) and CL/HW (males,  $r = 0.21$ ; females  $r = -0.20$ ). These variables along the second axis identify differences between the four *Myuchelys* species in shell depth, tail length, plastron width and head size. However, there was not a clear association for *M. latisternum* along this axis as it was intermediately positioned among the other taxa.

An analysis of similarity (ANOSIM) on the PCA resemblance matrix supports significant overall differences (0.05%) between the taxa (global  $R$  for males = 0.72 and females = 0.75) (Table 3.2). The ANOSIM reveals *M. latisternum* as the most distinct, with *M. bellii* and *M. georgesi* most similar (males  $r = 0.42$ ; females  $r = 0.54$ ).



**Figure 3.4** Principal components analysis of *M. bellii* (+), *M. georgesi* (●), *M. latisternum* (□) and *M. purvisi* (Δ): (a) males and unsexed, and (b) females. The morphometric ratios included in each PCA are listed in Table 3.1.

**Table 3.1** Correlations of variables with first two principal components (eigenvalues).

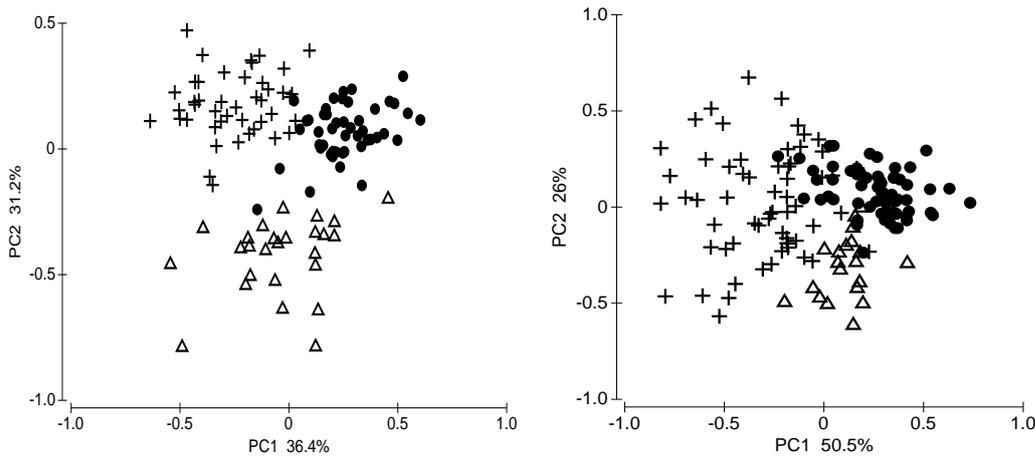
Variable	Males (n = 229)		Females (n = 257)	
	PC1 (60.8%)	PC2 (19.2%)	PC1 (72.8%)	PC2 (12.8%)
1. CL/PWP	0.09	0.35		
2. CL/HL			-0.36	-0.12
3. CL/HW	0.81	0.21	-0.72	-0.20
4. CL/SD	0.11	-0.51	-0.03	0.31
5. CL/TP			0.12	-0.90
6. CW/PWP	-0.01	0.12	0.00	0.10
7. CW/HW	0.52	-0.23	-0.52	0.16
8. CW/SD	-0.01	0.12		
9. HW/SD	-0.07	-0.11		
10. PWP/HW	0.24	-0.20	-0.25	-0.04

**Table 3.2** R statistic for PCAs calculated from ANOSIM using a resemblance matrix measured by Euclidean distance, with 2000 random sample permutations (0 >= observed). All pair wise comparisons between species for both male and female were significantly different at the 0.05% level.

Groups	R Statistic Males PCA	R Statistic Females PCA
<i>M. bellii</i> – <i>M. georgesi</i>	0.424	0.540
<i>M. bellii</i> – <i>M. purvisi</i>	0.663	0.571
<i>M. bellii</i> – <i>M. latisternum</i>	0.953	0.974
<i>M. purvisi</i> – <i>M. georgesi</i>	0.705	0.602
<i>M. latisternum</i> – <i>M. purvisi</i>	0.986	1
<i>M. latisternum</i> – <i>M. georgesi</i>	0.998	1
Global R	0.721	0.754

A second set of PCA's that excluded *M. latisternum* specimens was better able to discriminate between *M. bellii*, *M. georgesi* and *M. purvisi* (Figure 3.5a and 3.5b). Corresponding eigenvalues for the first two principal components are given in Table 3.3. PC 1 accounts for 36.4% of males and 50.5% of females among species variation. For males, this axis provides discrimination between *M. bellii* and *M. georgesi*, with *M. purvisi* having an intermediate position. For females, the *x* axis distinguishes *M. bellii* from the other two species, with variables correlated the most with PC 1 being CL/HW (males,  $r = 0.78$ ; females,  $r = 0.76$ ), CL/HL (females,  $r = 0.32$ ), CL/TP, (males,  $r = -0.46$ ), PWP/TP (males,  $r = -0.23$ ), PWP/HW (males,  $r = 0.14$ ; females,  $r = 0.30$ ) and CL/SD (females,  $r = -0.34$ ). PC 2 accounts for 31.2% of males and 26% of females among species variation. Along this axis, males of *M. purvisi* are separated from *M. bellii* and *M. georgesi* males. The females of *M. purvisi* are differentiated from *M. georgesi* with *M. bellii* females having a broader distribution along the *y* axis. The variables most correlated to this axis are CL/SD (males,  $r = 0.49$ ; females,  $r = 0.74$ ), CL/PWP (males,  $r = -0.48$ ), PWP/HW (males,  $r = 0.43$ ; females,  $r = 0.29$ ), PWP/SD (males,  $r = 0.39$ ; females,  $r = 0.41$ ), CW/HW (females,  $r = 0.31$ ) and CW/PWP (males,  $r = -0.32$ ; females,  $r = -0.14$ ). Shell depth, carapace width, plastron width posterior and head size are significant variables between *M. bellii*, *M. purvisi* and *M. georgesi*.

An analysis of similarity (ANOSIM) between the three species is provided in Table 3.4. Males of each species (excluding *M. latisternum*) significantly differ from each other by nearly equal distances ( $r = 0.74$ - $0.79$ ). In the absence of *M. latisternum* from the second PCA, female *M. georgesi* and *M. purvisi* were found to be the most dissimilar ( $r = 0.82$ ) while *M. bellii* and *M. purvisi* were most similar ( $r = 0.39$ ).



**Figure 3.5** Principal components analysis of (a) males, and (b) females: *M. bellii* (+), *M. georgesi* (●) and *M. purvisi* (Δ). The morphometric ratios included in each PCA are listed in Table 3.3.

**Table 3.3** Correlations of variables with first two principal components (eigenvalues) for Figure 3.5.

Variable	Males (n = 117)		Females (n = 144)	
	PC1 (36.4%)	PC2 (31.2%)	PC1 (50.5%)	PC2 (26%)
1. CL/PWP	0.18	-0.48		
2. CL/HL			0.32	0.00
3. CL/HW	0.78	0.15	0.76	0.11
4. CL/SD	-0.08	0.49	-0.34	0.74
5. CL/TP	-0.46	-0.08		
6. CW/PWP	-0.10	-0.32	-0.16	-0.14
7. CW/HW			0.24	0.31
10. PWP/HW	0.14	0.43	0.30	0.29
11. PL/PWP			-0.13	-0.27
12. PWP/SD	-0.12	0.39	-0.14	0.41
13. PWP/TP	-0.23	0.10		
14. CL/WT <sub>log</sub>	0.19	-0.23		

**Table 3.4** R statistic for PCAs (Figure 3.5) calculated from ANOSIM using a resemblance matrix measured by Euclidean distance, with 2000 random sample permutations ( $0 \geq$  observed). All species pair wise comparisons were significantly different at the 0.05% level.

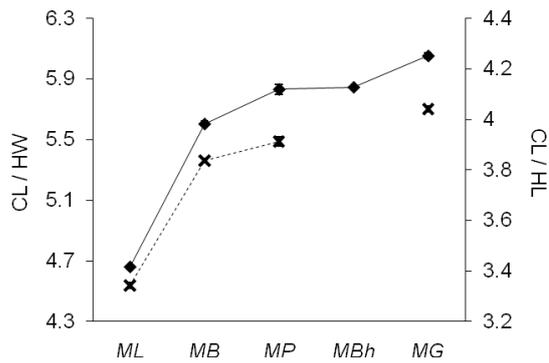
Groups	R Statistic	
	Males PCA	Females PCA
<i>M. bellii</i> – <i>M. georgesi</i>	0.747	0.587
<i>M. bellii</i> – <i>M. purvisi</i>	0.785	0.390
<i>M. purvisi</i> – <i>M. georgesi</i>	0.739	0.819
Global R	0.732	0.579

Correlated morphometric variables identified through the PCA's were selected for univariate analyses. Head width and length (CL/HW and CL/HL) between all four species were found to be significantly different ( $N = 748$ ,  $P < 0.001$ ; Figure 3.6). The larger relative head width (CL/HW =  $4.66 \pm 0.0147$ ;  $n = 349$ ; Figure 3.6) and length

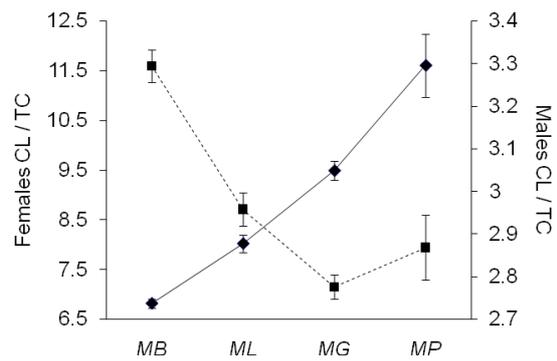
(CL/HL =  $3.34 \pm 0.012$ ;  $n = 349$ ; Figure 3.6) for *M. latisternum* separates it from its congeners and was a primary morphometric character for describing variation among species. Additionally, *M. bellii* has a broader head than *M. purvisi* which in turn has a broader head than *M. georgesi* (Figure 3.6). Tail length was also found to separate each species in univariate space with mature *M. bellii* males having relatively shorter tail lengths (CL/TC =  $3.28 \pm 0.04$ ;  $n = 65$ ; Figure 3.7) than their male congeners, and mature *M. bellii* females having correspondingly longer tail lengths (CL/TC =  $6.61 \pm 0.09$ ;  $n = 82$ ; Figure 3.7) than other *Myuchelys* species. In contrast, male *M. georgesi* and *M. purvisi* have the longest relative tail length and female *M. purvisi* the shortest relative tail length of the *Myuchelys* taxa.

Shell morphology was also diagnostic between species. The carapace width of males and females were found to have a similar pattern (Figure 3.8a and 3.8b) where *M. georgesi* was the most elongate (narrow), followed by *M. purvisi* and then *M. latisternum*. *Myuchelys bellii* exhibited an oval carapace that was generally wider than all other taxa for all size classes. Mean anterior plastron width (CL/PW) was significantly different between male *M. bellii*, *M. georgesi*, *M. latisternum* and *M. purvisi* ( $F_{3,283} = 74.77$ ,  $p < 0.001$ ). *Myuchelys latisternum* males were found to have the broadest anterior plastron and *M. purvisi* males the narrowest (Figure 3.9). In contrast, female *M. latisternum* and *M. bellii* possessed statistically similar anterior plastron widths, but were significantly different to *M. georgesi* and *M. purvisi* ( $F_{3,332} = 45.98$ ,  $p < 0.001$ ). Plastron width anterior means were significantly different between female *M. georgesi* and *M. purvisi*, which in turn were significantly different to female *M. bellii* and *M. latisternum*. Plastron width posterior (CL/PWP) means were significantly different between male *M. georgesi* and *M. purvisi*, which were significantly different to *M. bellii* and *M. latisternum* ( $F_{3,186} = 76.66$ ,  $p < 0.001$ ). Mean CL/PWP for female *M. purvisi* was significantly different to *M. bellii*, *M. georgesi* and *M. latisternum* ( $F_{3,259} = 54.34$ ,  $p < 0.001$ ).

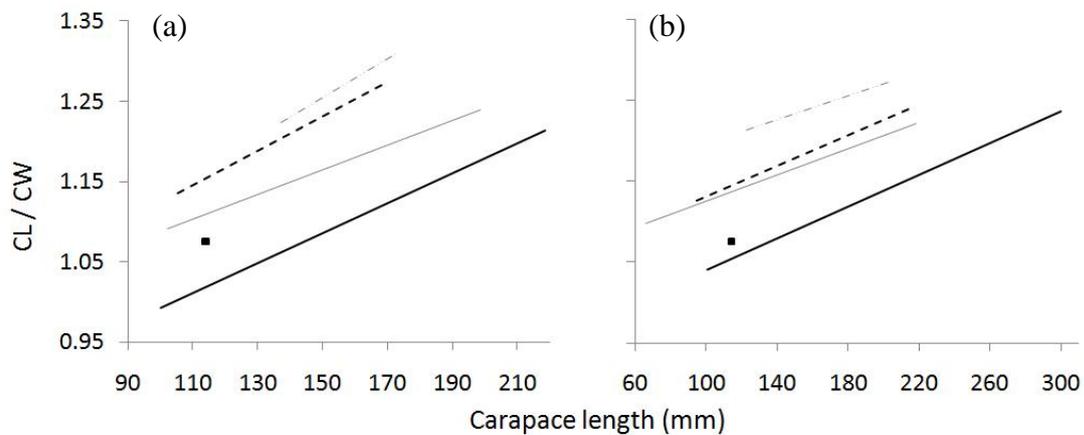
*Myuchelys latisternum* possess the broadest plastron (anterior and posterior) followed by *M. bellii*, then *M. georgesi*; *M. purvisi* has the narrowest plastron of all four taxa (Figure 3.9). Both male and female *M. purvisi* have a much greater shell depth than any other species and *M. bellii* has the most compressed shell profile of all species (Figure 3.10).



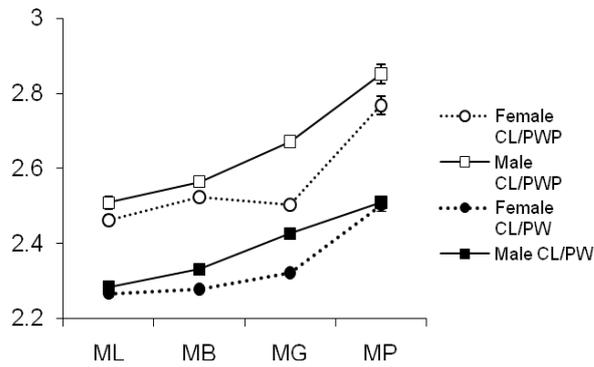
**Figure 3.6** Means  $\pm$  SE for carapace length to head width (CL/HW, solid line and diamond) and carapace length to head length (CL/HL, dashed line and x icons): *ML*, *M. latisternum*; *MB*, *M. bellii*; *MBh*, *M. bellii* holotype; *MG*, *M. georgesi*; and *MP*, *M. purvisi*. Mean CL/HW were significantly different for all taxa ( $F_{3,742} = 1282.85$ ,  $p < 0.001$ ).



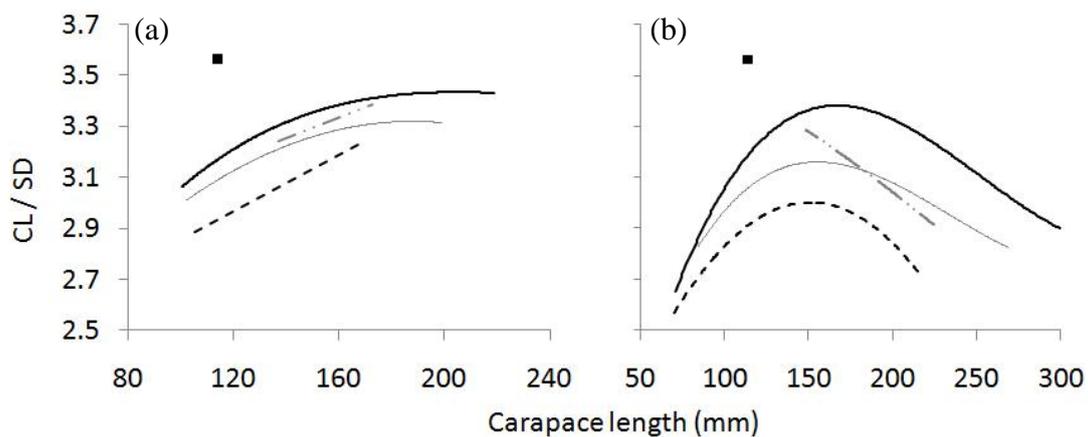
**Figure 3.7** Means  $\pm$  SE for carapace length to tail-to-carapace length (CL/TC) for sexually mature males (dashed line) and females (solid line). Refer to Figure 3.6 for species abbreviations. Male *M. bellii* were significantly different to *M. georgesi*, *M. latisternum* and *M. purvisi* ( $F_{3,232} = 16.03$ ,  $p < 0.001$ ). All four means for females were significantly different ( $F_{3,243} = 42.28$ ,  $p < 0.001$ ).



**Figure 3.8** Linear regression lines for carapace length to carapace width (CL/CW) for (a) males and (b) females and unsexed. Solid black line is *M. bellii*, thin grey line is *M. latisternum*, broken black line is *M. purvisi*, grey broken line is *M. georgesi* and a solid square represents *M. bellii* holotype specimen. All taxa (excluding holotype) were significantly different (Males: slopes  $p = 0.12$ , elevations  $p = 0.001$ ; 3, 283. Females: slopes  $p = 0.05$ , elevations  $p = 0.001$ ; 3, 474).



**Figure 3.9** Means  $\pm$  SE for carapace length to plastron width anterior (CL/PW) and carapace length to plastron width posterior (CL/PWP) for male and female *Myuchelys* species. Refer to Figure 3 for abbreviation of species names.



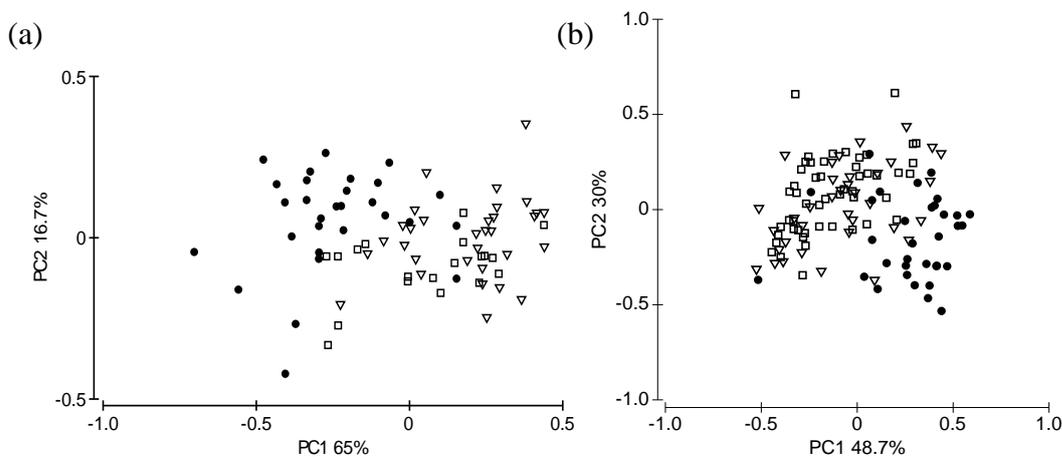
**Figure 3.10** Polynomials for carapace length to shell depth (CL/SD) for (a) males (N = 195) and (b) females and unsexed (N = 334). Solid black line is *M. bellii*, thin grey line is *M. latisternum*, broken black line is *M. purvisi*, grey broken line is *M. georgesi* and a solid square represents *M. bellii* holotype specimen.

#### *Multivariate analysis of the three extant M. bellii populations*

Multivariate analysis (PCA) of the three *M. bellii* populations revealed some segregation between the Bald Rock Creek population and the other two populations from the Gwydir and Namoi rivers based on the chosen morphometric variables (Figure 3.11). Bald Rock Creek males were most dissimilar to Gwydir River males ( $r = 0.528$ , Table 3.6) and Bald Rock Creek females were most dissimilar to Namoi River females

( $r = 0.56$ ). Male and female Gwydir and Namoi river populations were the most similar (males  $r = 0.089$ , females  $r = 0.064$ , Table 3.6). However, none of the ANOSIM results for either male or female were significant at the 0.05% level (Table 3.6).

On average, Bald Rock Creek males tended to have a marginally larger head for their size when compared to the Gwydir and Namoi populations (CL/HL,  $r = 0.47$  and CL/HW,  $r = 0.87$ , Table 3.5 and Figure 3.12). This ratio difference would account for approximately <2 mm width between the largest males of the respective populations. Females on the other hand varied among populations in the depth of their shell with Bald Rock Creek individuals more likely to have a compressed shell profile (CL/SD,  $r = 0.78$ , Table 3.5 and Figure 3.13). This observed difference between populations may have resulted from a proportionally greater number of 150-200 mm CL females and conversely lower number of larger individuals included from Bald Rock Creek compared to the other two study populations. Whilst there was some distinction in these variables, there were also large overlaps of values revealed in each PCA.



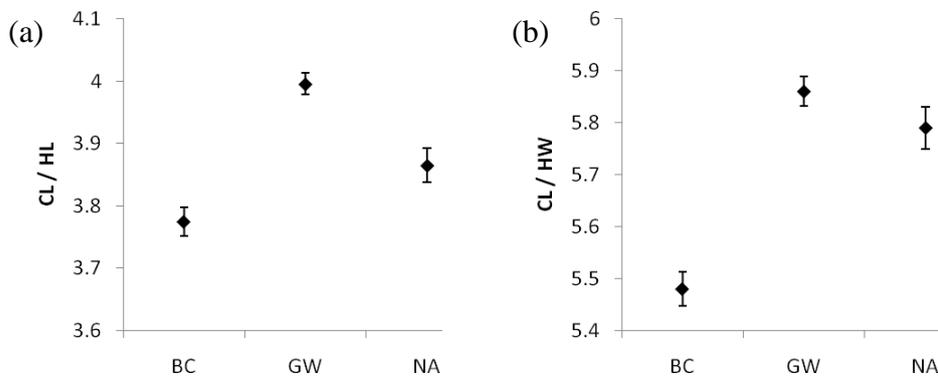
**Figure 3.11** Principal components analysis of *M. bellii* for the catchments of Bald Rock Creek (●), Gwydir River (▽) and Namoi River (□): (a) males, and (b) females. The morphometric ratios included in each PCA are listed in Table 3.5.

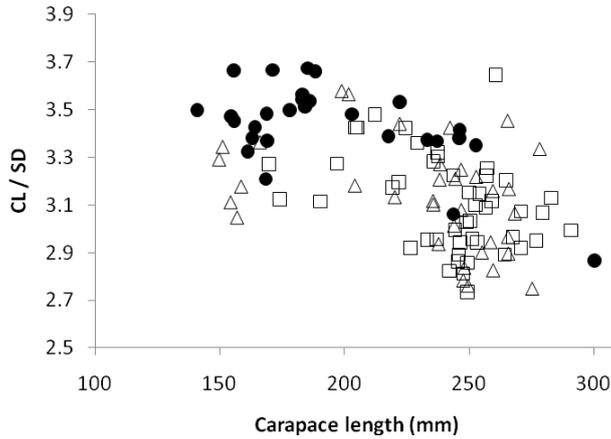
**Table 3.5** Correlations of variables with first two principal components (Eigenvalues) for each PCA conducted on the three *M. bellii* populations (see Figure 3.11).

Variable	Males (n = 80)		Females (n = 118)	
	PC1 (65%)	PC2 (16.7%)	PC1 (48.7%)	PC2 (30%)
1. CL/WTlog			0.58	-0.25
1. CL/CW	0.09	0.08	-0.12	0.10
2. CL/PW	-0.00	-0.03	0.12	0.08
3. CL/PWP	0.18	-0.08	-0.05	0.22
4. CL/HL	0.47	0.15	-0.02	0.42
5. CL/HW	0.85	-0.18	0.14	0.83
5. CL/SD	0.09	0.97	0.78	0.06

**Table 3.6** R statistic for PCAs (Figure 3.11) calculated from ANOSIM using a resemblance matrix measured by Euclidean distance, with 2000 random sample permutations ( $0 \geq$  observed). All results were non-significant at the 0.05% level. Abbreviations in the table are: Bald Rock Creek – BC, Gwydir River – GW, Namoi River – NA.

Groups	R Statistic Males PCA	R Statistic Females PCA
BC - GW	0.528	0.382
BC - NA	0.354	0.56
GW - NA	0.089	0.064
Global R	0.358	0.326

**Figure 3.12** Means  $\pm$  SE for (a) carapace length to head length and (b) carapace length to head width ratios for male *M. bellii* populations (CL > 160 mm,  $n = 77$ ). Study area abbreviations are explained in Table 3.6.



**Figure 3.13** Carapace to shell depth ratio for female *M. bellii* from Bald Rock Creek (●), Gwydir R. (△) and Namoi R. (□) ( $n = 111$ ).

**Table 3.7** Means  $\pm$  SD of selected variables for *M. bellii* populations including the holotype specimen. The Gwydir and Namoi data were pooled for comparison to the Bald Rock Creek population. Measurements given are in millimetres.

Sex	Size (mm)	<i>n</i>	CL	CW	HL	HW	SD
<b>Bald Rock Creek</b>							
Unsexed	70-150	19	111.7 $\pm$ 24.5	107.3 $\pm$ 19.7	30.4 $\pm$ 5.4	21.2 $\pm$ 3.9	34.8 $\pm$ 4.7
Male	>150	27	189.0 $\pm$ 14.5	164.1 $\pm$ 9.2	49.9 $\pm$ 3.7	34.2 $\pm$ 2.5	53.7 $\pm$ 5.0
Female	150-200	22	172.5 $\pm$ 12.9	155.3 $\pm$ 10.4	46.3 $\pm$ 3.7	31.4 $\pm$ 3.0	48.7 $\pm$ 3.2
Female	>200	12	236.6 $\pm$ 25.8	197.2 $\pm$ 16.1	61.4 $\pm$ 5.3	43.3 $\pm$ 5.6	71.7 $\pm$ 13.4
<b>Gwydir and Namoi rivers</b>							
Unsexed	70-150	16	125.1 $\pm$ 17.6	118.4 $\pm$ 14.7	33.4 $\pm$ 4.2	23.1 $\pm$ 2.7	39.9 $\pm$ 5.3
Male	>150	51	199.6 $\pm$ 12.5	169.1 $\pm$ 8.1	50.6 $\pm$ 2.7	34.2 $\pm$ 1.9	58.6 $\pm$ 3.3
Female	150-200	10	171.7 $\pm$ 17.9	154.4 $\pm$ 14.8	44.7 $\pm$ 4.6	30.6 $\pm$ 3.1	52.9 $\pm$ 5.2
Female	>200	76	248.6 $\pm$ 21.0	207.6 $\pm$ 16.1	64.8 $\pm$ 5.9	44.4 $\pm$ 4.5	80.7 $\pm$ 9.8
<b>Holotype Specimen</b>							
Unsexed	114		114	106		19.5	32

Sex	Size (mm)	<i>n</i>	CL/HW	CL/HL	CL/PL	CL/PWP	PW/PWP
<b>Bald Rock Creek</b>							
Unsexed	70-150	19	5.2 $\pm$ 0.3	3.6 $\pm$ 0.1	1.2 $\pm$ 0	2.6 $\pm$ 0.1	1.1 $\pm$ 0
Male	>150	27	5.5 $\pm$ 0.2	3.8 $\pm$ 0.1	1.2 $\pm$ 0	2.5 $\pm$ 0.1	1.1 $\pm$ 0
Female	150-200	22	5.5 $\pm$ 0.2	3.7 $\pm$ 0.1	1.2 $\pm$ 0	2.5 $\pm$ 0.1	1.1 $\pm$ 0
Female	>200	12	5.5 $\pm$ 0.2	3.9 $\pm$ 0.1	1.2 $\pm$ 0	2.5 $\pm$ 0.1	1.1 $\pm$ 0
<b>Gwydir and Namoi</b>							
Unsexed	70-150	16	5.4 $\pm$ 0.2	3.7 $\pm$ 0.1	1.2 $\pm$ 0	2.5 $\pm$ 0.1	1.1 $\pm$ 0
Male	>150	51	5.8 $\pm$ 0.2	4.0 $\pm$ 0.1	1.2 $\pm$ 0	2.6 $\pm$ 0.1	1.1 $\pm$ 0
Female	150-200	10	5.6 $\pm$ 0.2	3.8 $\pm$ 0.1	1.2 $\pm$ 0	2.5 $\pm$ 0.1	1.1 $\pm$ 0
Female	>200	76	5.6 $\pm$ 0.2	3.8 $\pm$ 0.1	1.2 $\pm$ 0	2.6 $\pm$ 0.1	1.1 $\pm$ 0
<b>Holotype Specimen</b>							
Unsexed	114		5.8		1.3	2.6	1.1

Selected summary data for the Bald Rock Creek population and the combined populations of Gwydir and Namoi River catchments are presented in Table 3.7. The largest individuals recorded during this study from each population were: female 300 mm (3250g) (QM 80571) and male 213 mm (1205g) from Bald Rock Creek; female 278 mm (2560g) and male 219 mm (1195g) from the Gwydir River; and female 298 mm (3240g) and male 213 mm (1025g) from the Namoi River.

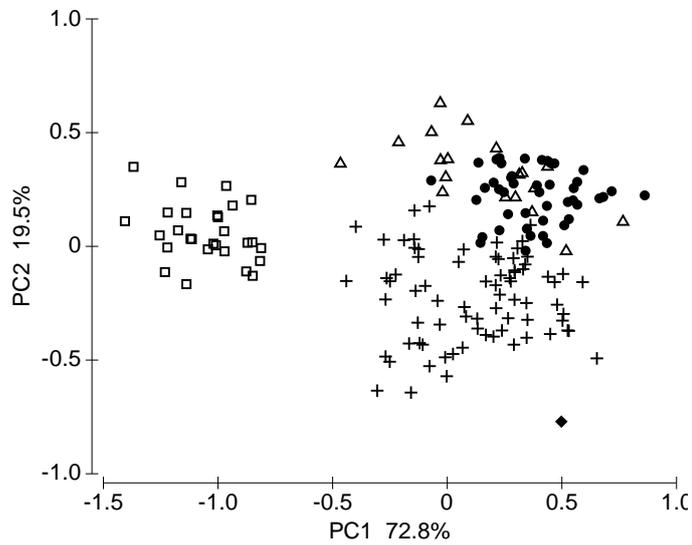
*Morphometric analysis of Phrynops bellii holotype specimen*

A PCA of mature males and the holotype specimen (*Phrynops bellii*, OUM 8460) was performed using the limited number of available variables for the holotype specimen (Figure 3.14). The holotype specimen is clearly discriminated being closest to *M. bellii*. PCA variables that most influence discrimination along the  $x$  axis were CL/HW ( $r = 0.82$ ), CW/HW ( $r = 0.55$ ) and along the  $y$  axis were CW/SD ( $r = -0.72$ ), CW/HW ( $r = -0.43$ ) and CL/CD ( $r = -0.43$ ) (Table 3.8). Thus, HW and SD are key morphological features to the holotype's identification.

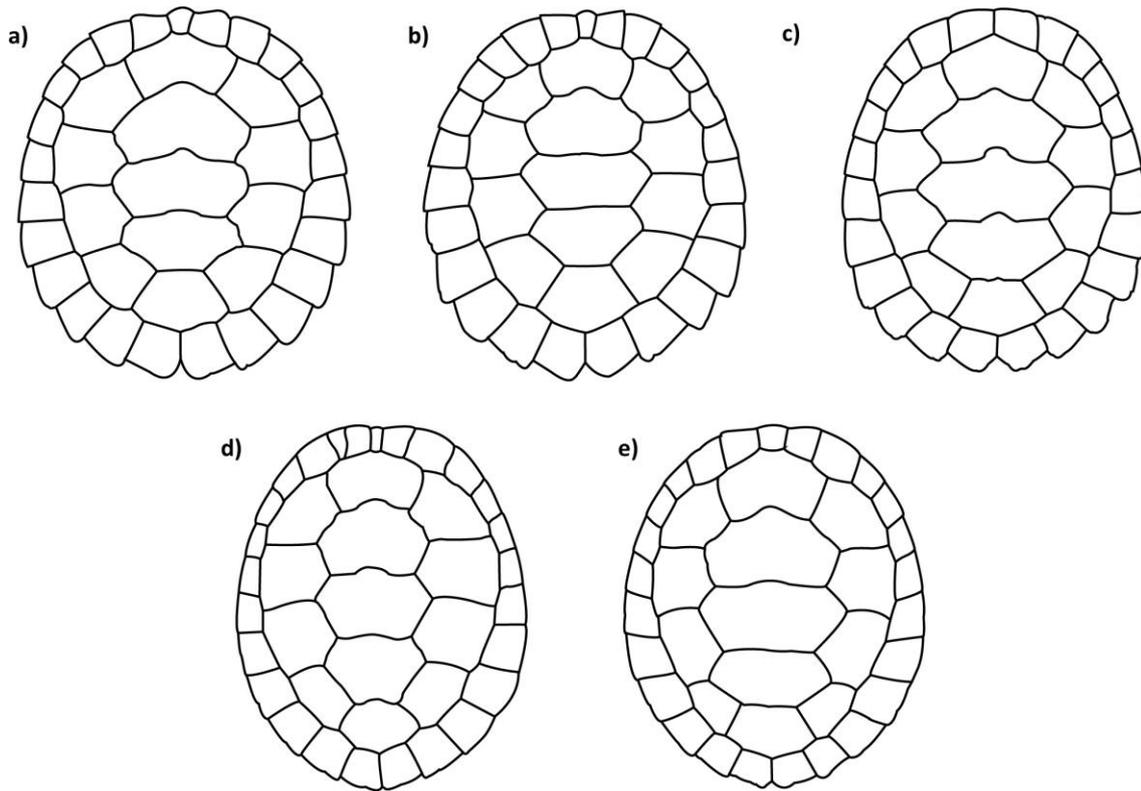
From univariate analysis, the holotype's head width relative to its carapace length of 5.8 (Figure 3.6) is closest to the geometric mean of *M. bellii* ( $5.6 \pm 0.016$ ;  $n = 228$ ; Figure 3.6) and *M. purvisi* ( $5.8 \pm 0.03$ ;  $n = 58$ ; Figure 3.6) and is consequently too narrow to be *M. latisternum*. The specimen's carapace length to carapace width ratio (CL/CW = 1.08) is intermediately located between *M. latisternum* and *M. bellii* (Figure 3.8). The anterior plastron width relative to its carapace length (CL/PW = 2.33; Figure 3.9) place it between the geometric mean of *M. bellii* ( $2.3 \pm 0.006$ ;  $n = 224$ ) and *M. georgesi* ( $2.37 \pm 0.008$ ;  $n = 113$ ). Additionally, the holotype has a very compressed dorso-lateral shell profile (like individuals of *M. bellii*); although it is a statistical outlier in comparison to all *Myuchelys* taxa (CL/SD = 3.56; Figure 3.10). A possible explanation for this may be that the 150 year old, dry preserved specimen has developed an artificial compression of the shell during its storage. Other characteristics of the holotype appear to place it most closely to *M. bellii* (Figure 3.15). These characteristics include the specimen retaining a cervical scute (98% of *M. bellii* possess one, and 15% of *M. latisternum*), moderately deep serrations on the posterior margin of the carapace, colouration patterns on the plastron and the soft tissue parts of the limbs and head.

**Table 3.8** Correlations of variables with first two principal components of a PCA conducted on mature males of *M. bellii*, *M. georgesi*, *M. latisternum* and *M. purvisi* including the *M. bellii* holotype specimen (see Figure 3.14).

Variable	Males (n = 175)	
	PC1 (72.8%)	PC2 (19.5%)
1. CL/PW	0.10	0.19
2. CL/HW	0.82	0.24
3. CL/CD	0.03	-0.43
4. CW/PW	0.03	-0.10
5. CW/HW	0.55	-0.43
6. CW/SD	-0.05	-0.72
7. HW/SD	-0.07	-0.09



**Figure 3.14** PCA of mature male *M. latisternum* ( $\square$ ), *M. bellii* (+), *M. georgesi* ( $\bullet$ ) and *M. purvisi* ( $\Delta$ ) including the *M. bellii* holotype specimen ( $\blacklozenge$ ). The morphometric ratios included in each PCA are listed in Table 7.



**Figure 3.15** Carapace outlines for a) *M. bellii* (126 mm CL) Gwydir R., b) *M. bellii* holotype (114 mm CL), c) *M. latisternum* (120 mm CL) Albert R., d) *M. georgesi* (137 mm CL) Bellinger R. and e) *M. purvisi* (102 mm CL) Barnard R. (tributary of the Manning).

#### *Additional observations of Myuchelys species*

Most *M. bellii* (98%,  $n = 247$ ) had a cervical scute, contrasting with only 15% of *M. latisternum* ( $n = 246$ ) in the Albert and Brisbane Rivers, 77% of *M. georgesi* ( $n = 382$ ) in the Bellinger River and 96% of *M. purvisi* ( $n = 74$ ) from the Manning River. All *M. bellii* specimens examined had an olive-grey iris and a black pupil encircled by a thin pale-white ring that becomes more pronounced with pupil dilation. This distinguishes it from *M. latisternum*, which have marked variation in eye colour from catchment to catchment but always with a leading and trailing dark spot, and *M. georgesi* and *M. purvisi*, which have a golden brown iris with specks. Both *M. bellii* and *M. latisternum* have well-defined dorsal neck tubercles and prominent scales in the temporal region behind the eyes. The numerous cornified tubercles distinguish them from *M. purvisi* and *M. georgesi* which have a reduced presence of neck tubercles. The head shield for *M. bellii* and *M. latisternum* is large and prominent, nearly extending down to the tympanum, and ridged including a distinct central furrow. In contrast, *M. purvisi* and *M. georgesi* head shields are smoother lacking a well defined central furrow.

Moderately deep double serration of the carapace for *M. bellii* and *M. latisternum* separate them from *M. purvisi* and *M. georgesi*, which exhibit minor or nil serrations on rear marginal scutes. Over 87% of sampled *M. bellii* turtles from all sizes and catchments were moderately or strongly serrated on the posterior marginal scutes with most having double serrations occurring on 8-12 or 9-12. Those that did not have noticeable double serrations were usually immature with double serrations yet to develop, or mature turtles whose margins had been worn smooth.

The head colouration of *M. bellii* is steel grey in colour with a conspicuous cream, golden yellow or apricot-pink lateral neck stripe extending from the lower jaw through the tympanum to the plastron. A reduced brightness and visibility of the neck stripe usually occurred with age. This lateral stripe was prominent in 59% of turtles ( $n = 209$ ), with noticeable markings persisting in some aged animals. Faded, nearly nonexistent neck stripe markings around the jaw and plastron occurred in 28% of turtles sampled with a further 13% having no visible lateral neck colouration. Similarly, a bright golden yellow or apricot-pink pigmentation of the ventral areas of the neck (encompassing the chin barbels through to the plastron) that occurs on all hatchlings and immature turtles (<120 mm) fades with age, persisting occasionally in mature individuals. Small irregular cream mottling can sometimes remain on the ventral surface of the neck throughout adulthood.

### **Key to *Myuchelys* species**

A key to Australian *Myuchelys* is provided here. The species included are the western sawshelled turtle, *M. bellii*, common sawshelled turtle, *M. latisternum*, the Bellinger River helmeted turtle, *M. georgesi* and the Manning River helmeted turtle, *M. purvisi*. The key excludes the New Guinea spotted turtle, *M. novaeguineae*, which was only tenuously included in *Myuchelys* pending further genetic studies (Georges and Thomson 2010). Common names follow that of Georges and Thomson (2009). The key is applicable to animals greater than 70 mm carapace length.

*Key to species*

- 1 Head broad with deeply furrowed head shield; prominent conical neck tubercles; carapace exhibits deep, double serrations to posterior marginal scutes 8-12 (except if worn smooth in older individuals); carapace oval with or without expanded marginal scutes 7-12; plastron broad ..... 2
- Head slender; head shield smooth; neck tubercles small and rounded or non-existent; carapace oval and smooth edged, sometimes exhibiting shallow serration of posterior marginal scutes; plastron narrow ..... 3
- 2 Carapace length to head width dimensions <5.2 (except as rare variant); iris with leading and trailing dark spots; cervical scute absent or narrow when present (15% of population); body size moderate with relatively narrow carapace width; plastron colour varies between catchments; shell profile moderately deep.....*M. latisternum*
- Carapace length to head width dimensions >5.2 (except as rare variant); iris clear with a uniform olive-grey colour; cervical scute present (except as rare variant); carapace oval with expanded posterior marginals; plastron becoming predominantly black with age; shell profile laterally compressed.....*M. bellii*
- 3 Carapace length to posterior plastron width dimensions <2.62 females and <2.72 males (except as rare variant); shell profile moderately shallow; cervical scute narrow (absent in 23% of population); olive colouring on ventral surface of carapace and plastron, neural bones absent..... *M. georgesi*
- Carapace length to posterior plastron width dimensions >2.62 females and >2.72 males (except as rare variant); shell profile moderately deep; broad cervical scute present (except as rare variant); bright yellow colouring of the ventral surfaces of the carapace and plastron (except in the oldest individuals); a bright yellow lateral stripe on the ventral surface of each limb, extending from the plastron to the first toe; central, bright yellow tail stripe from the plastron anal notch to the cloaca with two lateral yellow tail stripes meeting at the cloaca; ventral tail tip yellow; neural bones present.....*M. purvisi*

**Discussion**

From the morphological comparisons presented in this paper, *M. georgesi* and *M. purvisi* share a suite of characteristics including: being medium sized; having a relatively narrow oval carapace exhibiting minor serrations to the posterior margins; retaining a cervical scute (considered to be a primitive character state, Gaffney 1977); possessing a relatively small head for their body size; having small rounded neck tubercles and exhibiting a smooth head shield (not deeply furrowed). In contrast, the sister species pair of *M. bellii* and *M. latisternum* which are nested between *M. georgesi* and *M. purvisi* in the phylogeny (Figure 3.1) have: a larger overall size; laterally expanded carapace (particularly *M. bellii*); exhibit prominent conical neck tubercles (Legler and Winokur 1979); possess a significantly larger head (particularly *M. latisternum*); a furrowed head shield; expanded posterior carapace margins with moderately deep serrations; and have lost the cervical scute (*M. latisternum* only). This means that either a case of character convergence in allopatry has occurred between *M. georgesi* and *M. purvisi* or their shared characters are plesiomorphic (retained primitive traits). This latter interpretation is more likely and thus, the phenotype of the *Myuchelys* common ancestor would have expressed the plesiomorphic characters of the present day *M. georgesi* and *M. purvisi*.

Similarly, the shared characters of the sister species pair *M. bellii* and *M. latisternum* are considered to be synapomorphies (derived characters). However, the presence of cornified neck tubercles is believed to be a primitive trait, as evidenced in many fossil chelids from South America. This suggests that there was a reversal to the presence of prominent neck tubercles in the ancestral state of *M. bellii* and *M. latisternum* and after its derived loss in the *Myuchelys* ancestor. A reversal of morphological character traits has been observed in other chelid studies. For example, a secondarily derived character state (i.e., ancestral state subsequently lost and then reversed in present day species) is also believed to have occurred for short-necked chelid species of South America (Georges *et al.* 1998). These authors argue that the likely ancestral state for chelid species was a short neck with long necks being a derived state. Consequently, contemporary short-necks of South America are thought to have secondarily derived a short neck after having developed long-necks (three steps) (Georges *et al.* 1998).

For the first time, this research provides insights into the likely sequence of events in the *Myuchelys* dispersal across eastern and northern Australia. By examining the plesiomorphic and synapomorphic characters, the dispersal events that lead to today's biogeography of the *Myuchelys* can be elucidated. The distinctive characters possessed by *M. bellii* and *M. latisternum* – broader heads, conical neck tubercles and diagnostic shell morphology - developed prior to their radiation over a wide geographic area from coastal catchments in northern NSW to tropical Australia in Qld and the Northern Territory and also into the Murray Darling system. Furthermore, it is inferred that the *M. latisternum* and *M. bellii* split from their ancestral state occurred prior to *M. latisternum* developing a significantly larger head (considered here to be an apomorphy), and *M. bellii* developing a relatively more compressed shell profile and wider carapace. Hence, *M. bellii* dispersed into the Murray-Darling Basin system where it retained the primitive cervical scute and relatively smaller head than *M. latisternum*. Conversely, *M. latisternum* developed a robust head and an absence of a cervical scute (narrow when present) prior to its rapid radiation along the coastal drainages of eastern and northern Australia.

The cryptic species pair of *M. georgesi* and *M. purvisi*, endemics to single catchments of the Manning and Bellinger Rivers, retained plesiomorphic traits in allopatry. These plesiomorphic traits retained in allopatry by *M. georgesi* and *M. purvisi* may have resulted from the stability of the environments which they now occupy. The Manning and Bellinger River systems, separated by two coastal river catchments, are similar in their geological features and climatic conditions. Hence, their habitats are similar possibly allowing for particular plesiomorphic traits to be retained independently of one another over the millennia.

An aged *M. georgesi* specimen captured as part of a recent genetic study of the Bellinger River *Emydura* was mistakenly identified in the field as an *M. latisternum* (Arthur Georges, pers. comm.). However, it was later revealed that it had a *M. georgesi* mtDNA haplotype (Georges *et al.* 2007). The aged specimen had features that resembled *M. latisternum* including more prominent neck tubercles, robust head size and larger body size. This case of mistaken identity provides a possible insight into the selection processes for the speciation of *M. latisternum* from the *Myuchelys* ancestor.

The ontogeny of *M. georgesi* may allow for the oldest and largest of animals to have characters converging on those now possessed by *M. latisternum*.

In light of the present findings, the *Emydura* genus displaying minor tubercles, small head size, lack of a prominent head shield and smooth shell margins may be the neotenuous expression of *M. georgesi* and *M. purvisi*, and *M. latisternum* the reverse. Neoteny, that is, the maintenance of juvenile characteristics in adulthood, is a common phenomenon proposed to be responsible for major morphological changes (Gould 1977). *M. latisternum* may be an example of selection for early expression of traits normally expressed only in the very oldest, largest *M. georgesi*. This would allow rapid change of form and new opportunities in the directions of herbivory (*Emydura*) and carnivory (*M. latisternum*) that would have reinforced the morphology shift.

Prior to this research, the cryptic species pair *M. georgesi* and *M. purvisi* were recognised to be genetically divergent (Georges and Adams 1992; 1996; Georges *et al.* 1998), yet were considered to differ overtly only in the intensity of colouration with *M. georgesi* displaying an olive or blue tinge and *M. purvisi* bright yellow colouration on its neck, limbs and tail (Cann 1997a; Cann 1998; Thomson and Georges 2009; Georges and Thomson 2010). Furthermore, the osteological differences were thought to be limited to the presence of carapace neural bones in *M. purvisi* while being absent in *M. georgesi* (Thomson and Georges 1996). However, this present study reveals for the first time subtle, yet significant, external differences between the two species including *M. purvisi* having a comparatively deeper shell for both sexes (the deepest of all *Myuchelys* species examined) (Figure 3.10), significantly narrower plastron for both sexes (Figure 3.9), and a wider head and shorter tail for mature females (Figure 3.6 and Figure 3.7) than *M. georgesi*. In combination, these previously undocumented characters are diagnostic for the two species.

Furthermore, the morphological differences thought to be present in populations of *M. bellii* are unsupported by this study. Assertions made by others (Cann 1998; Wells 2002; Wells 2007) that the Queensland form have smaller heads, uniquely possess a second serration on posterior carapace scutes, reach a smaller overall size for mature

individuals, differ in central groove prominence and plastron form are not supported by analyses of over 233 individuals across all populations. The maximum potential size for individuals from each population is similar and this study revealed no difference in carapace serration depths or frequency of persistence on older individuals. Individuals from any population were likely to exhibit moderate to strong double serrations into adulthood. This study also observed that the lateral neck stripe colouration was consistent among *M. bellii* populations with yellow or cream stripes usually faded in older individuals. The combined weight of evidence of a shallow genetic structure (Chapter 2) and the lack of phenotypic variation among *M. bellii* populations preclude the existence of a cryptic taxon at the species or subspecies level.

Morphological assessment of the *M. bellii* holotype specimen (*Phrynops bellii*, OUM 8460) confirmed earlier assertions by Cann (1998) that its origins were from *M. bellii*. The strongest indication for this is the carapace length to head width ratio (CL/HW) for the holotype specimen being 5.85 (Figure 3.6) which excludes it from being a *M. latisternum* (<5.2). Other supporting evidence for it being a *M. bellii* is: the carapace width and presence of serrated marginal scutes exclude it from being *M. georgesi* or *M. purvisi*; its broad plastron excludes it from being *M. purvisi*; its compressed shell, the presence of a cervical scute and general colouration of the plastron and soft parts of the limbs and neck are consistent with *M. bellii*. Recommendations by Georges and Thomson (2010) for genetic testing of the 150 year dry preserved specimen would provide independent data to this conclusion. Thus, taxonomic validity of the binomial name for the biological entity of *M. bellii* is maintained.

This study of the Australian freshwater turtle genus, *Myuchelys*, provides for the first time a melding of morphology and a phylogenetic framework to gain a unique insight into the phenotype of the common ancestor to *Myuchelys*. In addition, it advances the understanding of the morphology, evolution and dispersal events of the *Myuchelys* species of freshwater turtles in Australia. A limitation on these conclusions was that most individuals of *M. latisternum* included here were from the southern extent of its current range (Brisbane and Albert river catchments). An assessment of morphological variation of *M. latisternum* specimens across its vast geographic range, including north Australian catchments, may reveal greater levels of intraspecific variation as suspected by Cann (1998).

**Chapter 4.      Reproduction and population ecology of the western  
sawshelled turtle *Myuchelys Bellii* in the Murray-Darling Basin,  
Australia.**



Picture: A *Myuchelys bellii* hatchling (two weeks old) released into microhabitat in Bald Rock Creek, Girraween National Park. Photo by Darren Fielder.

## **Chapter 4. Reproduction and population ecology of the western sawshelled turtle *Myuchelys Bellii* in the Murray-Darling Basin, Australia.**

### **Abstract**

Effective management decisions about the conservation of threatened species often rely on good information about their biology and life history traits. With turtle species worldwide facing rapid declines in abundance and loss of habitat, there is increasing concern that over half of all species could go extinct in the wild over the next century. The vulnerable western sawshelled turtle, *Myuchelys bellii*, from eastern Australia is one such species under threat. There is a pressing need for a greater understanding of the fundamental aspects of its reproductive traits, age at sexual maturity, population estimates and general health. In this study I found that the life history traits of *M. bellii* are similar to many other turtles by having delayed age at first breeding, low reproductive effort (14.3 eggs per adult female) and high survivorship with a predicted lifespan of over 40 years. Sexual maturity was determined using annual growth layers (annuli) and also from growth increment data from mark-recapture data. Increment data were determined to be the more reliable estimate of age at maturity with males taking nearly 10 years to mature and females approaching 20 years. Annual breeding rates varied greatly between years with only 78% of adult female *M. bellii* on average breeding in any one year. Of particular management concern for *M. bellii*, is the long term conservation of the small isolated Queensland population (<400 individuals in total). For the first time the life history traits of the nationally vulnerable western sawshelled turtle *M. bellii* have been quantified. The data presented in this study provide direction for future conservation strategies of this endemic turtle across jurisdictional borders in the Murray-Darling Basin of Australia.

### **Introduction**

With massive declines in species abundance and loss of habitat, nearly half (48.5%) of the world's modern chelonian fauna are threatened or are already extinct in the wild (Turtle Taxonomy Working Group 2010). Primary factors in the sharp decline of many turtle species are human consumption and unregulated trade markets across the globe along with habitat loss and degradation (Buhlmann *et al.* 2009) including likely

negative effects from climate change (Chaloupka *et al.* 2008). There is a high risk that without strategic conservation intervention, a significant proportion of turtle diversity could be lost over the next century (Buhlmann *et al.* 2009).

Approximately ten per cent or 32 of the world's 333 recognised species of tortoise and turtles occur in the Australasian region (Georges and Thomson 2010; Turtle Taxonomy Working Group 2010) and a recent assessment of global turtle priority areas identified coastal Australia as an additional focus of conservation effort (Buhlmann *et al.* 2009). Australia has six species of freshwater turtle listed nationally as threatened (Critically Endangered, Endangered or Vulnerable) under the *Environment Protection and Biodiversity Conservation Act 1999*. One of these, the vulnerable western sawshelled turtle, *Myuchelys bellii* (Gray 1844), occurs in just three small relict populations in the headwaters of the Darling River system in eastern Australia. This medium sized turtle is also listed as vulnerable under the respective New South Wales and Queensland state nature conservation legislation, and listed as endangered in the current 2010 IUCN Red List.

*Myuchelys bellii* was first described by Gray (1844) and its name later resurrected by Cann (1998) after confirmation from molecular analyses (Georges and Adams 1992; 1996). Until recently, *M. bellii* was suspected to contain a cryptic species whose characteristics were thought to be distinct morphologically from all other populations (Cann 1998). However, molecular and morphological studies (refer to Chapter 2 and Chapter 3) found no evidence to support this hypothesis. Consequently, all extant populations of *M. bellii* are regarded in this paper to be part of a single biological entity within *Myuchelys*.

Life history parameters have been generally well studied for several Australian chelids and representing most genera, including *Chelodina expansa* (Booth 2002; Spencer 2002b), *C. longicollis* (Parmenter 1976; Kennett and Georges 1990; Kennett *et al.* 2009), *C. rugosa* (Kennett, Georges *et al.* 1993; Kennett, Christian *et al.* 1993; Kennett 1994; Kennett *et al.* 1998; Kennett 1999), *Elusor macrurus* (Flakus 2002), *Emydura macquarii* (Limpus *et al.* 2002; Kennett, Georges *et al.* 1993; Kennett, Christian *et al.* 1993; Kennett 1994; Kennett *et al.* 1998; Kennett 1999), *Elseya albagula* (Hamann *et al.* 2007), *E. dentata* (Kennett 1999), *Myuchelys georgesi* (Allanson and Georges 1999;

Blamires *et al.* 2005) and *Pseudemydura umbrina* (Burbidge 1981; Kuchling and Bradshaw 1993). In contrast, the life history traits of *M. bellii* are poorly understood with only a few observations on diet and breeding biology reported from the literature (Cann 1998). A paucity of fundamental information on *M. bellii* makes decisions on conservation action difficult, or delayed indefinitely by management authorities.

This study provides the first detailed information on the life history traits of *M. bellii*. Long-term data from capture-mark-recapture surveys (2002-2009) were used to estimate population size and distribution of *M. bellii*. Size at sexual maturity and growth analysis based on growth rings (annuli) and direct capture-recapture measurements are used to estimate age at maturity. Reproductive biology and fecundity are also described.

## **Methods and Materials**

### *Taxonomy of M. bellii*

The nomenclature of the *Myuchelys latisternum* clade, incorporating the threatened *M. bellii*, has been a contentious subject with recent reviews by Thomson and Georges (2009) and Georges and Thomson (2010) rejecting proposed genus and species names raised in a series of articles (Wells 2002; 2007; 2009). Georges and Thomson (2010) argue that these documents do not meet the International Commission on Zoological Nomenclature publication guidelines. For the purposes of this paper, I have adopted the taxonomic and nomenclatural reviews by Thomson and Georges (2009) and Georges and Thomson (2010), as have other authors (see Nowak-Kemp and U. Fritz 2010).

### *Study area and climate*

*Myuchelys bellii* occurs in the temperate zone of eastern Australia on the New England Tableland (NET) which is a designated bioregion. The NET is a stepped plateau of hills and plains with elevations between 600 and 1500 metres above sea level (masl) on Permian sedimentary rocks, intrusive granites and extensive Tertiary basalts. The granite areas are steep with large areas of rock outcrop and rounded tors where streams flow through granite boulder screens and bedrock (Department of Environment, Climate Change and Water, NSW: [www.environment.nsw.gov.au](http://www.environment.nsw.gov.au)).

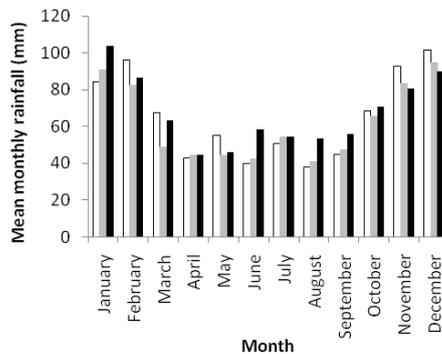
Bald Rock Creek in the Border Rivers catchment is a small headwater stream of the Murray-Darling Basin which has its beginnings in Girraween National Park. Bald Rock Creek flows through granite bedrock hills with an altitude range of 700-1100 masl. The climate is temperate with warm wet summers (November-February) and cold dry winters (April-September). Girraween National Park receives an average annual rainfall of 793 mm with a monthly average maximum of 103 mm in December and a minimum of 40 mm in June (weather station (WS) #41454; Table 4.1, Figure 4.1). December, January and February are the hottest months, with an average daily air temperature maximum of 25.3–26.4°C and an average minimum of 13.8–15.2°C, respectively (WS #41095; Table 4.1, Figure 4.2). The coldest month is July, with a mean daily maximum of 14.8°C and a mean minimum of 1°C.

The study area in the catchments of the Namoi and Gwydir Rivers has a similar climate to Girraween National Park. The Kingstown Post Office, located in the Gwydir River catchment, receives an average annual rainfall of 744 mm with a maximum monthly average of 94 mm in December and minimum monthly average of 40 mm in August (WS #56228; Table 4.1, Figure 4.1). The Walcha Post Office, located on the MacDonal River in the Namoi River catchment, receives an average annual rainfall of 808 mm with a maximum monthly average of 104 mm in January and a minimum monthly average of 45 mm in April (WS #56035; Table 4.1, Figure 4.1). Both the Gwydir and the Namoi River catchments experience similar daily mean air temperatures (WS #56006, #55136; Figure 4.2) to the Queensland catchment of Bald Rock Creek.

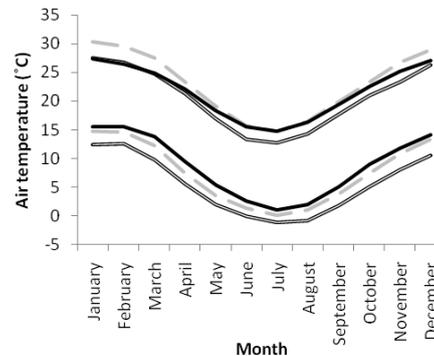
The habitat of *M. bellii* is permanent cold flowing upland streams above about 700 masl. Granite boulders and granite bedrock are common features of these streams, which are typically 30 - 40 m wide in the Namoi and Gwydir Rivers and 10 - 20 m wide in Bald Rock Creek. Coarse granite sand deposits in the stream occur in lower velocity areas and are usually covered in fine silt, algal growth or macrophytes (<2 m) and instream habitat is complex with underwater caverns formed by granite boulders. Large woody debris and overhanging banks are also common features in the three river drainages.

**Table 4.1** Australian Bureau of Meteorology (B.O.M.) long-term weather stations located in the study catchments. Their locations and the years of available rainfall and temperature data are provided. Source: “Climate Data Online” (www.bom.gov.au).

Weather Station	Station #	Years Open	Study Catchment	Altitude (masl)	Latitude	Longitude
Girraween National Park	41454	1973-2010	Border	950	28°49'60"S	151°55'60"E
Stanthorpe Leslie Parade	41095	1938-2010	Border	784	28°39'42"S	151°56'02"E
Kingstown Post Office	56228	1981-2010	Gwydir	711	30°30'28"S	151°6'51"E
Bundarra Post Office	56006	1914-1970	Gwydir	654	30°10'16"S	151°4'33"E
Walcha Post Office	56035	1880-1996	Namoi	1050	30°59'07"S	151°35'39"E
Woolbrook	55136	1970-2010	Namoi	910	30°58'02"S	151°20'42"E



**Figure 4.1** Long-term mean monthly rainfall histograms for selected weather stations. Hollow bar is Girraween National Park (WS #41454; Bald Rock Ck.), grey bar is Kingstown Post Office (WS #56228; Gwydir R.) and solid black bar is Walcha Post Office (WS #56035; Namoi R.). Refer to Table 4.1 for weather station details.



**Figure 4.2** Paired long-term mean monthly maximum and minimum (calculated daily) air temperatures for selected weather stations. Solid black lines are Stanthorpe Leslie Parade (WS #41095; Bald Rock Ck.); grey dashed lines are Bundarra Post Office (WS #56006; Gwydir R.); and hollow lines are Woolbrook (WS #55136; Namoi R.). Refer to Table 4.1 for weather station details.

The three river catchments have been subject to considerable water infrastructure and agricultural development. Copeton Dam (113 m wall height, 1364000 megalitres) and Keepit Dam (55 m wall height, 423000 megalitres) mark the known downstream extent of *M. bellii* populations in the Gwydir and Namoi rivers respectively. While the privately owned Anderson Weir (approximate 5 m wall height) marks the downstream extent of *M. bellii* in Bald Rock Creek. These, and other smaller impoundments, and

associated agricultural development have extensively modified *M. bellii* habitat across its known range. The main associated impacts are changed in-stream conditions from a lotic stream environment to a lentic system and loss of riparian vegetation reducing food resources and suitable habitat for the turtle. In addition, habitat in Bald Rock Creek is being adversely affected by the exotic goldfish which are present in large numbers throughout.

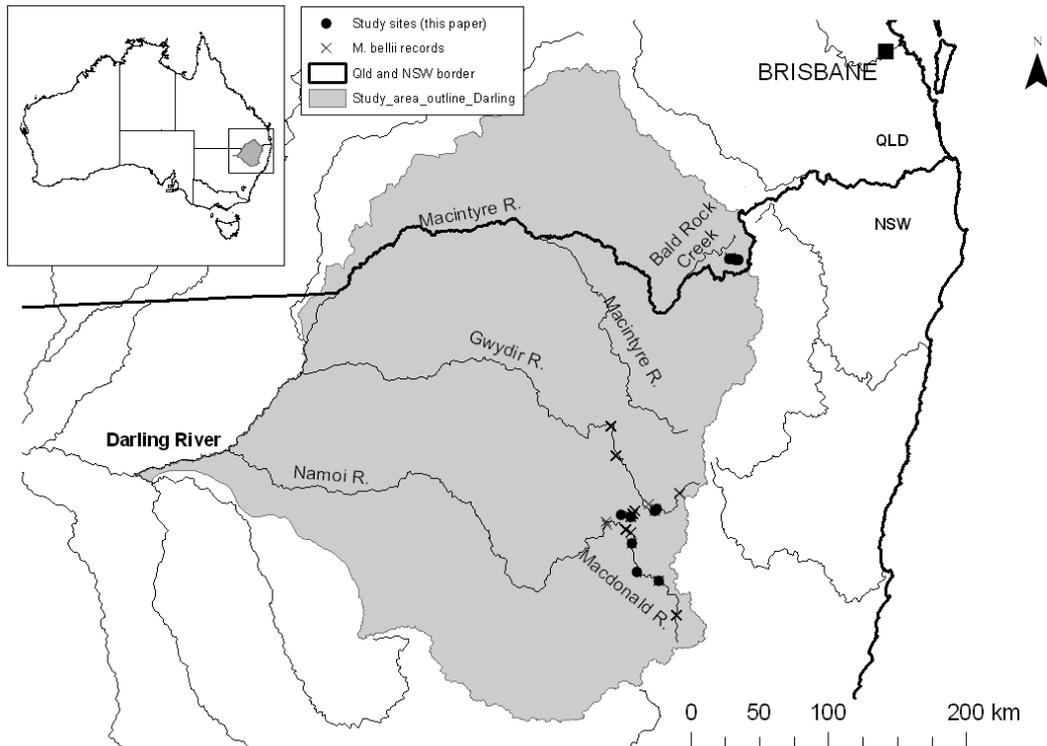
#### *Site characteristics*

Water temperatures at 30 cm depth in Bald Rock Creek were recorded using iButton technology for >30 days per season and at 15 minute intervals from spring 2006 to spring 2007.

#### *Capture and handling*

A capture-mark-recapture program was run with annual surveys across its natural range from 2002 until 2009 in Bald Rock Creek (located in the Border Rivers catchment) and from 2005 until 2007 in the Macdonald River (tributary of the Namoi River) and the Gwydir River of New South Wales, eastern Australia (Figure 4.3). Turtles were captured from several locations over an eight kilometre section of Bald Rock Creek which contained the only known population in Queensland. Survey locations were selected to cover the known geographical range in the Namoi and Gwydir river catchments.

Most specimens were live captured and released, either having been trapped using modified rectangular collapsible crab traps, or caught by hand and snorkel. Traps were baited daily with fresh beef heart or other meat. Fully submerged traps were checked every 2 - 3 hours and modified (“cathedral”) traps with a holding area that allowed trapped turtles to breath at the water surface were checked less regularly – at least once every 24 hours (Hamann *et al.* 2007). Turtles were typically processed and returned to the place of capture within 24 hours of being caught, or otherwise held in polyethylene tubs of water to prevent desiccation until their release (<72 hours, but very occasionally, isolated individuals were held for longer periods).



**Figure 4.3** Survey sites for *M. bellii* in the Border Rivers (Macintyre R. and Bald Rock Creek), Gwydir River and Namoi River catchments. Solid circles are site locations (this study) and crosses indicate previous records of *M. bellii* from the Australian National Museum and the Institute for Applied Ecology, University of Canberra. Inset map shows study area in relation to Australia.

Male and female sex and sexual maturity for individuals was determined by gonad examination using laparoscopy (Limpus *et al.* 2002), evidence of secondary tail elongation (Georges 1983) and sometimes through observed penis extension. Individuals were classified as unsexed when neither field observations nor statistical analysis could assist in sex identity. Information on male and female reproductive biology was collected via gonad examination using laparoscopy and ultra-sonography (Limpus *et al.* 2002; Limpus and Limpus 2003; Hamann *et al.* 2007: Pubescent males were identified by an epididymis within or enlarging from the body wall; adult males were identified by a epididymis fully distended from the body wall; immature females were identified by a compact ovary and a white straight oviduct; adult females were characterised by one or more of corpus luteum, corpora lutea, mature follicles, soft shelled oviducal eggs or hard shelled oviducal eggs), X-rays and palpation via the inguinal pocket to identify oviducal eggs and from egg collection and incubation.

Two clutches of eggs were collected for incubation during 2005/2006 and 2006/2007 summers to provide data on *M. bellii* hatchling size, fecundity and growth rates. On each occasion, the gravid female was administered Calicium Sandoz Syrup solution intramuscularly into the rear legs at a dose rate of 3 ml/kg to ensure follicles were calcified before being laid. Ilium Syntocin (active constituent 10 i.u./ml oxytocin) was intramuscularly injected into each rear leg at a dose rate of 2.2 ml/kg 48 hours after calcium injections. The Ilium Syntocin stimulated contractions and induced egg laying within 23-55 minutes of being administered. Each egg was measured immediately after being deposited. An X-ray post egg deposition confirmed that all eggs had been laid.

Clutches were incubated in a sterile vermiculite bed (1.1:1 parts water to vermiculite) in a plastic container (100 x 150 x 20 mm) and placed into an automatic temperature controlled incubator (Labec, Laboratory Equipment) set to 29°C. The incubator was housed in a constant temperature control room. The moisture level within the vermiculite was maintained using a hand spray container every four days during incubation (humidity was high, although not measured). A minimum estimate of fertility of eggs was determined by whether or not the incubating eggs developed a white patch during incubation (Thompson 1985). Fecundity was calculated as the product of the average number of eggs per clutch, the number of clutches laid per female within a breeding season and the proportion of females reproducing in any one year (Frazer *et al.* 1991).

Size at sexual maturity was determined using the results of laparoscopic examinations and then applying a logistic function analyses equation: proportion mature,  $y = 1/(1+\text{EXP}(-b*(CL-c)))$  (Limpus *et al.* 2002). This function of the ratio of adult to immature per 10 mm size increments by sex provides the carapace length when there is 50 per cent adult to immature ('adult size 50%', AS<sub>50</sub>).

### *Growth*

Growth curves were developed separately for males and females using the von Bertalanffy equation modified by Fabens (1965) being  $CL_t = a (1 - be^{-kt})$ , where CL is the carapace length,  $t$  is the age (in years),  $a$  is the asymptotic or maximum body size,  $b$  is related to hatchling length, where  $b = (1 - h)/a$  ( $h$  is hatchling length),  $e$  is the natural

logarithmic base, and  $k$  is the intrinsic growth rate. This growth equation was evaluated using the independent data of a) age estimated from annual growth rings (annuli), and b) growth increments measured from recaptured turtles of unknown age.

All annuli counts were verified by two experienced persons using photographs to limit possible counting of pseudoannual rings (Ewing 1939). Except for 13 individuals, all annuli counts had photographic evidence. Each individual turtle's "birth date" was determined by subtracting the total number of growth layers from its capture date, indexed to the 31<sup>st</sup> January of its hatching year. The 31<sup>st</sup> January is approximately in the middle of the Austral summer growing season when hatchlings are expected to emerge from their nests. As an example, a turtle captured on the 10/02/2006 exhibiting 5 growth layers would have its birth date at 31/01/2001. Thus, the indexed age for this animal is 5.03 years. Some individuals were captured more than once during the survey period (2002 – 2009) allowing multiple records from one animal to be used in the analysis. No sexually mature females were available for inclusion in the analysis as none exhibited discernable annuli.

I plotted the carapace length against age from annuli for all turtles which exhibited a full or near complete set of annuli. I fixed  $a$  to equal the maximum size for *M. bellii* (from this study: females 300.2 mm; males 218.9 mm). FiSAT II software (Gayaniilo *et al.* 2005) built in setting for analysis of 'length-at-age' was used to estimate  $k$  values. These values were estimated for male and females separately incorporating the same unsexed individuals in both analyses as in other studies (Spencer 2002*b*; Martins and Souza 2008).

Growth increment records from capture-recapture events for turtles of unknown age span seven years. Some individuals are included on more than one occasion to establish capture-recapture measurement pairs, as in other studies (Chen and Lue 2002; Martins and Souza 2008). A minimum of 365 days between capture events was imposed for growth increment analyses to ensure an entire growth period was observed. Negative growth rates were retained in the analysis as they were found to have no impact for a growth study of the Pacific angel shark (Cailliet *et al.* 1992). Growth rate ( $G$ ; mm/year) was calculated as carapace growth,  $G = CL_{recapt} - CL_{capt} / \Delta T$ , where  $CL_{recapt}$  is length at recapture,  $CL_{capt}$  is length at first capture and  $\Delta T$  is time interval in years between

$CL_{recapt}$  and  $CL_{capt}$ . The geometric mean size ( $GM$ ) was calculated as  $GM = \sqrt{(CL_{recapt} \times CL_{capt})}$  to minimise time interval effects between captures (Martins and Souza 2008). A regression analyses of growth rate (mm/year) and carapace length was done to determine the strength of the relationship (Spencer 2002b; Martins and Souza 2008).

A lack of juvenile turtles in growth models can over or underestimate ages depending on the species juvenile growth patterns (Spencer 2002b). To minimise this possible bias, five small unsexed individuals in their second and third years of life (<90 mm CL) based on annuli counts were included in both male and female analyses. The growth increments were measured by taking the mean size of 26.7 mm CL for *M. bellii* hatchlings (this study) from their capture size. Time between captures was estimated by applying a standardised hatch date of 31 January to their hatching year. Growth rings have been found to be reliable up to 3-9 years of age for other Australian chelids (Wilson *et al.* 2003). Seasonal variation on growth rates was ignored as in other studies (Spencer 2002b; Chen and Lue 2002; Martins and Souza 2008).

FiSAT II was employed to estimate the growth parameters  $a$  and  $k$ . The Fabens method in FiSAT II underestimated  $a$  for both females ( $a = 178.10$ ) and males ( $a = 202.68$ ), as for Martins and Souza (2008). The growth parameters  $a$  and  $k$  are inversely related so that underestimating  $a$  overestimates  $k$ . Consequently, I fixed  $a$  to equal the maximum size for *M. bellii* (from this study: females 300.2 mm; males 218.9 mm) and I also compared this to fixing  $a$  to equal the maximum size for a recaptured turtle used in the analysis (male 212.5 mm and female 259.8 mm). The growth parameter,  $k$ , was then computed using both estimates of  $a$  in FiSAT II. The maximum body size  $a$  and estimated  $k$  growth parameters were then used to plot the von Bertalanffy growth curve for males and females from the capture-recapture data of turtles of unknown age.

#### *Population estimate*

A capture-mark-recapture history profile for 79 individuals from Bald Rock Creek over seven sampling periods (winter to winter) from 2002 – 2009 was used to generate a population estimate using program Mark (G. White and Burnham 1999). Sexually mature females and males were one group and a second group was made up of sexed and unsexed immature turtles. Survival ( $\Phi$ ) and recapture probabilities ( $p$ ) of adult and

immature turtles were modelled and estimated using program MARK. Only a small set of candidate models were selected for comparison, based on the Cormack-Jolly-Seber (CJS) (Cormack 1964; Jolly 1965; Seber 1965) classic design of an open population in a long-term capture-recapture study that include recruitment, immigration, mortality and emigration over multiple periods.

To select the most appropriate model for describing demographic temporal variation, I used a bias-corrected version of Akaike's Information Criterion, AICc. Models were compared by the Delta AICc value (correction for overdispersion), and the most parsimonious model (lowest Delta AICc) was retained (Anderson *et al.* 1994). All turtles captured from Bald Rock Creek were assumed to be part of a single reproductive population because a male turtle was recorded to have traversed the full extent (~8 km) of known habitat in Bald Rock Creek within a year.

#### *Diet and health observations*

The diet observations reported in this study are from unquantified incidental records collected from visual examination of faecal matter from turtles held in isolation in tubs of water (usually overnight). Some vegetation (e.g., blackberry seeds) and animal parts (e.g., *Euastacus suttoni* exoskeleton) were able to be identified to species level. Though, predominantly observations were usually at the broader taxonomic levels (e.g., filamentous algae, leaves, stems or terrestrial insect material). Observations on turtle health including eye cataracts and shell damage were visually observed in the field. For instance, individuals were recorded to either have clear eyes, or one or both eyes infected with a milky clouding or complete opaque covering of the iris.

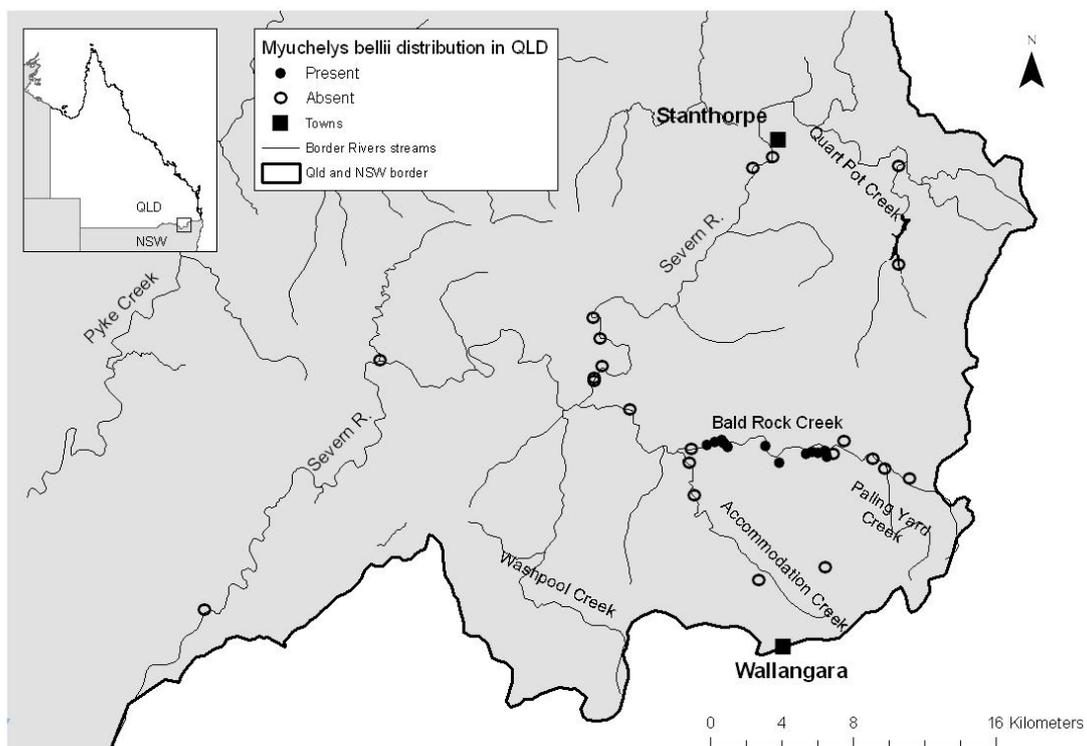
A standard set of measurements was taken for all turtles examined in the study (Limpus *et al.* 2002; Hamann *et al.* 2007; Hamann *et al.* 2008). All carapace, head and tail measurements were recorded to the nearest 0.1 mm using vernier callipers and weight recorded to the nearest 2 g using digital scales. Larger turtles (> 2.5 kg) were weighed to the nearest 25 g using a 3 or 6 kg spring balance. Straight carapace length (CL) is the minimum straight line distance between the cervical scute and the juncture of the posterior marginal scutes of the carapace. Field procedures for turtle capture in NSW were in accordance with the scientific permit S11000 and the University of New

England's Animal Ethics Committee (authority number: AEC04/158). Field procedures in Queensland were undertaken in accordance with the Department of Environment and Resource Management's (DERM) Animal Ethics Committee (EPA2006/11/20 – 22), and undertaken with supervision of staff from the Queensland Parks and Wildlife Service (QPWS).

## Results

### *Queensland distribution of M. bellii*

The Queensland population of *M. bellii* is restricted to about 8 km of stream length in Bald Rock Creek (Figure 4.4). Most individuals were recorded to occur in two permanent waterholes, each approximately 1.4 km in length, at either end of the stream section. The intermediate reach would appear to be less favourable habitat based on the limited numbers caught there. A variety of habitats (off-stream wetlands, private instream impoundments) at 22 other locations in the Border Rivers catchment, including above and below Nundubbermere Falls on the Severn River, were surveyed throughout the sampling period without *M. bellii* being encountered.



**Figure 4.4** The current distribution of *M. bellii* in the Queensland Border Rivers catchment. Survey sites are indicated by a solid circle (presence) or an open circle (absence). Inset map shows survey region in comparison to Queensland and New South Wales.

### *Seasonal water temperature in Bald Rock Creek*

Bald Rock Creek water temperatures at 30 cm depth ranged seasonally between 5 °C and 30 °C. Average temperatures are: winter  $8.6 \pm 0.02$  °C, spring  $20 \pm 0.04$  °C, summer  $23.2 \pm 0.04$  °C and autumn  $17.9 \pm 0.03$  °C.

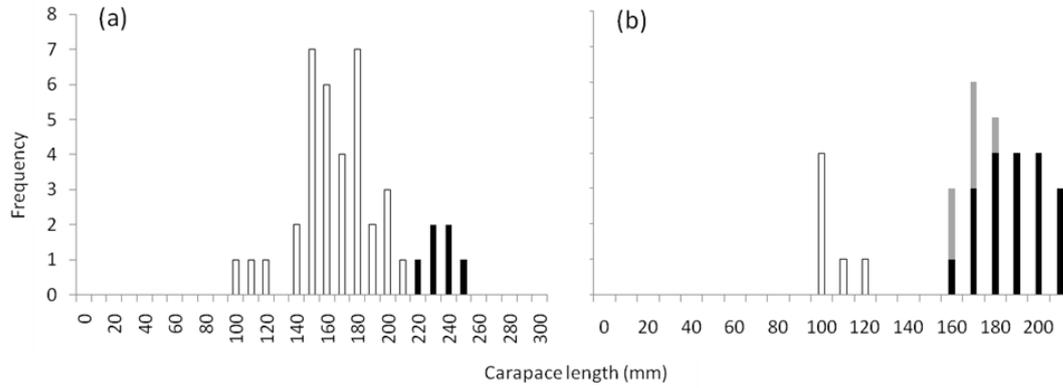
### *Sexual Maturity*

A total of 26 female *M. bellii* from Bald Rock Creek were examined by laparoscopy on 34 occasions during October 2004 to November 2008. Of these, six females were adult and 20 were immature (Figure 4.5a). No female transitioned from immature to mature during the study period. The maximum CL for an immature female was 211.8 mm and the minimum size for an adult female *M. bellii* from Bald Rock Creek was CL 222.1 mm. One adult female was recorded breeding for the first time at CL 233 mm. The size at sexual maturity for females was calculated to be 215 mm, AS<sub>50</sub> (Figure 4.6).

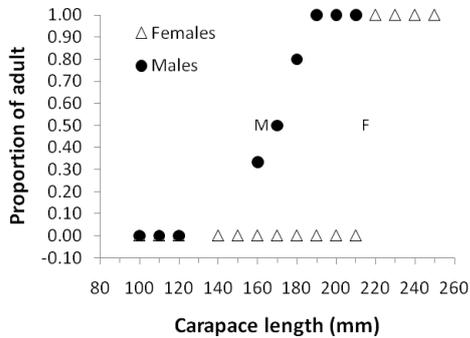
Similarly, 26 male *M. bellii* from Bald Rock Creek were examined by laparoscopy on 35 occasions during the same period. Of these, five males were immature, six were immature pubescent (sub-adult) and 18 were adult (Figure 4.5b). These totals include duplicate records from four males which transitioned from pubescent to mature during the period of the study. The maximum CL for an immature pubescent male was 187.3 mm and the minimum CL for an adult male was 168.0 mm. The size of four individuals observed to transition from immature pubescent males to mature adults were (time between captures in paranthesis): CL 167.5-170.5 mm (3.1 y), 178.4-179.5 mm (2.2 y), 178.1-180.8 mm (4.1 y) and 187.3-187.6 mm (1 y). The size at sexual maturity for males was calculated to be 161.6 mm, AS<sub>50</sub> (Figure 4.6).

One individual was identified as an hermaphrodite having both ovaries and an epididymis (CL 209.6 mm). Its data were excluded from analyses with respect to sex and maturity. The tail to carapace relationship for turtles examined by laparoscopy from Bald Rock Creek is presented in Figure 4.7. Sexual maturity for turtles from Bald Rock Creek and the Namoi and Gwydir rivers was identified based on the combined laparoscopy, AS<sub>50</sub> values and evidence of secondary tail elongation (Figure 4.8a, Figure 4.8b and Figure 4.9). Secondary tail elongation was evident for adult males when their tail to carpace length (TC) was >45 mm, and pubescent males exhibited a tail length

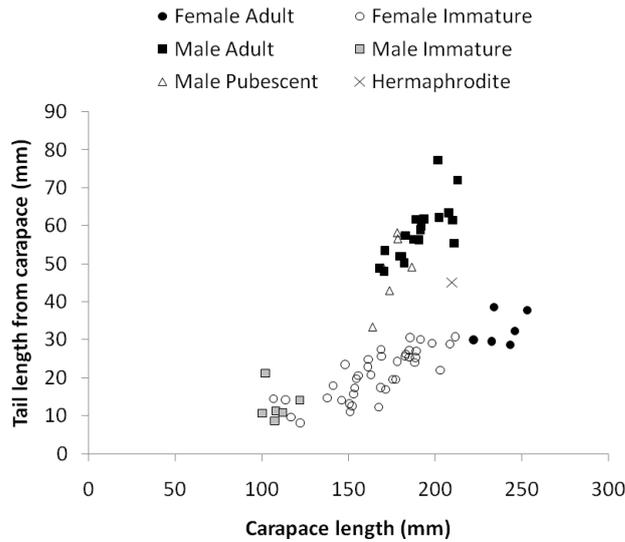
between 30-45 mm TC. It is acknowledged that no pubescent male with >45 mm TC could be identified in the absence of laparoscopic examination. Similarly, turtles <150 mm carapace length remained unsexed without the aid of laparoscopic examination or evidence of secondary tail elongation.



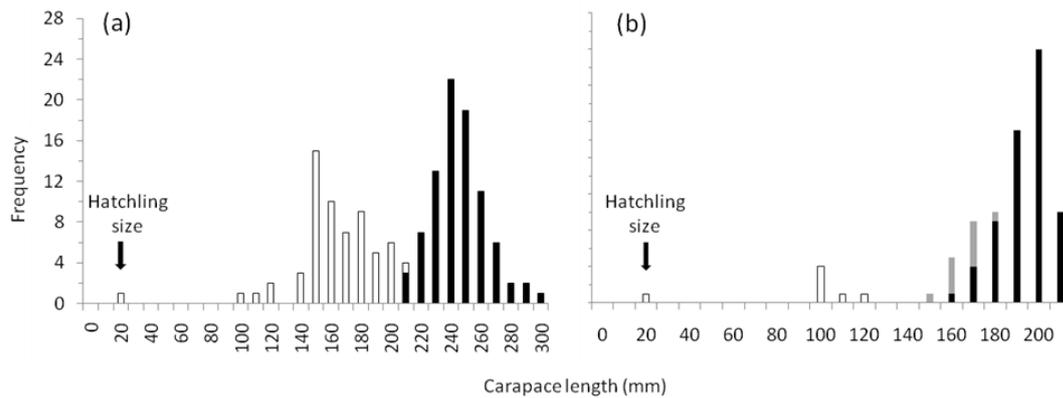
**Figure 4.5** *Myuchelys bellii* sexual maturity identified by laparoscopic examination for (a) females and (b) males from Bald Rock Creek. Hollow bars are sexed immature, grey bars are pubescent males and solid black bars are adults.



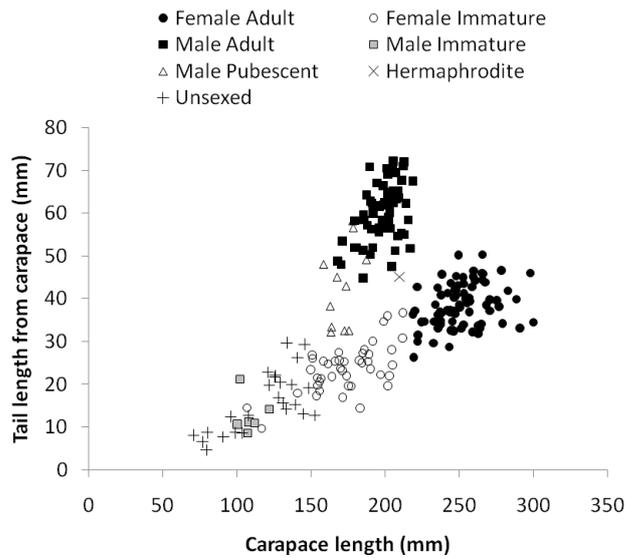
**Figure 4.6** Calculation of AS<sub>50</sub> values from laparoscopy data for adult male and female *Myuchelys bellii* from Bald Rock Creek (male logistic equation  $y = 1/(1+EXP(-1.7463*(CL-16.16)))$ ) and female logistic equation  $y = 1/(1+EXP(-21*(CL-21.5)))$ ). M and F correspond to the calculated AS<sub>50</sub> for each sex: M = 161.6 mm and F = 215 mm.



**Figure 4.7** Tail length from carapace and carapace length relationship based on laparoscopy examination for *M. bellii* ( $n = 52$ ). Measurements for recaptured immature individuals included.



**Figure 4.8** Graphs (a) females and (b) males are combined data from all three populations: Bald Rock Creek, Namoi and Gwydir River catchments. Graph (a) shows sexual maturity for all identified females using  $AS_{50}$  215 mm CL to distinguish between immature and adult; and graph (b) shows sexual maturity for all identified males using a combination of laparoscopy data,  $AS_{50}$  161.6 mm CL and evidence of secondary tail elongation (see Figure 4.7). Unsexed individuals ( $n = 27$ ) not shown.



**Figure 4.9** Tail length from carapace and carapace length relationship based on laparoscopy examination, AS<sub>50</sub> values and evidence of secondary tail elongation for *M. bellii* (n = 230).

### Sex Ratio

There are more adult males than females in the Gwydir and Bald Rock Creek populations (1:0.9 and 1:0.5), but there are nearly three times as many adult females than males in the Namoi River catchment (1:2.7) (Table 4.2). When sexed immature turtles are included, the sex ratio for all three populations has more females than males. Populations in the Gwydir and Bald Rock Creek have nearly equal numbers of males and females with 1:1.1 and 1:1.2 respectively. In contrast, the Namoi catchment sampled population has a heavy bias toward females of 1:2.8. The sex ratio for *M. bellii* across its range is 1:1.6 in favour of females (Table 4.2).

**Table 4.2** Male to female sex ratios of *M. bellii* populations in the Namoi and Gwydir Rivers and Bald Rock Creek. Included is an overall ratio for the combined populations.

	Gwydir	Namoi	Bald Rock Creek	Total
i) adults	1:0.9	1:2.7	1:0.5	1:1.2
ii) adults and sexed immature	1:1.1	1:2.8	1:1.2	1:1.6

### Age estimated from growth rings

Sixty two individuals, totalling 80 growth increments, from all three *M. bellii* populations, were used in developing a von Bertalanffy growth curve relationship between annual growth layers and straight carapace length (Figure 4.10). A hatchling

scale or first year growth layer was present in 46 individuals, absent in eight individuals and unknown for eight individuals. The growth pattern was best described by  $CL_t = 300.2 (1 - 0.911e^{-0.11t})$  for females, and  $CL_t = 218.9 (1 - 0.878e^{-0.20t})$  for males. Based on growth layers and the estimated  $AS_{50}$  values for males and females (Figure 4.6), males mature at 6.1 years and females 10.6 years (Figure 4.10).

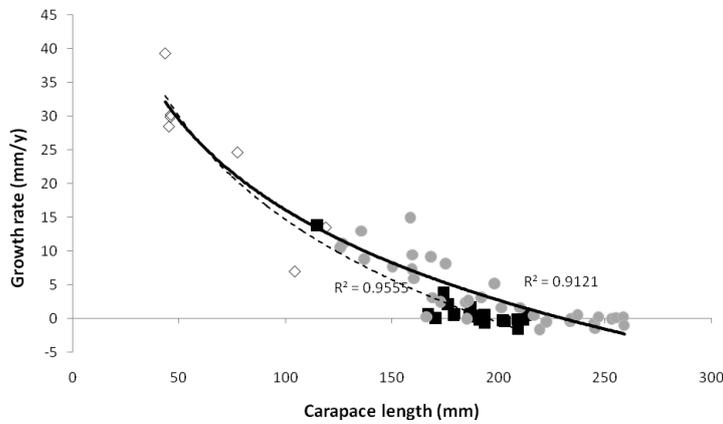


**Figure 4.10** Von Bertalanffy growth relationship between annuli and straight carapace length for immature females, males and unsexed immatures. All ages were indexed against a standardised hatching date of the 31<sup>st</sup> January for their first summer season. Thick solid line is the growth curve estimated for females and unsexed immatures,  $CL_t = 300.2 (1 - 0.911e^{-0.11t})$ , and the dashed line is the growth curve estimate for males and unsexed immatures,  $CL_t = 218.9 (1 - 0.878e^{-0.20t})$ . Grey circles represent females, black squares represent males and diamonds represent unsexed immatures.

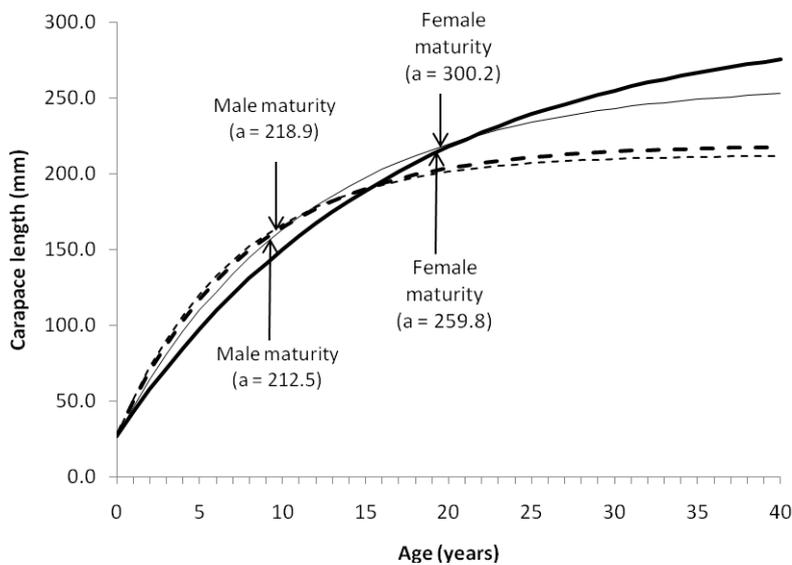
#### *Age estimated from recapture data*

Growth rates varied for male and female turtles with rapid growth of 28-39 mm in first year turtles reducing to <2 mm per year, or nil growth rates, after males and females reached approximately 180 mm and 220 mm carapace length respectively (Figure 4.11). A total of 42 growth increments were used for females and 28 growth increments for males with both growth models using the same unsexed immature dataset. The von Bertalanffy growth function was applied to females and males (Figure 4.12) using two fixed values for the parameter,  $a$  (Table 4.3). Using the male  $AS_{50}$  and female  $AS_{50}$  values (Figure 4.6), males mature at 9.25 y ( $a = 212.5$ ) and 9.5 y ( $a = 218.9$ ) and females mature at 18.8 y ( $a = 259.8$ ) and 19.5 y ( $a = 300.2$ ) years based on growth

increments from capture-recapture events (Figure 4.12). The von Bertalanffy curve fitted to growth increments predicts a lifespan for *M. bellii* of over 40 years (note: *x* axis not extended in graph, Figure 4.12).



**Figure 4.11** The relationship of growth rate *G* in mm/y to carapace length (GM, geometric mean size) fit to logarithmic least squares. Grey circles are females, black squares are males and diamonds are unsexed turtles. The solid line is females and unsexed, and the broken line is males and unsexed turtles.



**Figure 4.12** Faben’s von Bertalanffy growth curve relationship estimated using capture-recapture incremental growth data for turtles of unknown age. Thick solid line is the growth curve estimated for females and unsexed immatures,  $CL_t = 300.2 (1 - 0.911e^{-0.060t})$ , the thin solid line is females and unsexed immatures,  $CL_t = 259.8 (1 - 0.897e^{-0.088t})$ , thick dashed line is the growth curve estimate for males and unsexed immatures,  $CL_t = 218.9 (1 - 0.878e^{-0.127t})$ , and the dashed line is for males and unsexed immatures,  $CL_t = 212.5 (1 - 0.874e^{-0.140t})$ .

**Table 4.3** The Fabens modified von Bertalanffy growth function models for length-at-age growth rings and growth increments; *a*, asymptotic carapace length; *k*, growth parameter and estimated age at maturity.

von Bertalanffy Model	<i>a</i>	<i>k</i>	Age at maturity (years)
<i>Fabens using length-at-age growth rings</i>			
Females ( <i>n</i> = 71)	300.2 <sup>ii</sup>	0.110	10.6
Males ( <i>n</i> = 40)	218.9 <sup>ii</sup>	0.200	6.1
<i>Fabens using growth increments</i>			
Females – ( <i>n</i> = 42)	259.8 <sup>i</sup>	0.088	18.8
Females – ( <i>n</i> = 42)	300.2 <sup>ii</sup>	0.060	19.5
Males – ( <i>n</i> = 28)	212.5 <sup>i</sup>	0.140	9.25
Males – ( <i>n</i> = 28)	218.9 <sup>ii</sup>	0.127	9.5

i) maximum size in growth analysis; ii) maximum size caught in this study

### *Reproductive Biology*

Based on breeding success records encompassing 23 breeding seasons for six adult females spanning 2003-2010 in Bald Rock Creek, on average 78% of adult females ovulated in any one season (Table 4.4). The limited data indicate that the proportion of adult females breeding fluctuates between years: with perhaps all or most adult females breeding in some years, while in other years, a considerably lower proportion of the adult females may breed (Table 4.4). Females carrying hard shelled oviducal eggs were observed in November and December while soft shelled oviducal eggs were recorded in October and November. No females were observed with either hard shelled or soft shelled eggs in any other month during the survey period. Yet, one female was observed to have corpora lutea and no eggs on the 1<sup>st</sup> of October indicating it had already laid a clutch of eggs for that season. The observations indicate that the species lays only a single clutch of eggs per year.

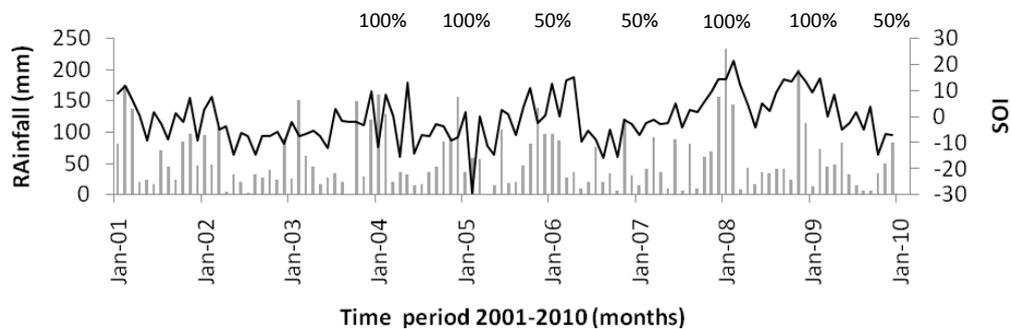
A disturbed natural nest of *M. bellii* was located on the banks of the MacDonald River at 'Watsons Creek' (30°41' 151°07'). This nest was located in loamy granite soil approximately eight metres from the water's edge on a relatively steep river bank. The nest had been dug up and appeared to have been predated on with scattered egg shells at its margins. The nest chamber was approximately 200 mm deep. No other natural *M. bellii* nests were located during this study.

**Table 4.4** Seasonal history (2003-2009) of follicle release for six female *M. bellii* from Bald Rock Creek based on laparoscopy examination of gonads. Y/N - observed follicle release. Annual proportion of adult females breeding was 0.78 (calculated across years).

Tag #	CL (mm)	Mass (g)	03/04	04/05	05/06	06/07	07/08	08/09	09/10
34001	246.3	1766		Y		Y			
34626*	233.8	1569		Y	N	N	Y	Y	
34733	245.2	1713			Y	N	Y	Y	N
34751	222.5	1255		Y					
34753	233.8	1541			N	Y	Y	Y	Y
34757	253.4	1926	Y	Y	Y		Y	Y	
Annual proportion breeding			1	1	0.5	0.5	1	1	0.5

\* First season of breeding was in 04/05.

Breeding data were plotted against the monthly rainfall recorded at Girraween National Park and the El Niño-Southern Oscillation (ENSO; Southern Oscillation Index: SOI) over the same period (Figure 4.13). There was no strong correlation with either La Niña (higher than average winter, spring and early summer rainfall) or El Niño (lower than average winter, spring and early summer rainfall) weather patterns.



**Figure 4.13** Percent annual breeding females compared to the average monthly (unweighted) Southern Oscillation Index (SOI) and monthly rainfall totals at Girraween National Park (#41454; Table 4.1). Solid line represents monthly SOI (-ve SOI indicates El Niño events and +ve SOI indicates La Niña events); grey histogram represents total rainfall (mm/month); and 100% or 50% represent recorded breeding females per summer period, marked to January each year (Table 4.4). Source: rainfall and SOI data were sourced from the Bureau of Meteorology ([www.bom.gov.au](http://www.bom.gov.au)).

Clutches taken from two females from Bald Rock Creek for artificial incubation were processed during 05/06 and 06/07 seasons. A clutch of 17 eggs was collected from the first female (245 mm CL; 1700 g) and a clutch of 18 eggs was collected from the second female (246 mm CL; 1785 g). The combined mean egg mass was  $8.6 \pm 0.1$  g,

with a mean maximum length of  $30.6 \pm 0.1$  mm and mean minimum width of  $21.9 \pm 0.1$  mm. 100% of the eggs in both clutches were fertile. Hatchlings emerged 51 and 49 days respectively after setting at  $29^\circ\text{C}$ . Hatchlings averaged  $26.7 \pm 0.3$  mm in carapace length and  $26.8 \pm 0.6$  mm in carapace width. These dimensions are similar to those reported elsewhere (Table 4.5). Hatchlings were later marked and released into micro-habitat of Bald Rock Creek in the vicinity of the respective females' capture sites.

*Myuchelys bellii* fecundity was calculated using the average clutch size of 18.3 eggs from data reported from the literature and from this study (excluding data given as a range, Table 4.5), a single clutch per season and the average proportion of females breeding annually (0.78, Table 4.4). Consequently, the best estimate of *M. bellii* fecundity is  $18.3 \times 1 \times 0.78 = 14.3$  eggs per adult female.

All adult males that were subject to laparoscopic examination ( $n = 18$  with a total number of annual examination = 24) were found to be in spermatogenesis during the months of September, October and November. No adult male was found to be non-breeding during these months. These observations include four adult males that were recorded breeding in successive years.

**Table 4.5** Comparison of clutch, egg and hatchling sizes, and incubation data for *Myuchelys* species. Mass is given in grams, measurements in mm and temperature in degrees Celsius. Averages for egg and hatchling data reported, except where a range was stated by previous studies.

Species	Eggs				Hatchlings			Incubation	
	Clutch	Length	Width	Mass	Length	Width	Mass	Days	Temp
<i>M. bellii</i> <sup>1</sup>	17	30.1	22.2	9	25.9	27.1		51	29
<i>M. bellii</i> <sup>1</sup>	18	31.1	21.6	8.3	27.5	26.6		49	29
<i>M. bellii</i> <sup>2</sup>	20	30.3	21.5	7.2	28.5-30.5	22.5-28	3.7-4.7	48	28-29
<i>M. bellii</i> <sup>2</sup>	8-23	30-34	20.5-23	9.3	30	28	5.5-6	80	27
<i>M. latisternum</i> <sup>3</sup>	17	26	24	12				60	30
<i>M. latisternum</i> <sup>2</sup>		33.5-35	22-23		32.5	22.5	7.6		
<i>M. latisternum</i> <sup>4</sup>	18	36.7	23	11.5					
<i>M. latisternum</i> <sup>4</sup>	18	34.6	24.1	11.8					
<i>M. georgesi</i> <sup>2</sup>	10-15	29-33.6	21-21.5	7.5-8.5	29.3-31	28.3-32	5.5-6.1	72	27
<i>M. georgesi</i> <sup>5</sup>	13.5								
<i>M. purvisi</i> <sup>6</sup>	7	30.6-33.5	22.5-24		28	26	4.5	59	26-27

Ref: 1 – this study; 2 – Cann (1998); 3 – Legler and Cann (1980); 4 – D. Limpus, unpublished data; 5 – Blamires *et al.* 2005; 6 – Dorrian (1986)

*Population Estimate*

The population size of *M. bellii*, including adults and immature, in Bald Rock Creek was  $319 \pm 89$  (95% CI) (Table 4.6). Annual survival for adults and immature turtles was 0.95 (with a 0.83-0.99, 95% CI). Due to the small dataset, data were pooled across all sites within Bald Rock Creek where *M. bellii* were captured and for each calendar year (winter to winter, seven periods). For the retained model ( $\phi(\cdot)p(c^*t)$ ), the survival of mature and immature turtles remained constant and capture probabilities were time dependent. Capture probabilities ranged for adults from 0.13-0.57 and for immature from 0.07-0.54. The low probabilities possibly reflect differences in capture effort between sampling periods. Some individuals were captured 10 or more times during the survey period (2002-2009), while over half (56%) were caught on more than one occasion.

**Table 4.6** Candidate models for the estimate of apparent survival and capture probability of grouped adult males and females, and immature *M. bellii* in Bald Rock Creek, Queensland (Ness = 132, chat = 1.23), where t = time, phi = survivability, p = capture rate. The selected model  $\phi(\cdot)p(c^*t)$  had the highest AICc Weights score.

Model	AICc	Delta AICc	AICc Weights	Model Likelihood	Num. Par	Deviance (95% CI)
$\phi(\cdot)p(c^*t)$	319.14	0	0.73455	1	13	88.7
$\phi(\cdot)p(t)$	321.37	2.23	0.24138	0.3286	14	88.4
$\phi(\cdot)p(\cdot)$ adults	327.01	7.87	0.01434	0.0195	9	106.1
$\phi(\cdot)p(t)$ immature						
$\phi(\cdot)p(t)$ adult	328.95	9.81	0.00544	0.0074	17	88.2
$\phi(t)p(t)$ immature						
$\phi(\cdot)p(\cdot)$	329.67	10.53	0.0038	0.0052	4	120
$\phi(\cdot)p(\cdot)$ adults	333.96	14.82	0.00044	0.0006	12	105.9
$\phi(t)p(t)$ immature						
$\phi(t)p(t)$	338.93	19.79	0.00004	0.0001	21	87.1
$\phi(t)p(\cdot)$	342.38	23.24	0.00001	0	14	109.4

*General observations on turtle diet and population health*

Faeces examination by this study reveal a varied diet consisting of aquatic plants (semi-emergent and submerged), filamentous green algae, freshwater sponge, terrestrial fruits overhanging the stream or floating in the water (including exotic blackberries), large numbers of emerging adults of an unidentified aquatic insect, shell fragments of *Euastacus suttoni* crayfish, carrion such as terrestrial insects in the water and sometimes

large amounts of sediment and terrestrial leaves. Hence, no indication from these observations would indicate a selective dietary requirement for *M. bellii*.

Ten per cent ( $n = 157$ ) of turtles sampled in the Gwydir and Namoi River catchments in NSW are suffering from an unknown eye disease which can cause blindness in adults. Only one adult male from the Gwydir River was found to have a cataract on its left eye while the remaining affected turtles were from a single location in the Namoi River catchment. On the MacDonal River at 'Watsons Creek' ( $30^{\circ}41' 151^{\circ}7'$ ), 37% of turtles caught were inflicted with cataracts, and of these, eight had cataracts in both eyes and seven in a single eye. Though untested, this affliction didn't seem to hinder their ability to find the baited trap and they all appeared to be otherwise healthy. In addition to cataracts, deaths of turtles from recreational fishing were observed in NSW.

Most turtles across all three populations exhibited no noticeable shell damage (75%) while a quarter of all sampled had one or more chipped marginal scutes, or damage to the costal or vertebral scutes of the carapace. Specifically, the per cent of individuals having some form of (mostly minor) shell damage in the Gwydir River, Namoi River and Bald Rock Creek populations was 21%, 15% and 23% respectively. Additionally, two adult females were missing the ends of their tails (approximately 20 mm missing) and one adult female was missing the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> digits on one of its rear foot. No other deformities to limbs were observed. One adult male was also observed to have the skin on all four soles of its feet worn smooth (about 10 mm diameter) exposing the dermis layer of the skin. It also had a central concave abrasion along the length of its plastron. This turtle was last captured 12 months prior to, and eight kilometres downstream, of where it was recaptured in Bald Rock Creek. These abrasions were most likely caused by upstream migration over flowing granite bed rock and granite cascades to reach the upstream large permanent waterhole. In subsequent recaptures, these lesions and abrasions were healed.

## **Discussion**

### *Reproductive biology and ecology*

Most freshwater turtles tend to share common features of low annual fecundity, low egg and hatchling survival, and a long lifespan due to high adult survival rates (type III

survivorship) (Iverson 1991*b*; Heppell 1998). Other studies have noted that annual survivorship in adults and delayed sexual maturity is correlated across different animal taxa (Shine and Iverson 1995). Not surprisingly then, this study demonstrates life history traits of *M. bellii* are similar to many other turtles by having delayed age at first breeding, low reproductive effort and high survivorship with a predicted lifespan of over 40 years. A general theory of life history evolution of turtles and other reptiles predicts that size at sexual maturity is typically around 70% of maximum size (Shine and Iverson 1995). Consistent with this theory, female and male *M. bellii* generally reach maturity at around 71% and 76% of maximum size respectively.

Age at sexual maturity is commonly reported for freshwater, terrestrial and marine turtles using growth curves developed from animals of 'known age' using growth rings (annuli) or growth increments from turtles of unknown age from recapture data. A recent review of the literature that reported turtles and tortoises ages from growth rings revealed a high number of studies (>100) that lacked a calibration of known actual ages of turtles to the number of growth rings deposited per year (Wilson *et al.* 2003). Wilson *et al.* (2003) therefore join other authors (e.g., Litzgus and Brooks 1998) in cautioning that this method requires the relationship between age and growth rings to be tested before its results can be applied with confidence. In an assessment of growth rings in an Australian chelid, *Emydura macquarii*, Spencer (2002*b*) recaptured released hatchlings of a known age and found that there was a 17% chance of error in growth ring counts for one year old turtles increasing to a 36% error for aging turtles at two years of age. Based on these findings, Spencer (2002*b*) argued that growth rings in this species were laid down too inconsistently to be of any value. Yet growth rings for three other Australian chelids were determined to be accurate to 4-6 years of age for *Pseudemydura umbrina*, 3 years for *Chelodina rugosa* and 9 years for *Elseya dentata* (Burbidge 1981; Kennett 1996). Furthermore, another review of aging turtles using growth rings argued this method's value for aging immature turtles (Germano and Bury 1998).

The *M. bellii* hatchlings of known age released into Bald Rock Creek in February 2006 and 2007 as part of this study were not recaptured during the following spring / summer seasons. Consequently, a direct relationship between growth rings and age of immature *M. bellii* was not ascertained. Nevertheless, based on the growth rates of 40 mm/y (mean) for first year *Emydura macquarii* (Spencer 2002*b*) and estimated growth rates of

28-39 mm/y for first year *M. bellii* turtles (this study), age derived via growth ring counts are reflective of actual age for juveniles up to about 100 mm CL or 4-5 years of age. For individuals greater than this size the relationship is less certain. However, a single growth ring (determined from photographs) was deposited during each 12 month period over three consecutive summers for an immature female turtle recaptured within days of the past year's capture date. This individual was assessed to have 6, 7 and 8 growth rings in consecutive years (CL 151.5 – 166.3 mm).

The von Bertalanffy growth curve model applied using growth ring data appears to overestimate growth rates of larger females and males with a poor fit to data after about 160 mm and 140 mm for females and males respectively (Figure 4.10). This result is possibly due to a lack of adult turtles included in the analysis (no adult female had a complete set of growth rings). Thus, the estimated age at sexual maturity based on growth rings for males (6.1 years) and females (10.6 years) are likely underestimates of true age at maturity (Spencer 2002b).

In contrast, the von Bertalanffy growth model using growth increment data from recaptured turtles estimated age at sexual maturity for *M. bellii* to be nearly 10 years for males and approaching 20 years for females. I compared the growth increment analyses derived from using the growth parameter,  $a$ , fixed to the maximum carapace length for male and female turtles recaptured to that derived when  $a$  was fixed to the absolute maximum size for females and males recorded in this study (Table 4.3). In both cases, using the maximum size for male and females recorded in this study for  $a$  estimated an older age at sexual maturity based on the calculated  $AS_{50}$  values. I recommend using the younger age at maturity estimates as few individuals are likely to reach the absolute maximum size reported here.

The von Bertalanffy growth model fitted to growth increment data is considered to have accurately predicted patterns of growth, and hence, age for other species of turtles (Litzgus and Brooks 1998b; Spencer 2002b; Blamires *et al.* 2005; Martins and Souza 2008). Spencer (2002b) also warned that growth rates for small immature turtles are required in any model to accurately predict patterns of growth in larger animals. I found that the von Bertalanffy model described well the growth observed in *M. bellii* populations using growth increment data from recaptures, which included small

immature turtles. For this reason, I believe that age at sexual maturity estimated from recapture growth increment data to be the more accurate assessment of growth patterns for *M. bellii* turtles. A more definitive relationship between growth rings and age (i.e., annual deposits) in future estimates of age at sexual maturity may be determined through recapture of released known age individuals.

The direct relationship of increasing clutch size and egg mass with increasing body size is documented for a number of freshwater turtles (e.g., Georges 1985; Kennett 1999; Zuffi *et al.* 1999), tortoises (e.g., Iverson 1991a; Mueller *et al.* 1998) and marine turtles (e.g., Van Burskirk and Crowder 1994). The small sample of clutches in this study prevented this relationship being tested for *M. bellii*. However if this assumption were true for *M. bellii*, 14.3 eggs per adult female would be an underestimate of fecundity because the average size of adult females in this study was 250 mm CL and a mass of 2018 g ( $n = 84$ ), and the two gravid females were 245 and 246 mm CL and 1700 and 1785 g respectively.

A comparison of reproductive outputs of clutch sizes and frequency of studied Australian chelids is provided by Kennett (1999). From these comparisons, my data show *M. bellii* has one of the lowest reproductive outputs of any Australian chelid with 14.3 eggs per female. The only other species with lower reproductive potential are *Pseudemydura umbrina* (3-4 eggs, Kuchling and Bradshaw 1993), *Elseya dentata* (8 eggs, Kennett 1999), *Chelodina steindachneri* (7-8 eggs, Kuchling 1988) and *C. oblonga* (12 eggs, Clay 1981, In: Kennett 1999). There is no clear comparison for congeners of *M. bellii* with little data on reproductive potential presented in the literature (see Table 4.6).

Annual breeding rates for Australian freshwater turtles have been reported at 100% for *C. rugosa* and *E. dentata*, 97% for *E. albagula* (Burnett R.), and 100% for *M. latisternum* and *Em. macquarii* (Burnett R.) (Kennett 1999; Hamann *et al.* 2007; Hamann *et al.* 2008). In contrast, only 78% of adult female *M. bellii* on average were recorded breeding in any one year in this study. The small number of females examined in this study means that confidence boundaries (not estimated) on this information would be low. Climatic conditions are thought to be implicated in highly variable breeding rates for Australian chelids with periods of drought preceding a nesting season

causing a decline in reproductive output (Limpus unpublished data). However, when *M. bellii* breeding data were compared to monthly rainfall recorded at Girraween and the El Niño-Southern Oscillation (ENSO) over the same period, there was no strong correlation evident with either La Niña or El Niño weather patterns. If a greater number of females were to be sampled in future studies, a relationship between breeding rates and climatic conditions may become apparent. Alternatively, other as yet unquantified environmental factors such as temporal variability in stream hydrology may have a greater affect on reproductive effort in any one year.

This study shows that the annual nesting season for *M. bellii* was September through to January each year which agree with earlier observations by Cann (1998). Not unlike other species in *Myuchelys* (Georges and Adams 1996; Cann 1998; Allanson and Georges 1999), *M. bellii* is omnivorous with a tendency toward being herbivorous (Cann 1998). Faeces examination by Cann (1998) and this study reveal a varied diet for *M. bellii* consisting of terrestrial leaves and fruits, terrestrial and aquatic insects, crayfish, algae and aquatic plants.

#### *Conservation threats*

The three disjoint populations of *M. bellii* have very restricted distributions with the Queensland population occurring in an 8 km stretch of Bald Rock Creek and the two NSW populations occurring in <100 km of stream length in each of the Namoi and Gwydir River catchments. All three populations are subjected to ongoing threatening processes including continued loss of riparian vegetation through grazing and clearing for agricultural development and loss of in-stream habitat from water infrastructure (impoundments). Ten per cent of turtles in NSW are suffering from an unknown eye disease which can cause blindness in adults. In addition, deaths of turtles from recreational fishing were observed in NSW. I also suspect probable predation of juvenile *M. bellii* from introduced fishes (e.g., redfin perch, trout spp.) and artificially translocated or stocked native fish predators such as the Murray cod and golden perch. Habitat modification from goldfish is an additional pressure to *M. bellii* survival in Bald Rock Creek. Predation by foxes on *M. bellii* nests is suspected to be high because of significant predation rates (>96%) reported for other turtle populations in the Murray-Darling Basin and adjoining catchments (Thompson 1983; Spencer 2002a). The effects

of climate change through prolonged drought or increasing ambient water temperatures is a gap in current understanding for this species. The effects of these threatening processes are yet to be determined, but are thought to be significant factors in the long-term survival and recovery of *M. bellii* populations.

### *Conclusion*

For the first time in this paper, the life history traits of the nationally vulnerable western sawshelled turtle *M. bellii* have been quantified. The molecular and morphological evidence of from Chapters 2 and 3 do not support the presence of a cryptic species within *M. bellii*. Consequently, the three major populations of *M. bellii* from the Gwydir, Namoi and Border Rivers need to be managed as a single species entity. The relatively small size and extent of each population also means that *M. bellii* is certainly worthy of its national vulnerable status and international endangered status. Of particular management concern for *M. bellii*, is the long term conservation of the small isolated Queensland population. Acknowledging the limited information developed from the present study, it is recommended that there be continuing research directed towards improving the management and conservation of this threatened and endemic turtle. In particular, research effort should be given to better understanding the current population size and distribution in NSW and to investigate possible predation impacts from feral animals on annual recruitment. Additionally, the Queensland population should be subjected to further genetic investigations to test for possible inbreeding within this small population. Lastly, predictions with respect to climate change and their potential impacts on the viability of the three disjoint populations into the future warrant attention.

**Chapter 5. Seasonal and diel dive performance and behavioural ecology of the bimodally respiring freshwater turtle *Myuchelys bellii* of eastern Australia.**

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Picture: Queensland Parks and Wildlife Service ranger John Cowburn releasing a female *Myuchelys bellii* into Bald Rock Creek at Girraween National Park with a time-depth recorder and radio transmitter attached to its carapace. Photo by Darren Fielder.

**Chapter 5. Seasonal and diel dive performance and behavioural ecology of the bimodally respiring freshwater turtle *Myuchelys bellii* of eastern Australia.**

**Abstract**

Freshwater turtles have an extraordinary physiological ability to endure dive times that can range from days to months by using aquatic respiration. In cryptodires (e.g., white-lipped mud turtle *Kinosternon leucostomum*) aquatic respiration is via buccal or cutaneous routes whereas in pleurodires (e.g., Fitzroy River turtle *Rheodytes leukops*), it is achieved primarily via specialized cloacal bursae. This study records the voluntary diving performance of the western sawshelled turtle *Myuchelys bellii* in Bald Rock Creek from the temperate zone of the Murray-Darling Basin of Australia. Despite *M. bellii*'s moderately specialized cloacal bursae morphology, it is second only to *R. leukops* in having the longest dive recorded for a freely diving freshwater or marine turtle at over 15 days. This is attributed to its ability to maintain aerobic dives via its cloacal bursae and low water temperatures in winter. *Myuchelys bellii* seasonal and diel diving performance, including its crepuscular habit, is comparable to *R. leukops* and *Eelseya albagula*. This study also recorded the first aquatic hibernation at depth (> 3 m) for any freshwater turtle; and only the second pleurodire to demonstrate aquatic hibernation as an overwintering strategy. Observed thermoregulation behavior in *M. bellii* is believed to provide multiple life history benefits.

## Introduction

The capacity of freshwater turtles to remain fully submerged in water for many hours to sometimes months, far eclipsing that of all other freely diving air-breathing vertebrates including sea turtles, mammals and birds, is now well established (Ultsch *et al.* 1984). They achieve these extraordinary dive lengths by means of several specialised adaptations such as depression of metabolic rates, tolerance of severe lactic acidosis through changes to their blood plasma, lactate uptake by skeletal bone (Jackson *et al.* 2007), reduced heart rate including bradycardia and aquatic respiration through cloacal, buccal (throat lining) or cutaneous (skin) gas exchange. Though, not all of these adaptations are equally available to all freshwater turtles (Ultsch *et al.* 1984). These remarkable adaptations have been well studied for many northern hemisphere criptodiran species (sea turtles, tortoises, and freshwater turtles) (e.g., Jackson *et al.* 1984; Ultsch *et al.* 1984; Ultsch 1988; Stone *et al.* 1992; Saunders *et al.* 2000; Reese *et al.* 2002; Maginniss *et al.* 2004; Jackson *et al.* 2007) and more recently for several southern hemisphere pleurodiran (side-necked turtles) species shown to have bimodal respiration capabilities including the Fitzroy River turtle *Rheodytes leukops*, the Mary River turtle *Elusor macrurus*, the white-throated snapping turtle *Elseya albagula*, the Murray turtle *Emydura macquarii*, the common sawshelled turtle *Myuchelys latisternum* and Georges helmeted turtle *M. georgesi* (King and Heatwole 1994a; King and Heatwole 1994b; FitzGibbon 1998; Gordos and Franklin 2002; Priest and Franklin 2002; Gordos *et al.* 2003b; Gordos *et al.* 2004; Mathie and Franklin 2006; Clark *et al.* 2008; Storey *et al.* 2008; Clark *et al.* 2008; Clark *et al.* 2009; FitzGibbon and Franklin 2010).

Several criptodirans such as the painted turtle, *Chrysemys picta*, rely on plasma ion changes in their blood to buffer the onset of anoxic acidosis to survive anoxic conditions for several months during winter hibernation (Ultsch *et al.* 1985; Jackson 2000). The Central American bimodal respiring species of the Mexican giant musk turtle, *Staurotypus triporcatus*, and the white-lipped mud turtle, *Kinosternon leucostomum*, utilise cutaneous gas exchange to extend their dive durations (Bagatto *et al.* 1997). In contrast, while many criptodiran species possess cloacal bursae, only the bimodally respiring criptodiran softshelled turtle *Apalone spinifera* is thought to use its cloaca (which lack cloacal bursae) for part of its aquatic oxygen uptake (Peterson and Greenshields 2001).

For pleurodires, however, bimodal respiration is predominantly via the cloacal bursae (Peterson and Greenshields 2001) and is most developed in the short-necked genera (Legler and Georges 1993b). Reliance on aquatic respiration for total oxygen consumption ( $\text{VO}_2$ ) in adults of species (at water temperature of 20°C) varies from 10% in *Em. macquarii* through to a maximum of 70% (mean 41.5%) in *R. leukops* (Priest 1997; Priest and Franklin 2002) with a number of species abilities falling in between (Clark *et al.* 2008; FitzGibbon and Franklin 2010). Cloacal respiration accounted for 48% and 32.6% of total aquatic oxygen uptake for *E. albagula* and *M. georgesi* respectively (King and Heatwole 1994b; FitzGibbon and Franklin 2010). The cloacal bursae of *M. georgesi* were found to contain many vascularised branched papillary processes protruding into the bursae lumen which were estimated to increase the surface area for gas exchange by about 200-fold (King and Heatwole 1994b). They also observed significant oxygen uptake in this species via the buccopharyngeal cavity (49%) and cutaneous surface areas (18%) (King and Heatwole 1994b). Surprisingly given these proportions, they found no specialised respiratory features or filamentous processes in the buccopharyngeal cavity to account for such a high value, and consequently considered them to be overestimates in relation to the cloaca (King and Heatwole 1994a).

The bench mark for the longest freely diving chelonian is *Rheodytes leukops* which has had its bimodal respiration capabilities through its cloacal bursae extensively studied (Priest 1997; Tucker *et al.* 2001; Gordos and Franklin 2002; Priest and Franklin 2002; Gordos *et al.* 2003b; Gordos *et al.* 2004; Clark *et al.* 2008). Gordos *et al.* (2003b) examined the diving performance of *Rheodytes* across the four seasons and the longest recorded dive was 21 days in winter (two turtles stayed submerged for the entire logging period) and 2.4 days in summer. These dive durations were attributed to its highly modified, vascularised branching papillae that line its cloacal bursae (Priest 1997). Extra pulmonary respiratory sites used for the uptake of aquatic  $\text{O}_2$  including the cloacal bursae are thought to also facilitate the diffusion of  $\text{CO}_2$  reducing the negative effects of hypercapnia in submerged turtles (Jackson 1976; Jackson *et al.* 1976; Crocker *et al.* 1999) and provide a pathway for nitrogen excretion, in addition to acid-base balance and ion regulation (Dunson 1967; Jeffree and Jones 1992). These physiological

functions of the cloacal bursae allowed *Rheodytes* to undertake prolonged dives while remaining aerobic (Priest 1997; Gordos *et al.* 2003b; Gordos *et al.* 2004).

In addition to the necessary physiology, the diving performance of a turtle is also determined by their behavioural response to seasonal fluctuating environmental conditions. For instance, as water temperatures transition from summer high average temperatures to winter low averages resulting in increased aquatic PO<sub>2</sub> availability and reduced turtle metabolic activity, there is a corresponding increase in reliance on aquatic respiration (Gatten 1980; Ultsch and Jackson 1982; Ultsch *et al.* 1985; King and Heatwole 1994b; Gordos *et al.* 2003b; Gordos *et al.* 2007). Consequently, bimodal respiring species switch from obligate air breathers in summer, due to increased metabolic activity rates, to facultative air breathers in winter (Gordos *et al.* 2003b). Similarly, diel trends in surfacing and diving behaviour can vary daily, and among seasons, depending on whether a species is predominantly nocturnal, diurnal or crepuscular in their habits (Gordos and Franklin 2002; Gordos *et al.* 2003a; Gordos *et al.* 2007).

The aim of this study was to investigate the seasonal and diel diving performance and behavioural ecology of the pleurodiran western sawshelled turtle *Myuchelys bellii* (Gray 1844; Cann 1998; Thomson and Georges 2009) in the wild, and determine its level of reliance on aquatic respiration via the cloacal bursae. The results are the first of this kind for a temperate zone pleurodiran. The morphology of the cloacal bursae of *M. bellii* is also described and compared to other species of turtle, and the behavioural characteristics of thermoregulation among seasons, and aquatic hibernation as a life strategy, are also discussed.

## **Methods and materials**

### *Study Area*

Dive investigations were conducted from Austral spring in 2006 to summer 2008 in two connected permanent pools of Bald Rock Creek in Girraween National Park, eastern Australia (28°49'60"S 151°55'60"E). Each pool was about 700 m in length and 10-20 m wide and generally 0.5 to 4 m deep. Ambient water temperature in one of the study pools was measured every 15 minutes at 30 cm, 1 m and 2 m depth intervals using Thermotron iButton technology ( $\pm 0.5^{\circ}\text{C}$ ) for >30 days per season for the duration of

the dive investigations. Water temperature was also measured every two minutes for the duration of the deployment directly from time-depth recorders (TDRs;  $\pm 0.1^\circ\text{C}$ ) attached to individual turtles. Aquatic  $\text{PO}_2$  was not measured. However,  $\text{PO}_2$  was considered to range within normoxic conditions because the upstream catchment of Bald Rock Creek is in a natural state and the two pool study sites experienced base flows for most of the deployment periods.

The climate at Girraween National Park is temperate with cold dry winters (April to September) and warm wet summers (November to February). On average, 793 mm of rain falls each year. Daily air temperatures for July range from a mean minimum of  $1^\circ\text{C}$  and a daily mean maximum of  $14.8^\circ\text{C}$ . For summer the mean minimum and maximum for January are  $15.2$  and  $26.4^\circ\text{C}$  respectively (Source: Australian Bureau of Meteorology, October 2010).

#### *Capture and handling*

Turtles were either trapped using modified rectangular collapsible crab traps, or caught by hand and snorkel (Hamann *et al.* 2007). Turtles were generally processed and returned to the place of capture within 24 hours of being caught, or otherwise held in polyethylene tubs of water to prevent desiccation until their release ( $< 72$  hours). Upon capture, the body mass, straight line carapace (CL), sex and sexual maturity (determined by laparoscopy) were recorded, and marginal scutes were notched and foot tags were applied to identify individuals in the study (Table 5.1) (Limpus *et al.* 2002; Hamann *et al.* 2007). One immature female (CL 210 mm) was used in the investigation and all others were adults.

**Table 5.1** Summary of biological information, duration of data logging and number of dives analysed for tagged turtles (values are given as mean  $\pm$  SE).

	No. of Turtles (males : females)	Mass (g) (range)	Straight carapace length (mm)	Deployment period	Logging duration (days)	No. of dives analysed
Autumn	3 (1:2)	$1202 \pm 519$ (835 – 1569)	$212 \pm 30$	2 March – 1 April 2007	$28 \pm 2$	$1264 \pm 271$
Winter	2 (1:1)	$1225 \pm 451$ (835 – 1719)	$213 \pm 28$	6 July – 5 August 2007	30.3	$24 \pm 8.5$
Spring	6 (3:3)	$1156 \pm 400$ (735 – 1719)	$211 \pm 23$	23 October – 22 November 2006	30.3	$1213 \pm 193$

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Summer	4 (2:2)	1154 ± 395 (835 -1719)	211 ± 24	20 December 2006 – 8 February 2007; 12 January – 5 February 2008	34 ± 11.3	2490 ± 984
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Attached to each turtle's post marginal carapace scutes was a pressure-sensitive time-depth recorder (TDR) (*DST milli*, 87100 measurements, 38 x 13 mm cylindrical capsule, mass of 9.2 g in air and 5 g in water, ± 4 cm depth accuracy, ± 0.1 °C accuracy; Star-Oddi, Iceland) and a single staged radio transmitter (40 x 15 x 15 mm, 150 mm whip antennae, Titley Electronics, Ballina, New South Wales, Australia). The TDR and radio transmitter were fastened to the shell (either side of the tail) using stainless steel bolts or plastic cable ties. The tags were then covered in a flexible hardening epoxy resin which was smoothed along the carapace to ensure that it did not pose a hazard to turtle movement or result in skin abrasion to the rear legs or tail. No TDR or transmitter came off a turtle's shell during the study. Unfortunately, all radio tags (including replacement tags) failed to transmit after 3-6 months of deployment and recapture then relied on chance trapping or snorkel capture.

The TDRs were programmed to start recording a minimum of five days after the turtle's release date to allow dive behaviour to stabilise after handling (thought to be less than 72 hrs) (Gordos and Franklin 2002). Once enabled, the TDR recorded temperature every two minutes and pressure every 40 seconds for a maximum period of 31 days. Following completion of each TDR sampling period the turtles were recaptured and the data downloaded, and the TDR was reset for the next seasons recording.

The analysis of TDR data follows closely that of Gordos *et al.* (2003b) including the use of a custom built program (M. A. Gordos) and applying the same assumptions. These assumptions incorporated setting a 40 cm dive threshold based on the maximum length of a turtles nares to the TDR (<30 cm with neck extended) and allowing for a 10 cm fluctuation in TDR measurements over a deployment period. This meant that any time spent above 40 cm water depth was considered to be a surfacing event and any time spent below this threshold was deemed to be a dive. An additional stipulation was that dives with a maximum depth of less than 45 cm were not scored but included in previous surfacing periods because of the inherent TDR manufacturer precision of ±4 cm.

The custom built program initially analysed the mean, maximum, frequency and proportion of time spent by a turtle in diving and surfacing events. The mean and maximum depth, and number of activity counts were also analysed. Activity counts were based on a  $\pm 6$  cm movement in the vertical plane, as per Gordos *et al.* (2003b). In the same way, seasonal mean activity counts and mean number of dives per day were averaged using daily totals starting at midnight each day (Gordos *et al.* 2003b). For diel analyses, individual *M. bellii* dive profiles of dive duration, surfacing events, water temperature, dive depth and activity counts were averaged across the total deployment period per hour. Each day was divided into five periods (T1, T2, T3, T4 and T5) for analysis (Table 5.2). These periods have been previously used for other Australian freshwater turtle investigations (Gordos and Franklin 2002; Gordos *et al.* 2003a; Gordos *et al.* 2007) and considered to be relevant for this study also. Dawn and dusk were determined to be the first and last two hours, respectively, of daylight each day.

**Table 5.2** Time periods for investigating diel trends in *M. bellii*.

Season	Night 1 (T1)	Dawn (T2)	Day (T3)	Dusk (T4)	Night 2 (T5)
Autumn	0000 – 0500	0500 – 0700	0700 – 1700	1700 – 1900	1900 – 2400
Winter	0000 – 0600	0600 – 0800	0800 – 1600	1600 – 1800	1800 – 2400
Spring	0000 – 0500	0500 – 0700	0700 – 1700	1700 – 1900	1900 – 2400
Summer	0000 – 0500	0500 – 0700	0700 – 1700	1700 – 1900	1900 – 2400

#### *Seasonal and diel trend data analysis*

A one-way ANOVA was used to determine significant values between seasons. In instances where assumptions of normality and equal variances were not met, a Kruskal-Wallis one-way ANOVA on ranks was employed. Following a significant relationship ( $P < 0.05$ ), a series of comparisons was undertaken using student's *t* test (or where assumptions of normality were not met, a Wilcoxon ranked sum test) to elucidate where the differences occurred. A MANOVA of diel trends between the five periods of night, dusk, dawn and day was applied for between and among season analysis. Following a significant relationship, similar comparisons were undertaken to determine differences using planned comparisons of a) dawn vs. dusk (T2 vs. T4), b) dawn/dusk vs. day (T2 and T4 vs. T3), c) daylight vs. night (T2, T3 and T4 vs. T1 and T5) and day vs. night (T3 vs. T1 and T5). Hourly dive and surfacing proportions were arcsine transformed ( $p'$

= arcsine ( $\sqrt{p}$ ) to ensure data were normally distributed before applying the above analyses (see Gordos *et al.* 2007). No comparison between the sexes was attempted due to the small sample size. All results are presented as mean  $\pm$  standard error (SE) unless otherwise stated.

#### *Cloacal bursae morphology investigations*

The cloacal bursae of *M. bellii* was photographed using a digital camera mounted on a laparoscopic probe. An adult female turtle (tag number 34733; 245 mm CL and 1700 g mass) was upended while securely attached to a flat board and the cloacal bursae cavity was filled with clean water using a small funnel. Photographs of the specialised cloacal bursae papillae processes and the bursae cavity were then taken.

## Results

### *Seasonal temperature*

Water temperatures in Bald Rock Creek range seasonally between 5 and 30 °C, with average temperatures provided in Table 5.3. Mean water temperature in winter was 15 °C cooler than the summer average with autumn and spring averages falling in between the other two seasons.

**Table 5.3** Seasonal water temperatures for Bald Rock Creek.

	Water temp. <sup>1</sup> (°C) depth			Water temp. <sup>2</sup> (°C) recorded From TDR
	30 cm	1 m	2 m	
Autumn	21.7 $\pm$ 0.02	20.6 $\pm$ 0.02		19.8 $\pm$ 0.3
Winter	8.6 $\pm$ 0.02			7.1 $\pm$ 0.06
Spring	18.9 $\pm$ 0.03	18.1 $\pm$ 0.03	17.6 $\pm$ 0.02	19 $\pm$ 0.3
Summer	23.2 $\pm$ 0.04	20.1 $\pm$ 0.02	19.7 $\pm$ 0.02	21.9 $\pm$ 0.5

<sup>1</sup> iButton technology for >30 days per season

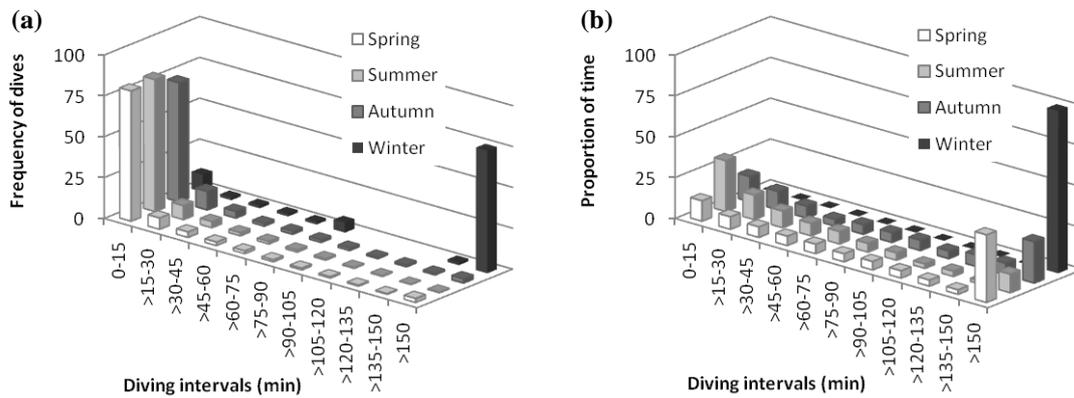
<sup>2</sup> averaged from individual turtles TDR deployment period

### *Seasonal diving performance*

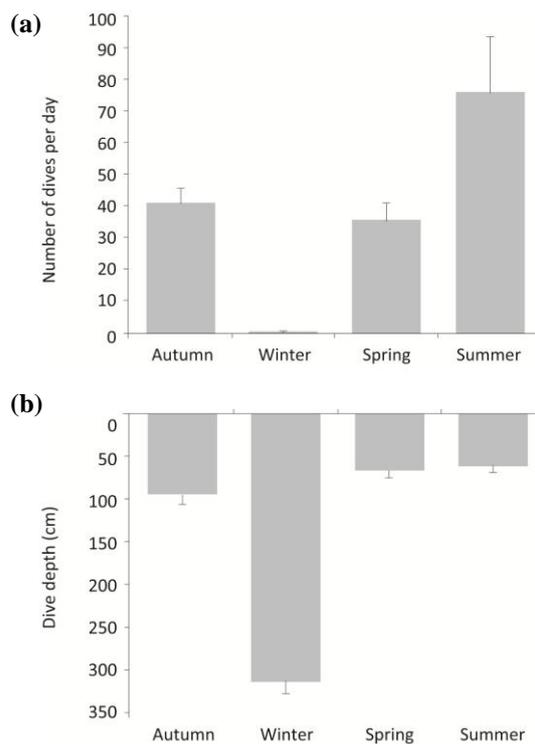
Most dives in spring, summer and autumn were of short duration with dives <15 min accounting for 73-81% of total submersions (Figure 5.1a). Dives >2.5 h during these seasons were relatively few in number representing only a small percentage of dives in spring (<3%), autumn (<3%) and summer (<1%). In contrast, only about 10  $\pm$  0.5% of dives in winter were <15 min duration, with a majority of dives (75  $\pm$  8.3%) in excess of

2.5 h. There was a similar disparity between winter and the other seasons when assessing the proportion of time spent in submergence within each dive interval analysed (Figure 5.1b). Nearly the entire submergence time ( $99.3 \pm 0.5\%$ ) in winter was spent in dives  $>2.5$  h, while in summer only  $11.3 \pm 5\%$  was spent submerged  $>2.5$  h. Dives  $>2.5$  h in spring and autumn accounted for  $41.3 \pm 8.5\%$  and  $25.2 \pm 9\%$  of total submergence time respectively (Figure 5.1b). Not surprisingly then, the overall proportion of time *M. bellii* spent diving in winter ( $97 \pm 1\%$ ) was significantly different to all other seasons ( $F_{[3, 11]} = 8.15$ ;  $P = 0.0039$ ). There was no significant difference in overall proportion of diving between spring ( $58 \pm 7\%$ ), summer ( $53 \pm 4\%$ ) or autumn ( $64 \pm 7\%$ ) results ( $F_{[2, 10]} = 0.50$ ;  $P = 0.6212$ ).

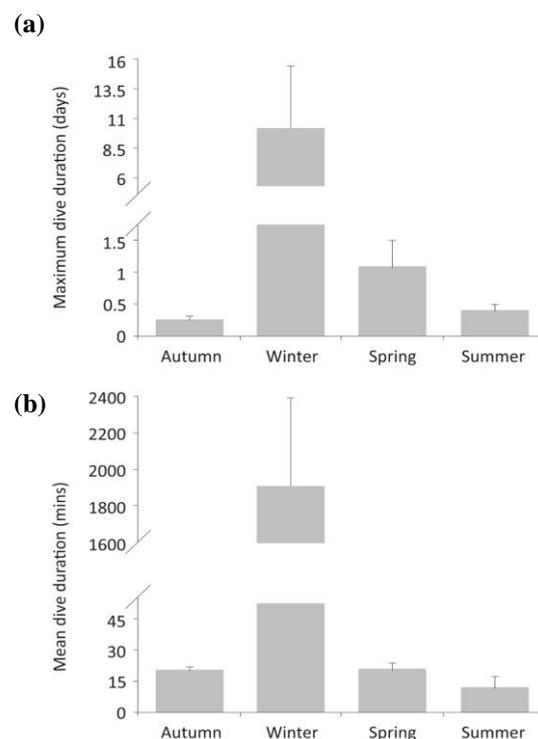
Significant differences in the diving performance of *M. bellii* were observed between winter and all other seasons. Mean dive time ( $F_{[3, 11]} = 3.78$ ;  $P = 0.0438$ ), average maximum dive duration ( $F_{[3, 11]} = 13.93$ ;  $P = 0.0005$ ), average number of dives per day ( $F_{[3, 11]} = 6.50$ ;  $P = 0.0086$ ) and average dive depth ( $F_{[3, 11]} = 7.12$ ;  $P = 0.0063$ ) were all significantly different among seasons (Figure 5.2 and Figure 5.3). During the winter deployment period, the *M. bellii* turtles averaged  $0.8 \pm 0.2$  dives per day which is a 95-fold difference to summer activity of  $76.1 \pm 17.7$  dives per day ( $t_{[4]} = -2.83$ ,  $p = 0.0474$ ). Similarly, mean dive duration in winter ( $1891 \pm 499$  min) was 152 times longer than in summer ( $12.4 \pm 4.3$  min) ( $t_{[4]} = 6.15$ ,  $p = 0.0035$ ) and the average maximum dive duration of 10.2  $\pm$  5.3 days during winter decreased to  $0.4 \pm 0.09$  days in summer ( $t_{[4]} = -3.02$ ,  $p = 0.0393$ ). The longest dive recorded in each season was 15.5 days in winter (see Figure 5.11d), 2.9 days in spring, 15.9 h in summer and 7.7 h in autumn. The average depth of *M. bellii* winter dives at  $315 \pm 11$  cm (Figure 5.2b) was significantly deeper than for spring ( $68 \pm 6$  cm,  $t_{[6]} = 21.16$ ,  $p = 0.0001$ ), summer ( $62$  cm,  $t_{[4]} = 22.71$ ,  $p = 0.0048$ ), and autumn ( $95 \pm 11$  cm,  $t_{[3]} = 13.67$ ,  $p = 0.0008$ ). In addition, the autumn average dive depth of  $95 \pm 11$  cm was significantly deeper than both the spring average  $68 \pm 6$  cm ( $t_{[7]} = -2.49$ ,  $p = 0.0415$ ) and the summer average of  $62 \pm 6$  cm ( $t_{[5]} = 2.94$ ,  $p = 0.0323$ ), though there was no statistical difference between spring and summer average dive depths ( $t_{[8]} = -0.72$ ,  $p = 0.4924$ ).



**Figure 5.1** Distribution of dive performance of *M. bellii* among seasons for a series of 15 min time intervals. (a) Frequencies of dives per period. (b) Proportions of total diving time per period. The total number of dives analysed per season were 7277 (spring), 9958 (summer), 1415 (autumn) and 48 (winter).



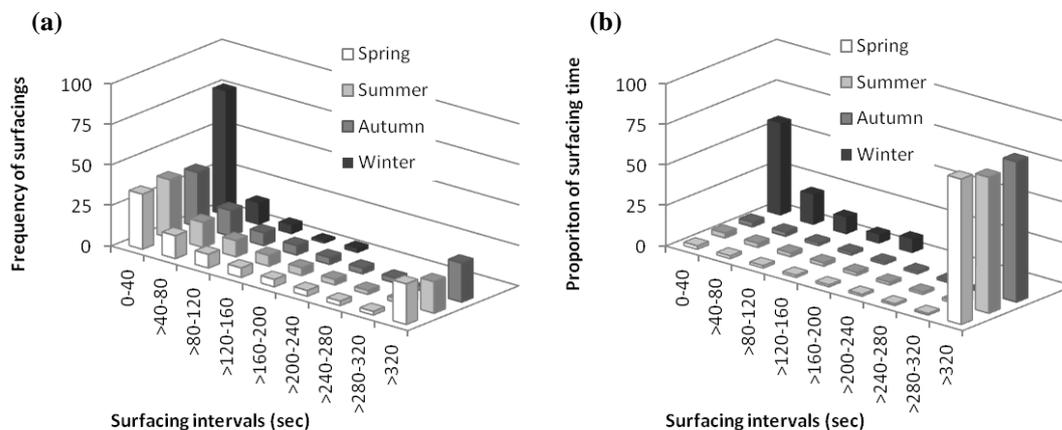
**Figure 5.2** A comparison of *M. bellii* dive performance among seasons with (a) number of dives per day and (b) average dive depth.



**Figure 5.3** A comparison among seasons for *M. bellii* of (a) maximum dive durations (days) and (b) mean dive times (min).

*Seasonal surfacing behaviour*

A majority of surfacing events (<40 cm depth) across all seasons were short in length (<40 s) (Figure 5.4a). The proportions of surfacing events <40 s were similar for spring, summer and autumn ranging between 34-36% but significantly increased to  $77 \pm 10\%$  in winter ( $F_{[3, 11]} = 8.97$ ;  $P = 0.0027$ ). No surfacing event in winter lasted longer than 200 s and most were <80 s ( $90 \pm 6.7\%$ ). In comparison, longer surfacing events lasting >5 min accounted for  $25 \pm 3.4\%$ ,  $19 \pm 1.7\%$  and  $24 \pm 9.5\%$  of surfaces in spring, summer and autumn respectively (Figure 5.4a). These values also correspond to a high proportion of the total surfacing time in spring ( $90 \pm 2.6\%$ ), summer ( $84 \pm 4.7\%$ ) and autumn ( $86\%$ ) (Figure 5.4b). Two *M. bellii* recorded a total surfacing time of  $21.7 \pm 2.3$  min (or 0.05%) over the entire winter deployment period. This compares to a total surfacing time of  $42.3 \pm 7.2\%$  in spring,  $46.7 \pm 4.2\%$  in summer and  $35.6 \pm 7.4\%$  in autumn.



**Figure 5.4** Distribution of surfacing performance of *M. bellii* among seasons for a series of 40 s time intervals. (a) Frequencies of surfacing events per period. (b) Proportions of total surfacing time per period.

*Diel diving and surfacing behaviour*

Diel trends in mean dive durations varied significantly within seasons. Individual turtles in spring and autumn were observed to have longer mean dive durations at night compared to daylight hours (Figure 5.6d, Figure 5.8d, Figure 11a and 11c). While for summer, this trend was reversed with turtles preferring longer dives during daylight hours with reduced dive duration at night (Figure 5.7d and Figure 11b). This was also reflected in the overall activity of turtles with reduced night activity in spring and autumn and correspondingly more activity in daylight hours with peaks in activity generally occurring around dawn and dusk (Figure 5.6b and Figure 5.8b). In the same way as dive duration, periods of greatest activity was reversed for summer with turtles being most active at night with a preference to being more active around dawn and dusk. Not surprisingly, the proportions of surfacing events follow closely individual turtles' activity levels (Figure 5.6a, 7a and 8a). That is, with increased activity there was a corresponding increase in the proportion of surfacing events particularly during the crepuscular hours of dawn and dusk. General trends in diel behaviour for the two turtles observed during winter are less clear due to the limited number (42 dives in total) and long durations of recorded dives (Figure 11d). Although during the day, there was an apparent increase in activity and surfacing events for these individuals compared to night (Figure 5.9).

Specifically, mean dive durations showed a four-fold increase in autumn from 16 min during the day to  $64 \pm 6$  min at night (T2/T3/T4 vs. T1/T5;  $W_{[42, 33]} = 6.639, p < 0.0001$ ) and a three-fold increase in spring mean dive duration from 16 min in daylight hours to  $49 \pm 5$  min at night ( $W_{[84, 60]} = 7.778, p < 0.0001$ ). Turtles were significantly more active and surfaced more often during the day than at night in autumn (activity:  $W_{[42, 30]} = 6.927, p < 0.0001$ ; surfacing:  $W_{[42, 30]} = 6.773, p < 0.0001$ ) and spring (activity:  $W_{[84, 60]} = 8.702, p < 0.0001$ ; surfacing:  $W_{[84, 60]} = 8.471, p < 0.0001$ ). In addition, turtles were more active and surfaced more often between the crepuscular hours of dawn and dusk in spring (T2/T4 vs. T3; activity:  $W_{[60, 23]} = 3.393, p = 0.0007$ ; surfacing:  $W_{[60, 24]} = 2.490, p = 0.0128$ ) and in autumn (T2/T4 vs. T1/T3/T5; activity:  $W_{[60, 12]} = 2.999, p = 0.0027$ ; surfacing:  $W_{[60, 12]} = 3.158, p = 0.0016$ ).

In contrast to autumn and spring results, mean dive durations in summer decreased from  $21 \pm 2$  min in the day to  $12 \pm 2$  min at night ( $W_{[56, 44]} = 2.309, p = 0.0209$ ). There was

no observed statistical differences in diel activity levels ( $W_{[56, 40]} = 0.602$ ,  $p = 0.5472$ ) or surfacing frequency ( $W_{[56, 40]} = 1.5162$ ,  $p = 0.1295$ ) between day (T2/T3/T4) and night (T1/T5). However, turtles were more active and surfaced more often in summer during the crepuscular hours of the day (T2/T4 vs. T3; activity:  $W_{[40, 16]} = 4.453$ ,  $p = 0.0001$ ; surfacing:  $W_{[40, 16]} = 4.235$ ,  $p < 0.0001$ ).

Finally, there was no significant difference in diel trends in mean dive depth within autumn ( $F_{[3, 68]} = 1.90$ ;  $P = 0.1382$ ) and winter ( $F_{[3, 44]} = 0.62$ ;  $P = 0.6089$ ). However, turtles mean dive depth in summer decreased significantly from an average depth of  $66 \pm 2$  cm during the day to  $57 \pm 3$  cm at night (T2/T3/T4 vs. T1/T5;  $W_{[56, 40]} = 2.739$ ,  $p = 0.0062$ ). In addition, the mean dive depth of  $87 \pm 5$  cm at dawn in spring was significantly deeper than the mean depth of  $57 \pm 4$  cm at dusk (T2 vs. T4;  $W_{[12, 12]} = 3.320$ ,  $p = 0.0009$ ).

#### *Diel temperature trends*

Water temperature at 30 cm depth recorded using iButtons fluctuated daily in each season. Daily mean temperatures in spring and summer varied  $< 3$  °C, while temperatures varied only 2 °C in autumn and winter with daily lows occurring at about 06:00 – 07:00 and daily highs occurring between 15:00 – 17:00. Daily mean temperatures logged across seasons from individual turtle TDRs varied in a similar trend to ambient water temperatures at 30 cm depth – though at a lower mean temperature because of average diving depths (Figure 5.2*b*). There was one exception to this result: spring TDR mean temperatures between 09:00 – 12:00 were on average higher than ambient water temperatures at 30 cm (Figure 5.10*a*).

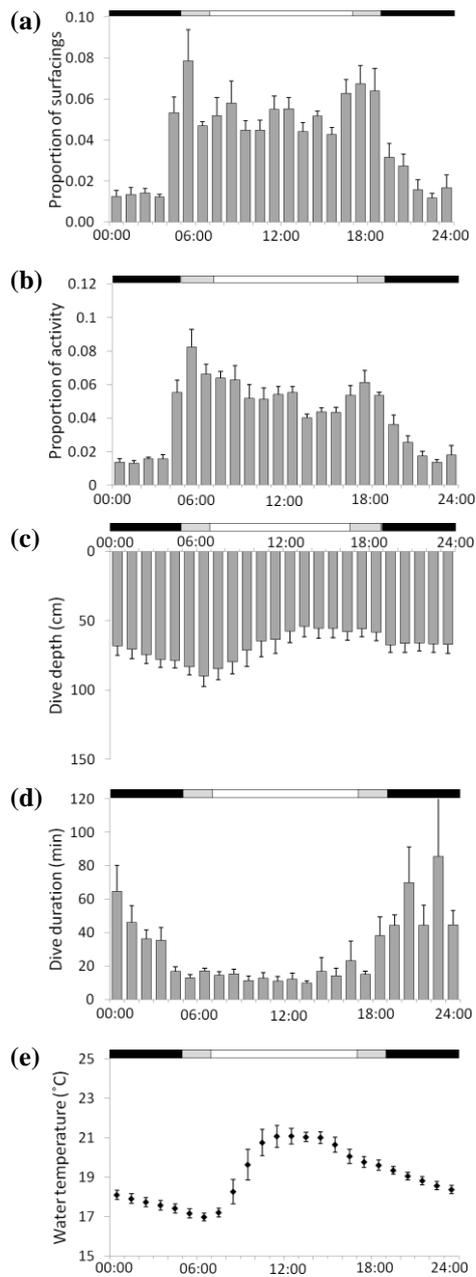
#### *Basking behaviour of M. bellii*

During this study, both male and female immature and mature turtles were observed to bask on granite boulders or logs on the edge of the stream bank or emerging from the water. One of the males with an attached TDR was observed basking on a boulder at about 10:00 in November 2007 along with three other adults. On this occasion, all of the turtles were completely out of the water and in full sun. Similarly, individuals or small groups of turtles were observed to bask on boulders in surveys of the Namoi and Gwydir rivers of New South Wales (Figure 5.5).

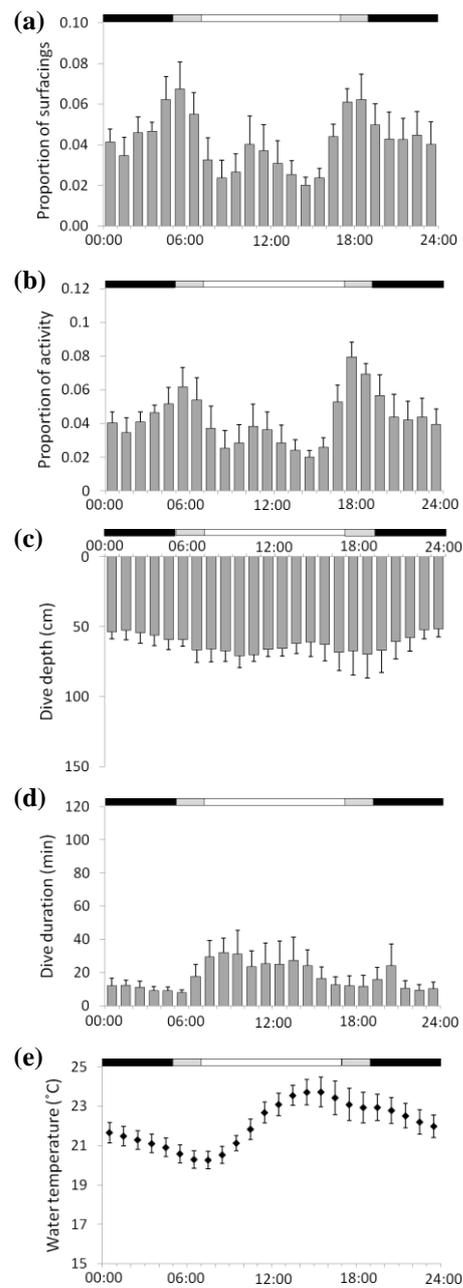
This basking behaviour was detectable in the logged data when the mean maximum hourly temperature recorded from individual turtle's TDRs was compared with the maximum hourly temperature recorded from iButton's at 30 cm depth (Figure 5.10). Differences among seasonal daytime (T3) maximum logged hourly temperatures recorded from TDRs were statistically significant ( $F_{[3, 142]} = 33.33; P < 0.0001$ ). The spring average maximum of  $33 \pm 0.8$  °C was significantly higher ( $W_{[60, 40]} = 2.600, p = 0.0093$ ) than summer average maximum temperatures of  $30 \pm 0.7$  °C which was significantly higher ( $W_{[40, 30]} = 2.914, p = 0.0036$ ) than autumn average maximum temperatures of  $27 \pm 0.8$  °C. Peaks in average maximum temperatures were between the hours of 09:00-11:00 (max. 42 °C), 10:00-13:00 (max. 39 °C) and 11:00-12:00 (max. 38 °C) for spring, summer and autumn respectively.



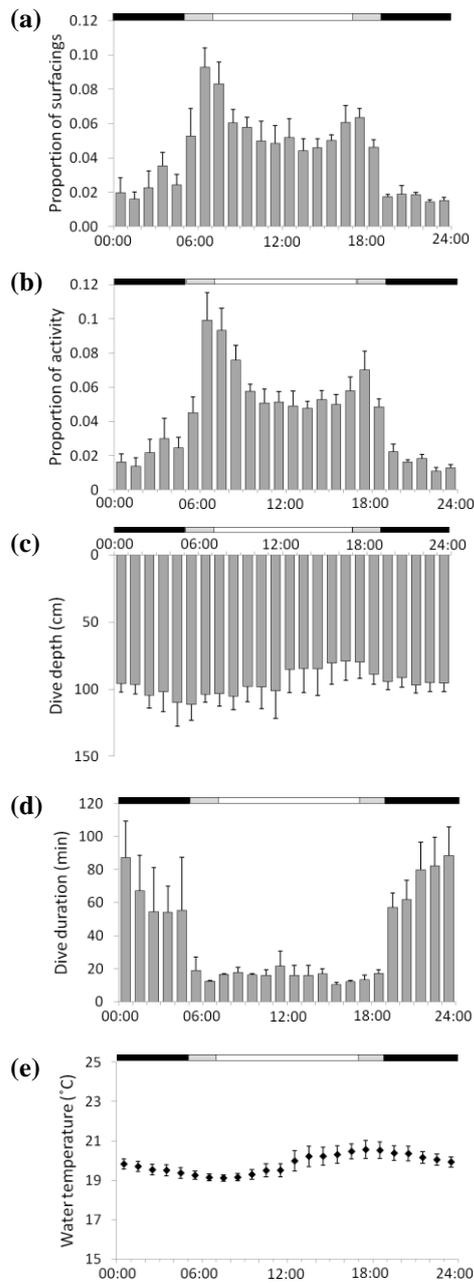
**Figure 5.5** Two adult *M. bellii* males basking on a granite boulder in the Namoi River, January 2007.



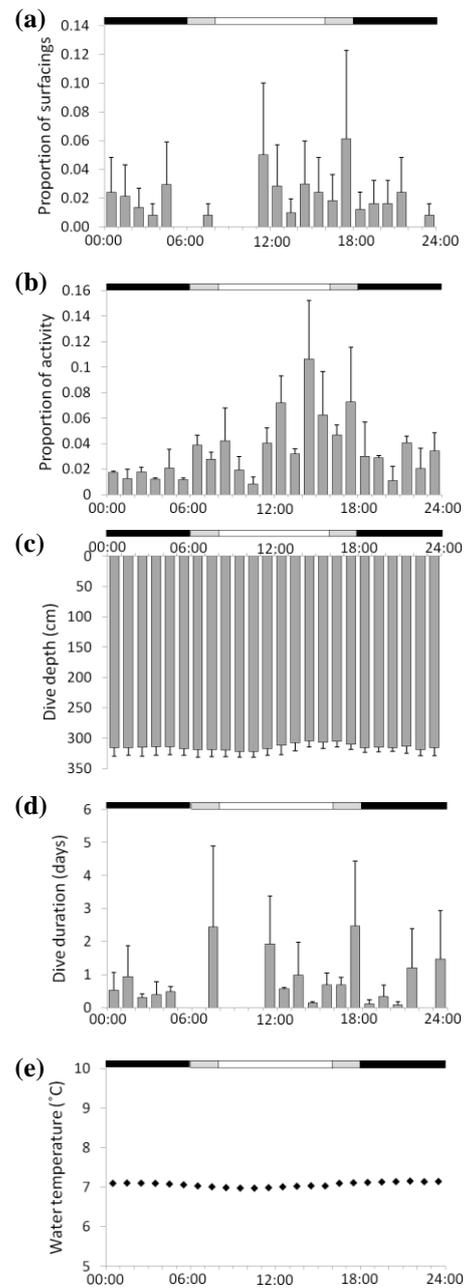
**Figure 5.6** Spring diel trends of *M. bellii* for (a) proportion of surfacings per hour, (b) proportion of activity per hour, (c) mean dive depth per hour, (d) mean dive duration per hour, and (e) mean water temperature per hour. The bar depicted for each graph represents the five periods for the purpose of analysis: dark bar is night (T1 and T5), grey bar is dawn and dusk (T2 and T4) and the open bar is day (T3) (refer Table 5.2).



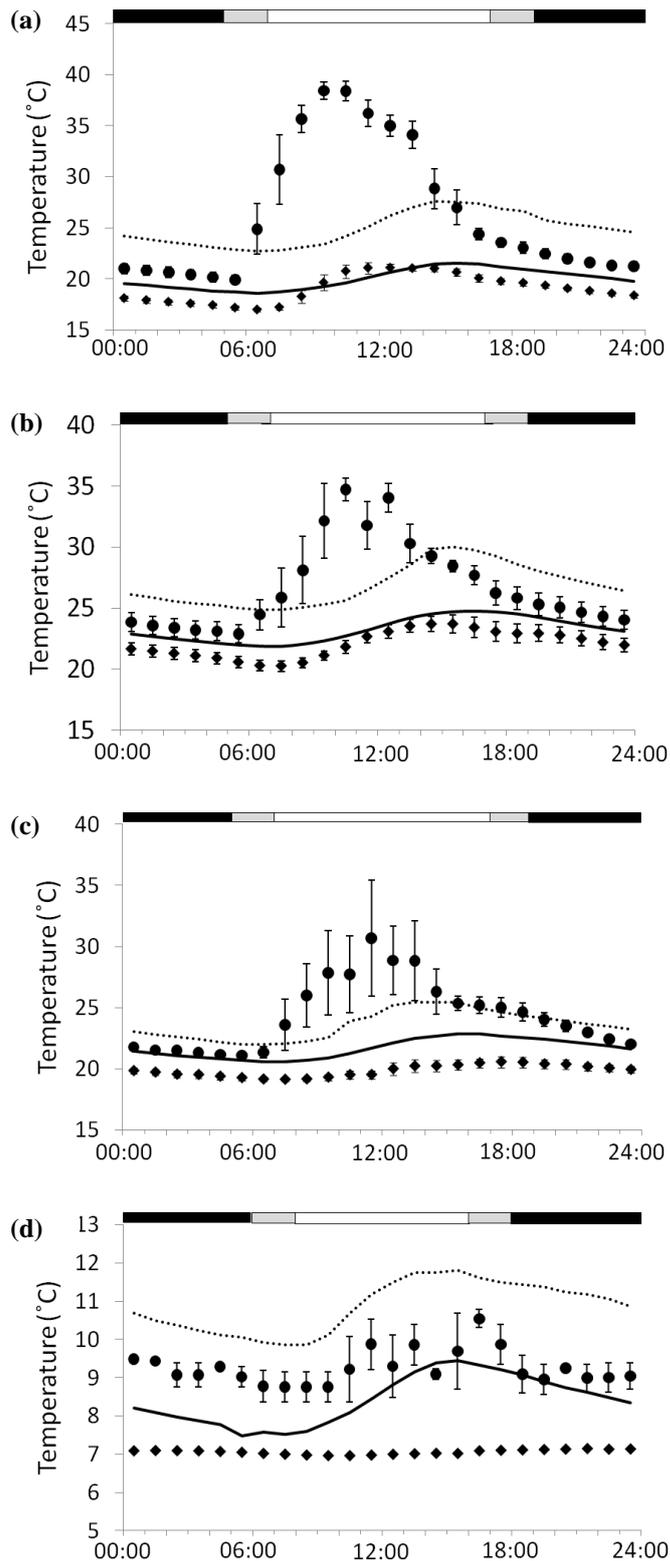
**Figure 5.7** Summer diel trends of *M. bellii* for (a) proportion of surfacings per hour, (b) proportion of activity per hour, (c) mean dive depth per hour, (d) mean dive duration per hour, and (e) mean water temperature per hour. The bar depicted for each graph represents the five periods for the purpose of analysis: dark bar is night (T1 and T5), grey bar is dawn and dusk (T2 and T4) and the open bar is day (T3) (refer Table 5.2).



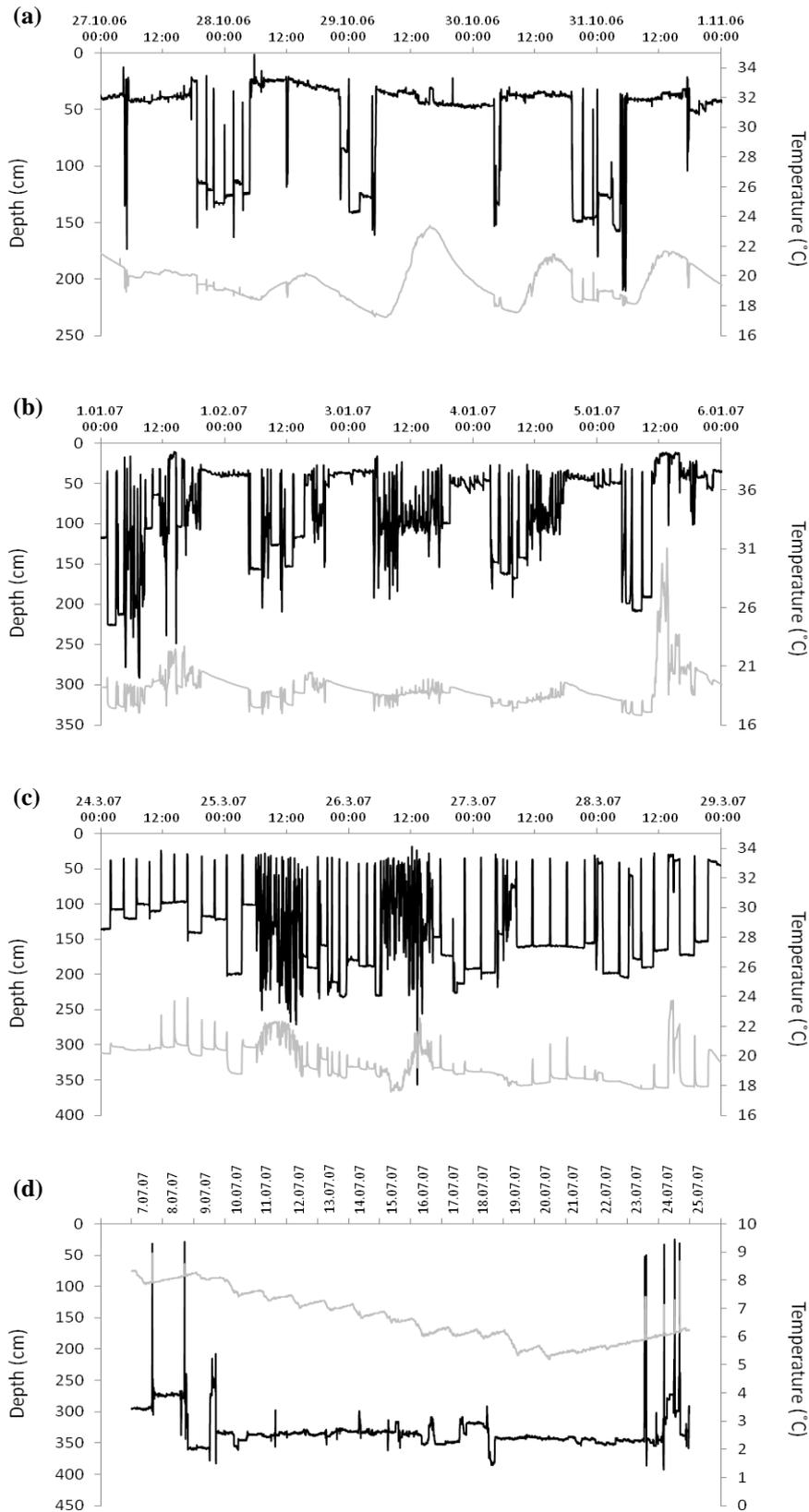
**Figure 5.8** Autumn diel trends of *M. bellii* for (a) proportion of surfacings per hour, (b) proportion of activity per hour, (c) mean dive depth per hour, (d) mean dive duration per hour, and (e) mean water temperature per hour. The bar depicted for each graph represents the five periods for the purpose of analysis: dark bar is night (T1 and T5), grey bar is dawn and dusk (T2 and T4) and the open bar is day (T3) (refer Table 5.2).



**Figure 5.9** Winter diel trends of *M. bellii* for (a) proportion of surfacings per hour, (b) proportion of activity per hour, (c) mean dive depth per hour, (d) mean dive duration per hour, and (e) mean water temperature per hour. The bar depicted for each graph represents the five periods for the purpose of analysis: dark bar is night (T1 and T5), grey bar is dawn and dusk (T2 and T4) and the open bar is day (T3) (refer Table 5.2).



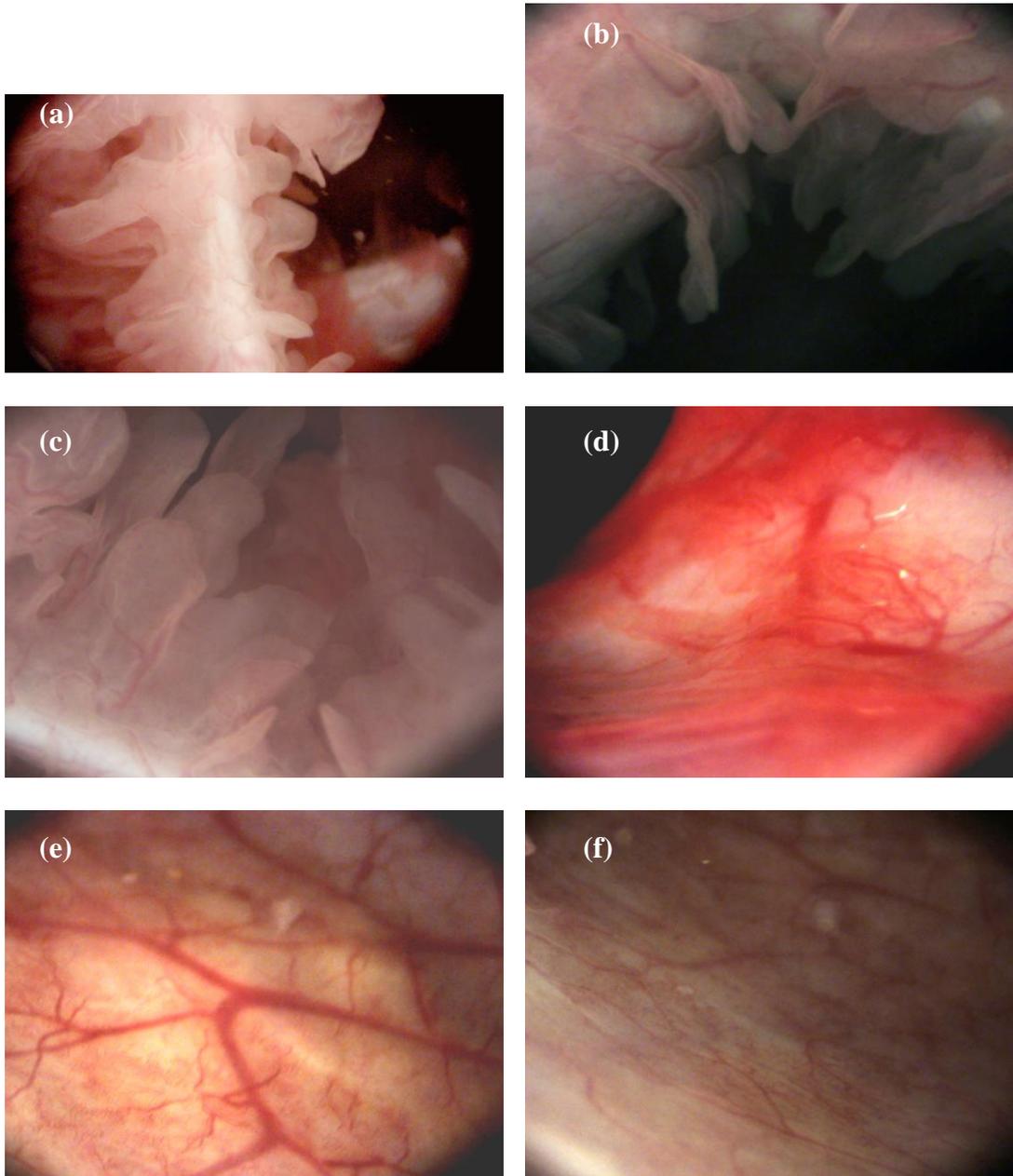
**Figure 5.10** Diel water temperature analysed hourly for (a) spring, (b) summer, (c) autumn and (d) winter. Solid line and broken line are the mean and maximum recorded temperature from iButtons (30 cm depth) respectively. Solid diamonds and solid circles are the mean and maximum recorded temperature from TDRs respectively. The bar depicted for each graph represents the five periods for the purpose of analysis: dark bar is night (T1 and T5), grey bar is dawn and dusk (T2 and T4) and the open bar is day (T3) (refer Table 5.2).



**Figure 5.11** Selected individual dive profiles for *M. bellii* during (a) spring, (b) summer, (c) autumn and (d) winter seasons. Dates given on the x axis are marked at midnight. The black line is the recorded depth and the grey line is the recorded temperature from the attached TDR.

*The cloacal bursae of M. bellii*

The two bursae of *M. bellii* lead off each side of the cloaca and are covered in highly vascularised flattened papillae lobes (Figure 5.12a). Numerous rows of these vascularised lobes align the mucosal lining of the bursae (Figure 5.12a, b and c). Their greatest density occurred at the bursae entrance (Figure 5.12a) and they were observed to move freely when water pulsed in and out of the bursae. Entering further into the lumen, the number of vascularised lobes decreased until none were attached to the mucosal wall at the cranial end of the bursae (Figure 5.12e and f). Both the lateral and cranial end of the mucosal wall appeared to be highly vascularised to the naked eye with an extensive network of surface capillaries. Similarly, when water was removed from the bursae the collapsed filamentous processes were showing intense pink colouration from the heavily vascularised papillae (Figure 5.12d). The flattened lobed papillae appear to have increased the total surface area of the bursae many-fold (though not measured).



**Figure 5.12** Photo plates of the cloacal bursae of *M. bellii*. (a) The many branched and flattened vascularised filamentous processes lining the entrance to the two cloacal bursae cavities (left and right). (b) Filamentous processes lining the mucosa, side view, extending into the lumen of the cloacal bursae. (c) Flattened vascularised processes, front view. (d) Collapsed vascularised processes after water removal. (e) Highly vascularised mucosa of the cloacal bursae, cranial end. (f) The highly vascularised lateral mucosa wall of the cloacal bursae.

## Discussion

### *Aquatic respiration of M. bellii*

The remarkable dive durations of *M. bellii* recorded in this study are second only to *R. leukops* for the longest reported freely diving marine or freshwater turtle (Gordos and Franklin 2002; Gordos *et al.* 2003a; Gordos *et al.* 2003b; Gordos *et al.* 2007). There were significant differences among seasons in the dive performance of *M. bellii* with winter mean and maximum dive durations far eclipsing other seasons' results, which is comparable with seasonal variations reported for *R. leukops* (Table 5.4). Water temperature is known to be a significant factor in the dive durations for freshwater turtles (Ultsch *et al.* 1984; Reese *et al.* 2002; Reese, Stewart *et al.* 2004; Gordos *et al.* 2003b; Clark *et al.* 2008). At lower temperatures dissolved oxygen is more readily available and at the same time, metabolic activity levels of ectothermic turtles decrease and lower demand for oxygen consumption, and reliance on aquatic respiration increases for bimodally respiring species (Butler *et al.* 1984; Bagatto and Henry 1999; Gordos and Franklin 2002; Gordos *et al.* 2003a; Gordos *et al.* 2007).

A commonly used method to determine whether a submersion remains aerobic is to examine the length of surfacing time required immediately after dives (Gordos *et al.* 2003b). Generally for aerobic dives, animals only require a short time at the surface to replenish oxygen stores and when an animal goes beyond its aerobic dive limit (ADL), longer surfacing times are needed for accumulated lactate to be oxidised (van Dam and Diez 1996; Butler and Jones 1997). For winter, *M. bellii* surfaced only 0.05% of the entire logged time and most (>80 per cent) of the surfacing events were less than 80 seconds even immediately following extended dives of 3-4 days in length and one dive of 15.5 days. Hence, the short surfacing times indicate that *M. bellii* remain aerobic for prolonged submersions during winter in Bald Rock Creek. Similarly for summer, *M. bellii* maximum dives can extend well beyond the ADL of 1.5 h after which non-hibernating turtles generally expend their O<sub>2</sub> supplies from their lungs (Jackson 1968; Caligiuri *et al.* 1981; Crocker *et al.* 1999). Like *R. leukops*, the ability of *M. bellii* to extend its dives to weeks at a time is attributed in this study to its specialised cloacal bursae and the very low temperatures (5-8 °C, Figure 5.11d). Although, extra-pulmonary gas exchange from buccal and cutaneous pathways as noted in *M. georgesi* (King and Heatwole 1994a; King and Heatwole 1994b) and for *E. albagula* (FitzGibbon

and Franklin 2010) cannot be ruled out as contributing to the overall aquatic oxygen uptake in *M. bellii*.

#### *Cloacal bursae morphology*

The morphology of cloacal bursae in Australian freshwater turtles differ substantially between species with *Emydura*, *M. georgesi* and *Elusor macrurus* the least developed having vascularised processes only partially covering the bursal lining (Legler and Georges 1993b; King and Heatwole 1994a; Cann and Legler 1994). In *Elseya albagula* and *E. dentata*, flattened branched papillae cover all of the bursal lining while *R. leukops* have the most specialised of all being highly vascularised and multi-branching (Legler and Cann 1980; Legler and Georges 1993b; Priest 1997; FitzGibbon and Franklin 2010). The cloacal bursae of *M. bellii* are only partially covered by moderately branching, flattened lobed papillae with the highest density being at the entrance to the cloacal bursae. Thus, the observed bursae structure of *M. bellii* positions it intermediately between *Emydura* and *Rheodytes* bursae morphology.

Currently, there is limited understanding about the relationship between the morphological structures of the bursae and aquatic respiration abilities in freshwater turtles (Clark *et al.* 2008). A general assumption from the literature is that there may be a linear function between the complexity of the bursae morphology and total aquatic oxygen uptake abilities of species: with the simplest forms (= lowest surface area to water ratio, low density of surface capillaries) being least effective in oxygen uptake, and conversely, the more elaborate structures (= greatest surface area to water ratio, high density of surface capillaries) being the most effective in oxygen uptake. However, no study has been able to categorically demonstrate this relationship in species with cloacal bursae. In fact, Clark *et al.* (2008) found a non-linear result in the diving abilities between hatchling, juveniles and adults of the same species, and also between species. Their results under laboratory conditions were unclear with no apparent correlation between the level of cloacal bursae specialisation and aquatic oxygen uptake apart from the standout species, *R. leukops*.

In addition, Gordos *et al.* (2003b) found that observed dive durations for *R. leukops* under laboratory conditions were gross underestimates of dive durations under natural conditions. These discrepancies were thought to possibly relate to the shallow artificial

pools and probable stressed state of the laboratory studied turtles (Priest 1997). No study has examined the level of oxygen uptake abilities of the cloacal bursae in *M. bellii*. However, the intermediate complexity of the bursae structure of *M. bellii* may allow for an unexpected level of aquatic oxygen uptake, particularly at low water temperatures and at depth, which may account for the prolonged submersions observed for winter in this study.

**Table 5.4** A comparison of seasonal performance for four freely diving Australian freshwater turtles with summary information on water temperature (recorded from TDRs deployed on turtles), number of dives per day, number of activity counts ( $\pm 6$  cm) in the vertical plane, dive depth and dive durations. Values are given as means unless otherwise stated.

Species	Season	Water Temp. ( $^{\circ}$ C)	Dives day <sup>-1</sup>	Activity counts day <sup>-1</sup>	Dive depth (cm)	Dive duration		Source
						mean (min)	max (days)	
<i>Myuchelys bellii</i>	Autumn	19.8	41	428	95	20.6	0.3	This study
	Winter	7.1	0.8	51	315	1891	15.5	This study
	Spring	19.0	36.3	383	68	21.0	2.9	This study
	Summer	21.9	76.1	612	62	12.4	0.7	This study
<i>Rheodytes leukops</i>	Autumn	>25	39.3		109	33.1	0.9	Gordos and Franklin 2002
	Autumn	24.7	24.2	898	111-183	88.6	3.5	Gordos <i>et al.</i> 2003a and 2003b
	Winter	16.5	4.3	370	132-142	10512	21.0	Gordos <i>et al.</i> 2003a and 2003b
	Spring	24.5	29.2	529	111-137	61.7		Gordos <i>et al.</i> 2003a and 2003b
	Summer	27.3	61	899	103	23.6	2.4	Gordos <i>et al.</i> 2003a and 2003b
<i>Elseya albagula</i>	Spring	23.5	101.7		136	7.4	0.10	Gordos <i>et al.</i> 2007
	Spring	24.1	74.2		71	19.2	0.13	Gordos <i>et al.</i> 2007
<i>Emydura macquarii</i>	Autumn	>25	112		120	9.6	0.06	Gordos and Franklin 2002

#### Seasonal dive behaviour

The seasonal diving behaviour of *M. bellii* corresponds closely to those observed for *R. leukops* (Gordos and Franklin 2002; Gordos *et al.* 2003b) with most dives in spring, summer and autumn being of less than 15 mins duration. Nearly the entire submergence time in winter was spent diving > 2.5 hours (99%) compared with 41% in spring, 25%

in autumn and 11% in summer. This compares to the reported time spent in dives >2.5 hours for *R. leukops* in winter of 80%, autumn and spring approximately 36% each and 11% for summer (Gordos *et al.* 2003b). In contrast, *E. albagula* in spring spent only 8-13% in dives greater than 90 min duration (Gordos *et al.* 2007) and no dive for *Em. macquarii* extended beyond 90 mins in autumn (Gordos and Franklin 2002).

There was considerable variation in individual *M. bellii* diving profiles between seasons and within a recording period. However, a general pattern of deeper resting dives >2 hours occurred in early spring and late autumn when water temperatures were colder. This was also reflected in differences between seasons in the number and the average length of dives with turtles having on average less than one dive per day in winter increasing 95-fold in summer with the number of dives in spring and autumn falling in between winter and summer levels (Table 5.4). On average, *M. bellii* dive more often per day than *R. leukops* (except for winter), but their daily activity levels in the vertical plane are significantly less than those reported for *R. leukops*. This result could indicate that *M. bellii* have more resting dives than *R. leukops* or that *M. bellii* spend more time in relatively shallower dives compared to *R. leukops* (as evidenced by the average seasonal dive depths) (Table 5.4). A combination of these behaviours is more likely though.

#### *Diel dive and surfacing behaviour*

Diel diving behaviour by *M. bellii* was similar in spring and autumn where they were characterised by significantly longer resting dives at night and shorter dives during the day, even though there was no noticeable difference in dive depth between night and day. Equally, they were more active during daylight hours than at night with significant peaks in activity and surfacing events around the crepuscular hours of dawn and dusk. This diurnal activity pattern was reversed in summer where *M. bellii* turtles had comparatively longer and deeper dives during the day than at night. This result corresponded with turtles in summer being more active at night compared to other seasons, but still showed a crepuscular habit of significantly increased activity around dawn and dusk. In addition, summer average dive depths for *M. bellii* were deeper during the day than at night indicating a similar diel behaviour observed for *E. albagula* during spring which was attributed to its dietary preference of ribbon weed (*Vallisneria*), filamentous algae and fallen fruits associated with shallower water depths

(Gordos *et al.* 2007). Interestingly, the observed crepuscular behaviour of *M. bellii* was not dissimilar to those reported elsewhere applying the same methods for *R. leukops*, *E. albagula* and *Em. macquarii* (Gordos and Franklin 2002; Gordos *et al.* 2003a; Gordos *et al.* 2007).

The observed behavioural change from spring and autumn where *M. bellii* were primarily diurnal in their habit to increased nocturnal surfacing behaviour in summer is novel. Hays *et al.* (2006) reported behavioural plasticity in leatherback sea turtles between geographic locations and attributed this to changes in prey patch density and prey diel activity patterns (Hays *et al.* 2006). However, it is unlikely that changes to food patchiness between seasons are the reason for changes in behaviour for *M. bellii*. It would seem more likely that it is related to increased ambient water temperatures and a corresponding increase in foraging activity in shallow water at night thought to account for similar diel trends in *E. albagula* (Gordos *et al.* 2007).

#### *Basking behaviour*

Thermoregulation behaviour in aquatic turtles has often been observed (e.g., Webb 1978; Morreale and Gibbons 1986; Chessman 1987; DonnerWright *et al.* 1999; Linderman 2000; Doody *et al.* 2001) with associated benefits thought to include raised body temperature resulting in higher growth rates (King *et al.* 1998), increased rates of food digestion in ectotherms (Spencer 1998; Pratt *et al.* 2009) and enhanced follicular development (Carrière *et al.* 2008), and health benefits from drying of the shell and skin to retard algal or fungal growth (Boyer 1965; Chessman 1987; Manning and Grigg 1997). Aquatic thermoregulation in the relatively warmer surface waters in marine turtles was thought to also be related to raising internal body temperature after prolonged cold dives (<7 – 10.5 °C from surface temperatures) and possible recovery from anaerobic dives (Hochsheid *et al.* 2010). In addition, loggerhead turtles microhabitat selection of warmer sea surface waters was presumed to speed up egg maturation rates before oviposition and allow turtles to initiate nesting at an earlier date (Schofield *et al.* 2009). While atmospheric basking was observed for *M. bellii* during this study and elsewhere (Cann 1998), it is uncertain if it exploits aquatic basking like some marine and freshwater turtles are known to do (King *et al.* 1998; Doody *et al.* 2001; Hochsheid *et al.* 2010).

The TDR hourly logged temperatures suggest an irregular, but characteristic, behaviour of atmospheric basking by *M. bellii*. This behaviour was prevalent in spring with the highest maximum temperatures recorded during the morning hours of 09:00-11:00 when mean temperatures peaked at 38 °C. One individual female *M. bellii* in spring appeared to bask on 15 days out of 31 logged days, with most individuals observed to alternate between basking and diving over the course of several hours of a day. Similarly, there was evidence of atmospheric basking in summer with recorded TDR mean maximum temperatures peaking mid-morning at 35 °C. Atmospheric basking in autumn was less frequent based on the logged data with mean temperatures peaking at 31 °C between 11:00 and 12:00. This basking behaviour generally reflects the increased activity, increased surfacing frequency and relatively shorter dive durations of *M. bellii* during the day for these seasons. Increased basking behaviour in spring when average water temperatures are <20 °C may also benefit the turtles' life history through higher body temperatures resulting in increased rates of growth and activity levels required for foraging, mating, reproductive effort (e.g., maturation of egg follicles) and earlier nesting dates to allow for hatchlings to emerge during the relatively shorter growing season. However, caution on interpreting the TDR maximum temperatures is required because they provide no indication on actual core body temperature of turtles or the prevailing weather conditions (e.g., cloud cover, radiant heat levels) on particular days.

### *Hibernation*

Aquatic hibernation has been extensively studied in the laboratory for cryptodiran species in North America where winter temperature of aquatic environments can decrease to 3-5 °C with surface ice forming on some habitats. The length of hibernation through prolonged submergence is dependent on the temperature, latitude of occurrence of the species, and the presence or absence of dissolved oxygen in the water (Ultsch 1988; Reese, Stewart *et al.* 2004). Simulated hibernation experiments via forced submergence saw the common snapping turtles *Chelydra serpentina*, the musk turtle *Sternotherus odoratus* and the painted turtles *Chrysemys picta picta* and *Chrysemys picta bellii* survive >100 days under normoxic conditions (3 °C) and approximately 20 to >100 days under anoxic conditions (3 °C) (Ultsch and Jackson 1982; Ultsch 1988; Ultsch *et al.* 1999; Reese *et al.* 2002; Reese, Ultsch *et al.* 2004). Overwintering sites for *S. odoratus* are thought to be buried in anoxic mud. However, it is unknown if they emerge for pulmonary or extra-pulmonary respiration during the winter period or if

indeed these reported submergence times from the laboratory are part of their natural ecology (Ultsch 1988).

A number of semi-aquatic species such as hibernating bog turtles, *Glyptemys muhlenbergii* and *G. guttata*, prefer the terrestrial habitats of bogs or borrows partially submerged in water for winter hibernacula (Ernst *et al.* 1989; Haxton and Berrill 1999). However, field studies on the behaviour and ecology of freshwater turtles in their natural aquatic environments over the duration of winter are few in number (Ultsch 1988) with in situ observations available for winter submergence times on *Rheodytes leukops* (Gordos *et al.* 2003b) and oxygen uptake in hibernating wood turtles, *G. insculpta* (Graham and Forsberg 1991). Wood turtles were observed to rest on the bottom of a stream bed (30 – 60 cm water depth) in open water next to submerged logs and rocks with minimal positional changes over 6-8 m<sup>2</sup> during winter months. The authors also noted that wood turtles presumably supplement aquatic respiration with air breathing as a turtle was seen to surface during their investigation period in winter. Other species such as the common snapping turtle *Chelydra serpentina* have been observed to overwinter in shallow water habitats buried in mud where they can extend their necks to take a breath of air, provided there was no ice layer on the surface (Meeks and Ultsch 1990; Reese *et al.* 2002).

Aquatic hibernation for *M. bellii* in Bald Rock Creek is only the second reported for any pleurodiran species (see Gordos *et al.* 2003b). Other Australian species of freshwater turtle such as eastern long-necked turtle *Chelodina longicollis* aestivate in terrestrial environments (Roe and Georges 2007; 2008; Kennett *et al.* 2009), or alternatively northern snake-necked turtle *C. rugosa* lie buried in dried mud with air holes to the surface until waterholes refill (Grigg *et al.* 1986; Kennett and Christian 1994). Two tropical short-necked freshwater species *M. novaeguineae* (formerly *Elseya novaeguineae*) and *Emydura subglobosa* from New Guinea rivers do not appear to hibernate (Crocker *et al.* 1999). In contrast, the diving profiles of *M. bellii* for winter (Figure 5.11) suggest a deliberate behavioural choice to hibernate at submerged depth (>3 m) with extended periods of inactivity when ambient water temperatures are at their lowest (range 5 – 8 °C).

It is not unusual for upland streams to freeze over and periodic winter snow falls across years to occur over *M. bellii*'s range. Therefore, aquatic hibernation as an overwintering life strategy in *M. bellii* is consistent with observed behaviour of aquatic turtle species from temperate regions in the northern hemisphere (Penney 1987; Crocker *et al.* 1999). The choice of moving to deep water habitat for winter hibernation appears atypical and may be linked to lower temperatures and higher aquatic oxygen capacitance at depth during winter months (Gordos *et al.* 2003a). There was a 7-fold decrease in activity in winter between *M. bellii* and *R. leukops* (Table 5.4). The diving profile of *M. bellii*, however, did not give an indication of movement in the horizontal plan and associated metabolic cost. Neither could the hibernacula site selections (e.g., submerged in mud, lying on the open stream bed or under objects) be determined from this study.

### *Summary*

Observed changes in the reliance on aquatic respiration among seasons for *M. bellii* follows that of other studies of ectothermic turtles where low water temperatures and low turtle metabolic activity translates to an increased reliance on aquatic respiration and vice versa for warmer periods. The cloacal bursae of *M. bellii* is only moderately specialised when compared to other freshwater turtles. Yet despite this, the bursae appear to demonstrate a high level of aquatic respiration capacitance at low water temperature allowing *M. bellii* to sustain prolonged dives in the same way as *R. leukops*. Any species that can stay submerged for weeks without apparent detrimental effects (based on short surfacing times) can most likely survive for an indefinite period of time given aerobic conditions and low relative water temperatures are maintained. The evolutionary forces of the temperate climate experienced by *M. bellii* across its range have meant that highly specialised bursae like those in *R. leukops* are not required to undertake aquatic hibernation during winter. However, a comparison of aquatic respiration levels at higher temperatures might reveal significant differences in dissolved oxygen uptake capabilities between *M. bellii* and other species. Finally, I anticipate that there are a number of other pleurodiran species (e.g., other *Myuchelys* spp) with modest bursae structures from sub-tropical and temperate climates that will exhibit seasonal diving behaviour analogous to *M. bellii*, and these species should be the focus of future studies of this nature.

## Chapter 6. Synopsis



Picture: Andersons Weir pool represents the most downstream extent of the Queensland *Myuchelys bellii* population in Bald Rock Creek. Photo by Col Limpus.

## Chapter 6. Synopsis

### Conservation considerations for *Myuchelys bellii*

Georges (1993) identified rarity, distinctiveness, intrinsic vulnerability to population decline and current threats facing a species as criteria to assess the conservation priorities and status for freshwater turtles. Often though, not enough is known about species and their individual requirements to make unqualified assessments (Georges 1993). These criteria provide a good measure for assessing the conservation priorities for the western sawshelled turtle *M. bellii* and its intrinsic value to the Australian and global chelonian fauna. But before these can be applied with confidence, there were a number of questions that needed addressing, and these formed the framework of this thesis. Accordingly, there were three major themes to my work: (i) seeking taxonomic and nomenclatural stability for populations of *M. bellii*; (ii) establishing baseline data on the life history traits of the reproductive biology and population ecology of *M. bellii*; and (iii) investigating the aquatic respiration ability of *M. bellii* as a life strategy.

Nomenclatural stability and the identification of taxa are crucial to biological conservation because without a clear understanding of taxa, conservation outcomes can be stifled. Legislative instruments for conserving biodiversity rely on good taxonomic knowledge at the species level, yet alpha taxonomy for Australian freshwater turtles has lagged scientific discoveries and has been confused with erroneous descriptions or misidentification of a biological entity (Georges 1993; Georges and Thomson 2010). In addition, numerous variations on species concepts are often debated in the literature (Simberloff 1974; Bush 1975; Templeton 1981; Sites and Marshall 2004; Rubinoff 2006; Bickham *et al.* 2007; Georges and Thomson 2010). Consequently, species boundaries can become blurred without adequate information on poorly known species and unresolved taxonomies.

Identification of biological entities at the specific and sub-specific taxon levels are best attained from more than one line of investigation, as morphology and molecular divergence can be uncoupled leading to potentially conflicting interpretations of relationships among taxa (Georges and Thomson 2010). Discordance between morphology and genetic data has been reported in a number of animal groups including

anurans (Ohmer *et al.* 2009; Guayasamin *et al.* 2010), birds (Phillimore *et al.* 2008; Murphy *et al.* 2010), mammals (Newbound *et al.* 2008), crustaceans (Finston 2000) and turtles (Uwe Fritz *et al.* 2005; Spinks and Shaffer 2005; Uwe Fritz *et al.* 2006; Uwe Fritz *et al.* 2008; Stuart and Uwe Fritz 2008). For this reason, I used phylogenetics (Chapter 2) and morphological comparisons (Chapter 3) to resolve taxonomic issues relevant to *M. bellii*.

The mitochondrial DNA phylogeny of the *Myuchelys* genus revealed a shallow genetic structure of 0.1 - 0.3% divergence across *M. bellii*'s range in the Namoi, Gwydir and Border Rivers indicating only recent separation. Similarly, no diagnostic morphological differences between *M. bellii* populations were found but the holotype specimen was confirmed to be an *M. bellii*. Consequently, the genetic and morphological data were concordant in finding no support for the taxonomic distinction of the Queensland population of *M. bellii* to its southern congeners, thus rejecting earlier assertions by Cann (1998) of a cryptic species in *M. bellii*. Additionally, because of *M. bellii*'s close phylogenetic relationships to other *Myuchelys* species, in particular its sister taxon *M. latisternum* (3.4% mtDNA, Chapter 2), it cannot be considered highly distinctive in Australian or global terms; unlike the pig-nosed turtle *Carettochelys insculpta* which is Australia's most distinctive turtle (Georges 1993; Georges and Wombey 1993; Georges *et al.* 1993). Resolving these taxonomic issues contributes to our understanding of species boundaries and provides a basis for the collection and interpretation of the biology and ecology of *M. bellii* across its distribution (Chapter 4). Taxonomic certainty is also foundational for assessing conservation priorities because without it, the concepts of rarity and distinctiveness could be erroneously applied to sub-populations of *M. bellii*.

In his criteria for assessing conservation priorities, Georges (1993) relates rarity to the geographic distribution and local population size with species locally abundant, but having a restricted distribution, vulnerable to extinction through habitat destruction, climate change or disease. For habitat specialists, local and regional connectivity between populations is important in an overall species strategy to persist in the landscape (Fahrig and Merriam 1994). However, when populations become isolated from each other the chance for local extinctions without recolonisation increases (Blaustein *et al.* 1994). Thus, local factors, such as a large population size, habitat

heterogeneity and within-population dispersal become important determining factors in species persistence (Harrison 1991). The only reported local extinction event for an Australian freshwater turtle was the extinction of the critically endangered western swamp tortoise *P. umbrina* population at ‘Twin Swamps’ in Western Australia leaving only a single population remaining in the wild near Perth (Kuchling and de Jose 1989; Burbidge *et al.* 1990).

In the biological sense, *M. bellii* is certainly rare (vulnerable to extinction) with only three isolated populations occurring in the Darling River catchment of the Murray-Darling Basin. Of the three populations, the most significant management unit is from Bald Rock Creek because of its very restricted distribution (8 km section) and small population size (<400 individuals, Chapter 4). The two NSW populations from the Namoi and Gwydir Rivers also have restricted distributions (<100 linear kilometres each) and are suffering from an eye disease (Chapter 4) which is having an unknown effect on population demographics. Surveys of the current distribution and population sizes of *M. bellii* in NSW are now needed to inform conservation managers in developing strategies for its protection and recovery into the future.

The intrinsic vulnerability of a species, the third criteria described by Georges (1993) for determining conservation status, is related to its life history traits where its biology renders it more or less vulnerable to population decline and ultimate extinction. *Myuchelys bellii* have delayed age at first breeding, low annual fecundity and high survivorship with a predicted lifespan of over 40 years (Type III survivorship; Chapter 4). Long-lived species such as *M. bellii* can be susceptible to a sustained lack of recruitment which can take decades for the effects to show in population declines and subsequently decades for a species to recover following successful intervention (see loggerhead turtle example in Limpus 2008). A lack of recruitment into the adult population for a number of Australian freshwater species has been attributed to high predation rates by feral and native animals (foxes, pigs and goannas) (Hamann *et al.* 2007). Predation by foxes on *M. bellii* populations is suspected to be high in areas where fox control is limited or non-existent because of the significant predation rates reported for other turtle populations in the Murray-Darling Basin and adjacent catchments (Thompson 1983; Spencer and Thompson 2005). In addition, exotic fish may be having a detrimental impact on hatchling survival. Thus, these threatening

processes in conjunction with low annual fecundity and delayed sexual maturity make *M. bellii* intrinsically vulnerable to extinction.

The fourth criteria that Georges (1993) provides for guidance in setting conservation priorities for turtles is an assessment of current threats with a key requirement being the demonstration of population declines (whether from human or natural causes). Population trends for the long-lived *M. bellii* are not easily gained from a relatively small timeframe of a few years – the duration of this study. Nonetheless the information gained by my work on its reproductive biology and demographics (Chapter 4) provides a good baseline for future monitoring efforts of its population in Bald Rock Creek, and more generally, for the Namoi and Gwydir populations. The data for the Bald Rock Creek population seem to indicate a healthy, but small, population with numerous immature turtles recorded from different cohorts across years. A possible concern though for the health of *M. bellii* populations is the apparent lack of adult females in the Bald Rock Creek population (sex ratio 2:1 in favour of males) and a lack of adult males in the Namoi population (ratio 0.4:1 in favour of females). However, no attempt was made by me to determine if these observations were artefacts of trapping effort (trap shy animals, sampling biases) or reflect real population structure (for a detailed discussion on turtle sex ratios, refer to Georges *et al.* 2006). In addition to the pressing need to determine the distribution and population size of *M. bellii* in NSW, a monitoring program that extends over many years for the Bald Rock Creek population would be required to elucidate any declines or increase in numbers and extent.

Another significant threat to *M. bellii* populations is the continued loss and alteration of its habitat across its known distribution. In Bald Rock Creek, one of the large permanent natural waterholes (approximately 1.4 km in length) was impounded in 1987 resulting in a significant proportion of available habitat being changed to a lacustrine environment. Existing impoundments (e.g., Copeton Dam and Keepit Dam) on the Namoi and Gwydir Rivers have similarly caused loss and alteration of habitat for *M. bellii* populations in NSW. Decreased oxygen levels are thought to negatively impact on turtle species that rely on aquatic respiration with dive durations substantially reduced under hypoxic conditions (Gordos *et al.* 2004; Clark *et al.* 2009). Given the propensity of *M. bellii* to undertake aquatic hibernation at depth during winter and rely on cloacal ventilation to remain aerobic (Chapter 5), much of the water column in the stagnant

weir pool in Bald Rock Creek would be hypoxic resulting in unsuitable habitat (Tucker 2000; Tucker *et al.* 2001; Gordos *et al.* 2004; Clark *et al.* 2009).

In addition to the changes to the water quality, impoundment of the channel and the mechanical removal of vegetation and modification of the stream bed and banks within reservoirs results in the loss of heterogeneity. A reduction in overall productivity and dietary changes in depauperate environments has been shown to result in reduced growth rates and decreased fecundity of freshwater turtles in comparison to their congeners in more productive waters (Georges 1982; Georges 1985; Parmenter and Avery 1990; Kennett *et al.* 2009). Habitat specialists are potentially the most affected by a loss of habitat heterogeneity (e.g., pool riffle sequences) (Gordos 2003). These larger impoundments may also act as significant barriers to dispersal and gene flow between *M. bellii* populations.

### **Ecophysiology and diving behaviour of *Myuchelys bellii***

Not unlike *Rheodytes leukops* which is considered to be a habitat specialist associated with well oxygenated riffle zones in the Fitzroy River (Legler and Cann 1980; Georges 1993), *M. bellii* is only found above about 700 masl in presumably well oxygenated cold flowing streams in the headwaters of the Murray-Darling Basin. It is restricted to riverine habitats and requires permanent aquatic refugia to persist at the local scale (Chapter 4). My study was the first to describe the extraordinary aquatic respiration abilities of *M. bellii* which allow for extended aerobic dives of up to weeks during winter hibernation which I attribute to its cloacal bursae and temperate climate across its range (Chapter 5). Criptodirans from the northern hemisphere aquatically respire via buccal or cutaneous pathways whereas in pleurodires, the cloacal bursae are the primary site for aquatic oxygen uptake (Peterson and Greenshields 2001). Despite its moderately specialised bursae morphology in comparison to other Australian chelids, *M. bellii* is second only to *Rheodytes leukops* in having the longest recorded dive for any freely diving freshwater or marine turtle at 15.5 days (maximum) (Chapter 5).

The evolutionary significance of this finding relates to the current paradigm in the literature that only highly specialised bursae allow for extended aerobic dives (see Chapter 5). Yet *M. bellii* dives recorded across all seasons can extend well beyond the aerobic dive limit (ADL) of 1.5 h after which non-hibernating turtles generally expend

their O<sub>2</sub> supplies in their lungs (Jackson 1968; Caligiuri *et al.* 1981; Crocker *et al.* 1999). In fact the seasonal diving behaviour of *M. bellii* corresponds closely to those observed for the habitat specialist *R. leukops*, albeit at lower ambient water temperatures. Consequently, the intermediate complexity of the bursae structure for *M. bellii* may allow for an unexpected level of aquatic oxygen uptake, particularly at low temperatures and at depth. Furthermore, the evolutionary forces of a temperate climate have meant that highly specialised bursae like those in *R. leukops* are not required to undertake aquatic hibernation during winter.

The observed behaviour of aquatic hibernation at depth has implications for the conservation management of *M. bellii* across its distribution. Water depth appears to be a significant factor in the selection of aquatic hibernacula and consequently, maintaining these oxygenated deeper waterholes is an important consideration for decisions regarding winter drawdown for human uses. In addition, any further instream water developments within the distribution of *M. bellii* would most likely have a detrimental impact on its populations for the reasons given above.

#### **Future directions for the conservation of *Myuchelys bellii***

Populations of the western sawshelled turtle in the Namoi and Gwydir River catchments are listed as vulnerable under the New South Wales *Threatened Species Conservation Act 1995* and the Commonwealth government *Environment Protection and Conservation Act 1999*. The Queensland population is currently listed as common under the *Nature Conservation Act 1992* because of the historical uncertainty around its taxonomic identity. However, in 2009 I submitted a threatened species nomination to the Queensland Department of Environment and Resource Management (DERM) scientific advisory committee (SAC). The SAC endorsed a listing of a vulnerable conservation status for the Bald Rock Creek population of *M. bellii* which is now awaiting formal endorsement from the Queensland Parliament. In February 2009, I also participated in an expert panel for the International Union for the Conservation of Nature (IUCN) Red List for Australian freshwater turtles. At this meeting, my preliminary research data were presented and it was determined that *M. bellii* met the endangered criteria B1 and B2c. These criteria relate to the species distribution occupying an area <5000 km<sup>2</sup> and the species suffering declines from habitat modification or loss. Consequently, the current IUCN Red List has *M. bellii* as

internationally endangered (IUCN 2010). By having a legislative conservation status accorded to all populations of *M. bellii*, it provides an official mandate for conservation agencies to allocate limited resources to its protection and recovery.

I regard the small, disjunct population in Bald Rock Creek to be of particular management concern for *M. bellii* (see Chapter 4) that warrants a greater conservation focus including implementation of a long-term monitoring program. The Queensland DERM, Southern Downs Regional Council, the local community of Stanthorpe and the smaller townships located in the granite belt of the New England Tablelands and landholders are key stakeholders to any efforts for restoration and protection of *M. bellii* and its habitat. A number of conservation measures to sustain the population in Girraween National Park have already been implemented including monitoring of water levels in Bald Rock Creek and applying restrictions on the water usage of recreational campers during drought. An active feral animal control program for goldfish and foxes is also in place. These measures need to continue as well as the restoration of riparian vegetation and instream habitats that were previously lost from impoundment and mechanical removal are required to recover, and increase, its potential range. In the same way, targeted restoration of riparian zones through grazing management (off-stream watering points, fenced river banks) and regeneration of native vegetation cover in the Namoi and Gwydir catchments is needed to consolidate and extend its range in these catchments. No western sawshelled turtle was located within Accommodation Creek in Queensland during this study. However, I observed suitable habitat which may sustain a small population or offer future sites for relocation or expansion of its distribution in Queensland. Thus protection of remaining native vegetation and instream habitats in areas outside protected estate should be a focus of conservation efforts.

The affect of feral animals (e.g, foxes and pigs) on nest predation and recruitment into the adult population is unknown and should be a target of future studies into *M. bellii*, particularly for NSW populations which have a vast majority of their distributions outside of protected estate. Also, the active control of exotic goldfish, redfin and trout species would also benefit the conservation of *M. bellii* through improved recruitment of hatchlings into adult populations. Education and awareness of this turtle's habitat requirements and limited distribution is needed, particularly for local governments, recreational fishers and landholders. Interpretive signs at public locations to inform

local fishermen and landholders of the turtle's presence and need for protection would be beneficial.

Areas of future research to better understand *M. bellii* and its overall life strategies include investigation of its aquatic respiration abilities across all size classes (juveniles and adults) and its behavioural and ecological responses to artificial impoundments, and the significance of aquatic hibernation at depth. This would include research into the effects (or lack thereof) of impoundments on life history traits of annual fecundity and dietary requirements of *M. bellii* by comparing between populations in the free flowing and impounded sections. Research effort should also be given to better understanding the current population size and distribution in NSW. The Queensland population should also be subjected to further genetic investigations to test for possible inbreeding within this small population.

In addition, the zone of overlap between *M. bellii* and *Emydura macquarii* is restricted to the contact points at the margins of their distributions and it is unknown if there is a negative interspecific effect with only a few isolated individuals recorded to venture either up or downstream into the other species range. This observation may also relate to the varying tolerances of each species to water temperatures with *Emydura* possibly preferring the warmer waters in lowland streams to the higher altitude cold water streams and vice versa for *M. bellii*. This is one area of future research worthy of study to determine barriers to *M. bellii* movement and possible implications for climate change should average ambient water temperatures increase. Lastly, I anticipate that a number of similar pleurodiran species with modest bursae structures from sub-tropical and temperate climates will exhibit seasonal diving behaviour analogous to *M. bellii*, and these species should be the focus of future studies of this nature.

## References

- Alacs, E.A., 2008. *Forensics, Phylogeography and Population Genetics: A case study using the Northern Snake-necked turtle, Chelodina rugosa*. PhD Thesis. Canberra, Australia: University of Canberra.
- Alacs, E.A. and Georges, A., 2008. Wildlife across our borders: a review of the illegal trade in Australia. *Australian Journal of Forensic Sciences*, 40, pp.107-123.
- Allanson, M. and Georges, A., 1999. Diet of *Elseya purvisi* and *Elseya georgesii* (Testudines: Chelidae), a sibling species pair of freshwater turtles from eastern Australia. *Chelonian Conservation and Biology*, 3(3), pp.473-477.
- Anderson, B.J., Armsworth, P.R., Eigenbrod, F., Thomas, C.D., Gillings, S., Heinemeyer, A., Roy, D.B. and Gaston, K.J. 2009. Spatial covariance between biodiversity and other ecosystem service priorities. *Journal of Applied Ecology*, 46(4), pp.888-896.
- Anderson, D.R., Burnham, K.P. and White, G.C., 1994. AIC model selection in overdispersed capture-recapture data. *Ecology*, 75, pp.1780-1793.
- Artner, H., 2008. The world's extant turtle species, Part 1. *Emys*, 15, pp.4-32.
- Awise, J.C., Bowen, B.W., Lamb, T., Meylan, A.B. and Bermingham, E., 1992. Mitochondrial DNA evolution at a turtle's pace: evidence for low genetic variability and reduced microevolutionary rate in the Testudines. *Molecular Biology and Evolution*, 9(3), pp.457-473.
- Bagatto, B., Guyer, C., Hauge, B. and Henry, R.P., 1997. Bimodal respiration in two species of central American turtles. *Copeia*, 1997(4), pp.834-839.
- Bagatto, B. and Henry, R.P., 1999. Aerial and aquatic respiration in the snapping turtle, *Chelydra serpentina*. *Journal of Herpetology*, 33, pp.490-492.
- Baumgartner, S., 2007. The insurance value of biodiversity in the provision of ecosystem services. *Natural Resource Modelling*, 20(1), pp.87-126.
- Boulenger, G.A., 1889. Catalogue of the Chelonians, Rhynchocephalians, and Crocodiles in the British Museum (Natural History). British Museum of Natural History.
- Bickham, J.W., Iverson, J.B., Parham, J.F., Philippen, H.D., Rhodin, A.G.J., Shaffer, H.B., Spinks, P.Q. and Dijk, P.P., 2007. An annotated list of modern turtle terminal taxa with comments on areas of taxonomic instability and recent change. *Chelonian Research Monographs*, 4, pp.173-199.
- Blamires, S.J., Spencer, R.J., King, P. and Thompson, M.B., 2005. Population parameters and life-table analysis of two coexisting freshwater turtles: are the Bellinger River turtle populations threatened? *Wildlife Research*, 32, pp.339-

- Blaustein, A.R., Wake, D.B. and Sousa, W.P., 1994. Amphibian declines: judging stability, persistence, and susceptibility of populations to local and global extinctions. *Conservation Biology*, 8(1), pp.60-71.
- Booth, D., 2002. The breaking of diapause in embryonic broad-shell river turtles (*Chelodina expansa*). *Journal of Herpetology*, 36(2), pp.304-307.
- Boyer, D.T., 1965. Ecology of the basking habit in turtles. *Ecology*, 46, pp.99-118.
- Brown, W., George, M. and Wilson, A., 1979. Rapid evolution of animal mitochondrial DNA. *Proceedings from the National Academy of Science USA*, 76, pp.1967-1971.
- Buhlmann, K.A., Akre, T.S.B., Iverson, J.B., Karapatakis, D., Mittermeier, R.A., Georges, A., Rhodin, A.G.J., van Dijk, P.P. and Gibbons, J.W., 2009. A Global Analysis of Tortoise and Freshwater Turtle Distributions with Identification of Priority Conservation Areas. *Chelonian Conservation and Biology*, 8(2), pp.116-149.
- Burbidge, A.A., 1981. The ecology of the western swamp tortoise *Pseudemydura umbrina* (Testudines: Chelidae). *Australian Wildlife Research*, 8, pp.203-223.
- Burbidge, A.A. and Kuchling, G., 1994. *Western swamp tortoise recovery plan*, Como, WA.
- Burbidge, A.A., Kuchling, G., Fuller, P.J., Graham, G. and Miller, D., 1990. *The Western Swamp Tortoise*, PO Box 104, Como, WA 6152, Australia: Western Australian Department of Conservation and Land Management.
- Bush, G.L., 1975. Modes of animal speciation. *Annual Review of Ecology and Systematics*, 6, pp.339-364.
- Butler, P.J. and Jones, D.R., 1997. Physiology of diving birds and mammals. *Physiological Reviews*, 77, pp.837-899.
- Butler, P.J., Milsom, W.K. and Woakes, A.J., 1984. Respiratory, cardiovascular and metabolic adjustments during steady state swimming in the green turtle, *Chelonia mydas*. *Journal of Comparative Physiology B.*, 154, pp.167-174.
- Cailliet, G.M., Mollet, H.F., Pittenger, G.G., Bedford, D., and Natanson, L.J., 1992. Growth and demography of the Pacific angel shark (*Squatina californica*), based on tag returns of California. *Australian Journal of Marine and Freshwater Research*, 43, pp.1313-30.
- Cairns, J., 1997. Protecting the delivery of ecosystem services. *Ecosystem Health*, 3(3), pp.185-194.
- Caligiuri, M., Robin, E.D., Hance, A.J., Robin, D.A., Lewiston, N. and Theodore, J.,

1981. Prolonged diving and recovery in the freshwater turtle, *Pseudemys scripta* - II magnitude of depression of O<sub>2</sub> requirements and the relation to body O<sub>2</sub> stores. *Comparative Biochemistry and Physiology Part A: Physiology*, 70(3), pp.365-369.
- Cann, J., 1998. *Australian Freshwater Turtles*, Singapore: Beaumont Publishing.
- Cann, J., 1997a. Georges short-necked turtle. *Monitor (Victorian Herpetological Society, Melbourne)*, 9(1), pp.18-23, 31, 32.
- Cann, J., 1997b. Irwin's Turtle. *Monitor (Victorian Herpetological Society, Melbourne)*, 9(1), pp.36-40, 31-32.
- Cann, J., 1997c. The northern yellow-faced turtle *Emydura tanybaraga* sp. nov. Holotype A.M. R125498 Collected by Dr. A. Georges. *Monitor (Victorian Herpetological Society, Melbourne)*, 9(1), pp.24--29, 31-32, 34-35.
- Cann, J., 1978. *Tortoises of Australia*, Sydney: Angus and Robertson.
- Cann, J. and Legler, J.M., 1994. The Mary River Tortoise: A new genus and species of short-necked chelid from Queensland, Australia (Testudines: Pleurodira). *Chelonian Conservation and Biology*, 1, pp.81-96.
- Carrière, M., Rollison, N., Suley, A.N. and Brooks, R.J., 2008. Thermoregulation when the growing season is short: sex-biased basking patterns in a northern population of painted turtles (*Chrysemys picta*). *Journal of Herpetology*, 42(1), pp.206-209.
- Chaloupka, M., Kamezaki, N. and Limpus, C.J., 2008. Is climate change affecting the population dynamics of the endangered Pacific loggerhead sea turtle? *Journal of Experimental Marine Biology and Ecology*, 356, pp.136-143.
- Chen, T.H. and Lue, K.Y., 2002. Growth patterns of the Yellow-margined Box Turtle (*Cuora flavomarginata*) in Northern Taiwan. *Journal of Herpetology*, 36(2), pp.201-208.
- Chessman, B.C., 1987. Atmospheric and aquatic basking of the Australian freshwater turtle *Emydura macquarii* (Gray) (Testudines: Chelidae). *Herpetologica*, 43(3), pp.301-306.
- Clark, N.J., Gordos, M.A. and Franklin, C.E., 2008. Diving behaviour, aquatic respiration and blood respiratory properties: a comparison of hatchling and juvenile Australian turtles. *Journal of Zoology*, 275, pp.399-406.
- Clark, N.J., Gordos, M.A. and Franklin, C.E., 2009. Implications of river damming: the influence of aquatic hypoxia on the diving physiology and behaviour of the endangered Mary River turtle. *Animal Conservation*, 12, pp.147-154.
- Clark, N.J., Gordos, M.A. and Franklin, C.E., 2008. Thermal plasticity of diving behaviour, aquatic respiration, and locomotor performance in the Mary River turtle *Elusor macrurus*. *Physiological and Biochemical Zoology*, 81(3), pp.301-

- Clay, B.T., 1981. Observations on the breeding biology and behaviour of the long-necked tortoise, *Chelodina oblonga*. *Journal of the Royal Society of Western Australia*, 4, pp.27-32.
- Coin, L. and Durbin, R., 2004. Improved techniques for the identification of pseudogenes. *Bioinformatics*, 20(Suppliment 1), pp.94-100.
- Cormack, R.M., 1964. Estimates of survival from the sighting of marked animals. *Biometrika*, 51, pp.429-438.
- Crocker, C.E., Ultsch, G.R. and Jackson, D.C., 1999. The physiology of diving in a north-temperate and three tropical turtle species. *Journal of Comparative Physiology B.*, 169, pp.249-255.
- Cullen, P., 2003. *The Heritage River Proposal - Conserving Australia's undamaged rivers*, University of Queensland Printery, St Lucia, Queensland, Australia.
- van Dam, R.P. and Diez, C.E., 1996. Diving behaviour of immature hawksbills (*Eretmochelys imbricata*) in a Caribbean cliff-wall habitat. *Marine Biology*, 127, pp.171-178.
- de Vis, C.W., 1897. The extinct freshwater turtles of Queensland. *Annals of the Queensland Museum*, 3, pp.3-7.
- Doherty, R.M. *et al.*, 2010. Implications of future climate and atmospheric CO<sub>2</sub> content for regional biogeochemistry, biogeography and ecosystem services across East Africa. *Global Change Biology*, 16, pp.617-640.
- DonnerWright, D.M., Bozek, M.A., Probst, J.R. and Anderson, E.M., 1999. Responses of turtle assemblage to environmental gradients in the St. Croix River in Minnesota and Wisconsin, U.S.A. *Canadian Journal of Zoology*, 77(6), pp.989-1000.
- Doody, J.S., Sims, R.A. and Georges, A., 2001. Use of thermal springs for aquatic basking by the pig-nosed turtle, *Carettochelys insculpta*. *Chelonian Conservation and Biology*, 4, pp.81-87.
- Dunson, W.A., 1967. Sodium fluxes in freshwater turtles. *Journal of Experimental Zoology*, 165, pp.171-182.
- Engstrom, T.N., Shaffer, H.B. and McCord, W.P., 2004. Multiple data sets, high homoplasy, and the phylogeny of softshell turtles (Testudines: Trionychidae). *Systematic Biology*, 53(5), pp.693-710.
- Engstrom, T.N., Shaffer, H.B. and McCord, W.P., 2002. Phylogenetic diversity of endangered and critically endangered southeast Asian softshell turtles (Trionychidae: Chitra). *Biological Conservation*, 104(2), pp.173-179.

- Ernst, C.H., Zappalorti, R.T. and Lovich, J.E., 1989. Overwintering sites and thermal relations of hibernating bog turtles, *Clemmys muhlenbergii*. *Copeia*, 1989(3), pp.761-764.
- Ewing, H.E., 1939. Growth in the eastern box-turtle, with special reference to the dermal shields of the carapace. *Copeia*, 1939(2), pp.87-92.
- Fabens, A.J., 1965. Properties and fitting of the von Bertalanffy growth curve. *Growth*, 29, pp.265-289.
- Fahrig, L. and Merriam, G., 1994. Conservation of fragmented populations. *Conservation Biology*, 8(1), pp.50-59.
- Faith, D.P., 1992. Conservation evaluation and phylogenetic diversity. *Biological Conservation*, 61, pp.1-10.
- Finston, T., 2000. Morphology and molecules conflict to confound species boundaries in salt lake ostracodes of the genus *Mytilocypris* (Crustacea : Ostracoda). *Australian Journal of Zoology*, 48, pp.393-409.
- FitzGibbon, S.I., 1998. *The diving physiology and dive behaviour of an undescribed turtle from the Mary River, Queensland (Elseya sp.)*. University of Queensland Editor.
- FitzGibbon, S.I. and Franklin, C.E., 2010. The importance of the cloacal bursae as the primary site of aquatic respiration in the freshwater turtle, *Elseya albagula*. *Australian Zoologist*, 35(2), pp.276-282.
- FitzSimmons, N.N., Moritz, C. and Moore, S.S., 1995. Conservation and dynamics of microsatellite loci over 300 million years of marine turtle evolution. *Molecular Biology and Evolution*, 12(3), pp.432-440.
- Flakus, S., 2002. *The ecology of the Mary River turtle, Elusor macrurus*. MSc Thesis. University of Queensland.
- Frazer, N.B., Gibbons, J.W. and Greene, J.L., 1991. Life history and demography of the common mud turtle *Kinosternon subrubrum* in south Carolina, USA. *Ecology*, 72(6), pp.2218-2231.
- Fritz, U., Ayaz, D., Buschbom, J., Kami, H., Mazanaeva, L.F., Aloufi, A.A., Auer, M., Rifai, L., Silic, T. and Hundsdoerfer, A.K., 2008. Go east: phylogeographies of *Mauremys caspica* and *M. rivulata* - discordance of morphology, mitochondrial and nuclear genetic markers and rare hybridization. *Journal of Evolutionary Biology*, 21(2), pp.527-540.
- Fritz, U., d'Angelo, S. and Pennisi, M.G., 2006. Variation of Sicilian pond turtles, *Emys trinacris* - What makes a species cryptic? *Amphibia-Reptilia*, 27(4), pp.513-529.
- Fritz, U., Fattizzo, T., Guicking, D., Tripepi, S., Pennisi, M.G., Lenk, P., Loger, U. and Wink, M., 2005. A new cryptic species of pond turtle from southern Italy, the

- hottest spot in the range of the genus *Emys* (Reptilia, Testudines, Emydidae). *Zoologica Scripta*, 34(4), pp.351-371.
- Fritz, U. *et al.*, 2008. Diversity of the Southeast Asian leaf turtle genus *Cyclemys*: how many leaves on its tree of life? *Zoologica Scripta*, 37(4), pp.367-390.
- Fritz, U., Guicking, D., Auer, M., Sommer, R.S., Wink, M. and Hundsdorfer, A.K., 2007. Phenotypic plasticity leads to incongruence between morphology-based taxonomy and genetic differentiation in western *Palaeartic tortoises* (Testudo graeca complex; Testudines, Testudinidae). *Amphibia-Reptilia*, 28(1), pp.97-121.
- Gaffney, E.S., 1979. Fossil chelid turtles of Australia. *American Museum Novitates*, 2681, pp.1-23.
- Gaffney, E.S., 1981. A review of the fossil turtles of Australia. *American Museum Novitates*, 2720, pp.1-38.
- Gaffney, E.S., 1977. The side-necked turtle family Chelidae: A theory of relationships using shared derived characters. *American Museum Novitates*, 2620, pp.1-28.
- Gatten, R.E., 1980. Aerial and aquatic oxygen uptake by freely-diving snapping turtles (*Chelydra serpentina*). *Oecologia*, 46(2), pp.266-271.
- Gayanilo, F.C., Sparre, P. and Pauly, D., 2005. *FAO\_ICLARM Stock Assessment Tools II (FiSAT II) User's Guide*. *FAO Computerized Information Series (Fisheries)*, 8, Revised Version, FAO, Rome.
- Georges, A., 1982. Diet of the Australian freshwater turtle *Emydura krefftii* (Chelonia : Chelidae), in an unproductive lentic environment. *Copeia*, 2, pp.331-336.
- Georges, A., 1985. Reproduction and reduced body size of reptiles in unproductive insular environments. In *Biology of Australasian frogs and reptiles*. Royal Zoological Society of New South Wales, pp. 311-318.
- Georges, A., 1983. Reproduction of the Australian freshwater turtle *Emydura krefftii* (Chelonia: Chelidae). *London Journal of Zoology*, 201, pp.331-350.
- Georges, A., 1993. Setting conservation priorities for Australian freshwater turtles. In D. Lunney and D. Ayers, eds. *Herpetology in Australia: A Diverse Discipline*. Sydney: Royal Zoological Society of New South Wales, pp. 49-58.
- Georges, A. and Adams, M., 1992. A phylogeny for Australian chelid turtles based on allozyme electrophoresis. *Australian Journal of Zoology*, 40, pp.453-476.
- Georges, A. and Adams, M., 1996. Electrophoretic delineation of species boundaries within the short-necked freshwater turtles of Australia (Testudines: Chelidae). *Zoological Journal of the Linnean Society*, 118, pp.241-260.
- Georges, A., Birrell, J., Saint, K.M., McCord, W.P. and Donnellan, S.C., 1998. A

- phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation. *Biological Journal of the Linnean Society*, 67, pp.213-246.
- Georges, A. and Cottingham, P., 2002. *Biodiversity in inland waters - priorities for its protection and management. Recommendations from the 2001 Fenner Conference on the Environment.*, Canberra, Australia: Cooperative Research Centre for Freshwater Ecology.
- Georges, A., Guarino, F. and White, M., 2006. Sex-ratio bias across populations of a freshwater turtle (Testudines:Chelidae) with genotypic sex determination. *Wildlife Research*, 33, pp.475-480.
- Georges, A., Limpus, C.J. and Parmenter, C.J., 1993. Natural history of the Chelonia. In *Fauna of Australia*. Canberra: Australian Government Publishing Service, p. Chapter 17.
- Georges, A. and Thomson, S., 2010. Diversity of Australasian freshwater turtles, with an annotated synonymy and keys to species. *Zootaxa*, 2496, pp.1-37.
- Georges, A. and Thomson, S., 2005. Evolution and Zoogeography of Australian Freshwater Turtles. In *Freshwater Turtles*.
- Georges, A., Walsh, R., Spencer, R.J., Welsh, M. and Shaffer, H.B., 2007. *The Bellinger Emydura. Challenges for Management*. Report to NSW National Parks and Wildlife Service, Sydney, by the Institute for Applied Ecology, University of Canberra., p. 1-20, Canberra
- Georges A, Spencer, R.J., Welsh, M, Shaffer, H.B., Walsh, R, and Zhang, X., 2011. Application of the precautionary principle to taxa of uncertain status – the case of the Bellinger River Turtle. *Endangered Species Research* submitted.
- Georges, A. *et al.*, 2002. *National River Health Program: Modelling dry season flows and predicting the impact of water extraction on a flagship species.*, Canberra: Department of Lands, Planning and Environment, NT.
- Georges, A. and Wombey, J.C., 1993. Family Carettochelyidae. In *Fauna of Australia, Volume 2: Amphibia, Reptilia, Aves*. Canberra: Australian Government Publishing Service, p. 153-156.
- Germano, D.J. and Bury, R.B., 1998. Age determination in turtles: evidence of annual deposition of scute rings. *Chelonian Conservation and Biology*, 3(1), pp.123-132.
- Goode, J., 1967. *Freshwater Tortoises of Australia and New Guinea (in the Family Chelidae)*, Melbourne: Lansdowne Press.
- Gordos, M.A., 2003. *Diving physiological ecology of the bimodally respiring freshwater turtle, Rheodytes leukops*. PhD Thesis. Brisbane: University of Queensland.

- Gordos, M.A. and Franklin, C.E., 2002. Diving behaviour of two Australian bimodally respiring turtles, *Rheodytes leukops* and *Emydura macquarii*, in a natural setting. *Journal of Zoology, London*, 258, pp.335-342.
- Gordos, M.A., Franklin, C.E. and Limpus, C.J., 2004. Effect of water depth and water velocity upon the surfacing frequency of the bimodally respiring freshwater turtle, *Rheodytes leukops*. *Journal of Experimental Biology*, 207, pp.3099-3107.
- Gordos, M.A., Franklin, C.E. and Limpus, C.J., 2003a. Seasonal changes in the diel surfacing behaviour of the bimodally respiring turtle *Rheodytes leukops*. *Canadian Journal of Zoology*, 81, pp.1614-1622.
- Gordos, M.A., Franklin, C.E. and Limpus, C.J., 2003b. Seasonal changes in the diving performance of the bimodally respiring freshwater turtle *Rheodytes leukops* in a natural setting. *Canadian Journal of Zoology*, 81(4), pp.617-625.
- Gordos, M.A., Franklin, C.E., Limpus, C.J. and Wilson, G., 2004. Blood-respiratory and acid-base changes during extended diving in the bimodally respiring freshwater turtle *Rheodytes leukops*. *Journal of Comparative Physiology B.*, 174, pp.347-354.
- Gordos, M.A., Hamann, M., Schauble, C.S., Limpus, C.J. and Franklin, C.E., 2007. Diving behaviour of *Elseya albagula* from a naturally flowing and hydrologically altered habitat. *Journal of Zoology*, 272, pp.458-469.
- Gould, S.J. 1977. *Ontogeny and Phylogeny*. Cambridge, MA: The Belknap Press of Harvard University Press.
- Graham, T.E. and Forsberg, J.E., 1991. Aquatic oxygen uptake by naturally wintering wood turtles *Clemmys insculpta*. *Copeia*, 1991(3), pp.836-838.
- Gray, J.E., 1841. A catalogue of the species of reptiles and amphibia hitherto described as inhabiting Australia, with a description of some new species from Western Australia. In *Journals of Two Expeditions of Discovery in North-west and Western Australia, during the years 1837, 38 and 39, under the Authority of Her Majesty's Government*. London: T. and W. Boone, pp. 422-449.
- Gray, J.E., 1830. A synopsis of the species of the class Reptilia. In *The Animal Kingdom Arranged in Conformity with its Organization by the Baron Cuvier*. London: Whittaker, Treacher and Co., pp. 1-110.
- Gray, J.E., 1844. Catalogue of the Tortoises, Crocodiles and Amphibianians in the Collection of the British Museum. In London: Edward Newman.
- Gray, J.E., 1867. Description of a new Australian tortoise (*Elseya latisternum*). *Annals and Magazine of Natural History*, 20, pp.43-45.
- Gray, J.E., 1856. On some new freshwater tortoises from North America, Ceylon and Australia, in the collection of the British Museum. *Proceedings of the*

- Zoological Society of London*, 1855, pp.197-202.
- Gray, J.E., 1863. On the species of *Chelymys* from Australia, with the description of a new species. *Annals and Magazine of Natural History*, 12, pp.98-99, 246.
- Gray, J.E., 1872. On the genus *Chelymys* and its allies from Australia. *Proceedings of the Zoological Society of London* 1872:504–514.
- Grigg, G.C., Johansen, K., Harlow, P., Beard, L.A. and Taplin, L.E., 1986. Facultative aestivation in a tropical freshwater turtle *Chelodina rugosa*. *Comparative Biochemistry and Physiology Part A: Physiology*, 82, pp.321-323.
- Guayasamin, J.M., Bonaccorso, E., Duellman, W.E. and Coloma, L.A., 2010. Genetic differentiation in the nearly extinct harlequin frogs (Bufonidae: *Atelopus*), with emphasis on the Andean *Atelopus ignescens* and *A. bomolochos* species complexes. *Zootaxa*, 2574, pp.55-68.
- Hamann, M., Schauble, C.S., Emerick, S.P., Limpus, D.J. and Limpus C.J., 2008. Freshwater turtle populations in the Burnett River. *Memoirs of the Queensland Museum*, 52(2), pp.221-232.
- Hamann, M., Schauble, C.S., Limpus, D.J., Emerick, S.P. and Limpus C.J., 2007. *Management Plan for the conservation of Elseya sp. [Burnett River] in the Burnett River Catchment.*, (Queensland Environmental Protection Agency: Brisbane).
- Harrison, S., 1991. Local extinction in a metapopulation context: an empirical evaluation. *Biological Journal of the Linnean Society*, 42, pp.73-88.
- Hasegawa, H., Kishino, H. and Yano, T., 1985. Dating the human-ape split by a molecular clock by mitochondrial DNA. *Molecular Evolution*, 22, pp.160-174.
- Haxton, T. and Berrill, M., 1999. Habitat selectivity of *Clemmys guttata* in central Ontario. *Canadian Journal of Zoology*, 77(4), pp.593-599.
- Hay, J., Sarre, S. and Daugherty, C., 2004. Nuclear mitochondrial pseudogenes as molecular outgroups for phylogenetically isolated taxa: a case study in *Sphenodon*. *Heredity*, 93, pp.468-475.
- Hays, G.C., Hobson, V.J., Metcalfe, J.D., Righton, D. and Sims, D.W., 2006. Flexible foraging movements of leatherback turtles across the north Atlantic Ocean. *Ecology*, 87(10), pp.2647-2656.
- Heppell, S.S., 1998. Application of life-history theory and population model analysis to turtle conservation. *Copeia*, 1998(2), pp.367-375.
- Hochscheid, S., Bentivegna, F., Hamza, A. and Hays, G.C., 2010. When surfacers do not dive: multiple significance of extended surface times in marine turtles. *Journal of Experimental Biology*, 213, pp.1328-1337.

- IUCN, 2010. *IUCN Red List of Threatened Species. Version 2010.4.*  
<<http://www.iucnredlist.org>>. Downloaded on 16 December 2010.
- Iverson, J.B., 1991a. Life history and demography of the yellow mud turtle, *Kinosternon flavescens*. *Herpetologica*, 47(4), pp.373-395.
- Iverson, J.B., 1991b. Patterns of survivorship in turtles (order Testudines). *Canadian Journal of Zoology*, 69(2), pp.385-391.
- Jackson, D.C., 2000. Living without oxygen: lessons from the freshwater turtle. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, 125(3), pp.299-315.
- Jackson, D.C., 1968. Metabolic depression and oxygen depletion in the diving turtle. *Journal of Applied Physiology*, 24, pp.503-509.
- Jackson, D.C., 1976. Non-pulmonary CO<sub>2</sub> loss during diving in the turtle, *Pseudemys scripta elegans*. *Comparative Biochemistry and Physiology Part A: Physiology*, 55(3), pp.237-241.
- Jackson, D.C., Allen, J. and Strupp, P.K., 1976. The contribution of non-pulmonary surfaces to CO<sub>2</sub> loss in 6 species of turtles at 20 °C. *Comparative Biochemistry and Physiology Part A: Physiology*, 55(3), pp.243-246.
- Jackson, D.C., Herbert, C.V. and Ultsch, G.R., 1984. The comparative physiology of diving North American freshwater turtles. II. Plasma ion balance during prolonged anoxia. *Physiological Zoology*, 57(6), pp.632-640.
- Jackson, D.C., Taylor, S.E., Asare, V.S., Villarnovo, D., Gall, J.M. and Reese, S.A., 2007. Comparative shell buffering properties correlate with anoxia tolerance in freshwater turtles. *American Journal of Regulatory, Integrative and Comparative Physiology*, 292, pp.R1008-R1015.
- Jeffree, R.A. and Jones, M.K., 1992. Accumulation of radiocalcium from the aquatic medium via the cloaca and buccopharynx of Australian freshwater turtles (Chelidae). *Comparative Biochemistry and Physiology Part A: Physiology*, 102(1), pp.85-91.
- Jolly, G.M., 1965. Explicit estimates from capture-recapture data with both death and immigration stochastic model. *Biometrika*, 52, pp.225-247.
- Kartavtsev, Y.P., Zaslavskaya, N.I., Svinyna, O.V. and Kijima, A., 2006. Allozyme and morphometric variability in the dogwhelk, *Nucella heyseana* (Gastropoda: Muricidae) from Russian and Japanese waters: evidence for a single species under different names. *Invertebrate Systematics*, 20, pp. 771-782.
- Kennett, R., 1996. Growth models for two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet-dry tropics of northern Australia. *Herpetologica*, 52(3), pp.383-395.
- Kennett, R., 1994. *Ecology of two species of freshwater turtle, Chelodina rugosa and*

*Elseya dentata*, from the wet-dry tropics of northern Australia. University of Queensland.

Kennett, R., 1999. Reproduction of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet-dry tropics of northern Australia. *London Journal of Zoology*, 247, pp.457-473.

Kennett, R. and Christian, K., 1994. Metabolic depression in estivating long-necked turtles (*Chelodina rugosa*). *Physiological Zoology*, 67, pp.1087-1102.

Kennett, R., Christian, K. and Bedford, G., 1998. Underwater nesting by the Australian freshwater turtle *Chelodina rugosa*: effect of prolonged immersion and eggshell thickness on incubation period, egg survivorship, and hatchling size. *Canadian Journal of Zoology*, 76(6), pp.1019-1023.

Kennett, R., Christian, K. and Pritchard, D., 1993. Underwater nesting by the freshwater turtle *Chelodina rugosa* from tropical northern Australia. *Australian Journal of Zoology*, 41, pp.47-52.

Kennett, R., Georges, A. and Palmer-Allen, M., 1993. Early developmental arrest during immersion of eggs of a tropical freshwater turtle, *Chelodina rugosa* (Testudinata: Chelidae), from northern Australia. *Australian Journal of Zoology*, 41, pp.37-45.

Kennett, R., Roe, J.H., Hodges, K. and Georges, A., 2009. *Chelodina longicollis* (Shaw 1794) - Eastern Long-necked Turtle, Common Long-necked Turtle, Common Snake-Necked Turtle. *Chelonian Research Monographs*, 5, pp.31.1-31.8.

Kennett, R. and Georges, A., 1990. Habitat utilization and its relationship to growth and reproduction of the eastern long-necked turtle, *Chelodina longicollis* (Testudinata: Chelidae). *Herpetologica*, 46(1), pp.22-33.

King, J.M., Kuchling, G. and Bradshaw, S.D., 1998. Thermal environment, behavior, and body condition of wild *Pseudemydura umbrina* (Testudines: Chelidae) during late winter and early spring. *Herpetologica*, 54(1), pp.103-112.

King, P. and Heatwole, H., 1994a. Non-pulmonary respiratory surfaces of the chelid turtle *Elseya latisternum*. *Herpetologica*, 50, pp.262-265.

King, P. and Heatwole, H., 1994b. Partitioning of aquatic oxygen uptake among different respiratory surfaces in a freely diving pleurodiran turtle, *Elseya latisternum*. *Copeia*, 1994(3), pp.802-806.

Klemens, M.W. ed., 2000. *Turtle conservation*, Washington and London: Smithsonian Institution Press.

Kuchling, G., 1988. Gonadal cycles of the Western Australian long-necked turtles *Chelodina oblonga* and *Chelodina steindachneri* (Chelonia: Chelidae). *Records of the Western Australian Museum*, 14(2), pp.189-198.

- Kuchling, G., 2010. Taxonomy and nomenclature of the longneck turtle (genus *Chelodina*) from south-western Australia. *Records of the Western Australian Museum*, 25, pp.449-454.
- Kuchling, G. and Bradshaw, S.D., 1993. Ovarian cycle and egg production of the western swamp tortoise *Pseudemydura umbrina* (Testudines: Chelidae) in the wild and in captivity. *Journal of Zoology, London*, 229(405-419).
- Kuchling, G. and de Jose, J., 1989. A captive breeding operation to rescue the critically endangered western swamp turtle *Pseudemydura umbrina*. *International Zoo Yearbook*, 28(103-109).
- Legler, J., 1960. A simple and inexpensive device for trapping aquatic turtles. *Proceedings of the Utah Academy of Science, Arts, and Letters*, 37, pp.63-66.
- Legler, J.M. and Cann, J., 1980. A new genus and species of chelid turtle from Queensland, Australia. *Contributions to Science (Natural History Museum of Los Angeles County)*, 324, pp.1-18.
- Legler, J.M. and Georges, A., 1993a. Biogeography and phylogeny of the Chelonia. In *Fauna of Australia. Vol. 2A Amphibia and Reptilia* (Ed. C.J. Glasby, G.J.B. Ross, and P.L. Beesley). Australian Government Publishing Service. Canberra. pp. 129-132.
- Legler, J.M. and Georges, A., 1993b. Family Chelidae. In *Fauna of Australia. Vol. 2A Amphibia and Reptilia* (Ed. C.J. Glasby, G.J.B. Ross, and P.L. Beesley). Australian Government Publishing Service. Canberra. pp. 142 – 152.
- Legler, J.M. and Winokur, R.M., 1979. Unusual neck tubercles in an Australian turtle, *Elseya latisternum*. *Herpetologica*, 35(4), pp.325-329.
- Lerat, E. and Ochman, H., 2005. Recognizing the pseudogenes in bacterial genomes. *Nucleic Acids Research*, 33(10), pp.3125-3132.
- Lieberman, B.S., 2003. Paleobiogeography: the relevance of fossils to biogeography. *Annual Review of Ecology and Systematics*, 34, pp.51-69.
- Limpus, C.J., 2008. *A biological review of Australian marine turtles. 1. Loggerhead turtle Caretta caretta (Linnaeus)*, Brisbane: Queensland Environmental Protection Agency.
- Limpus, C.J. and Limpus, D.J., 2003. The biology of the loggerhead turtle, *Caretta caretta*, in southwest Pacific Ocean foraging areas. In B. Witherington and A. Bolten, eds. *Biology and Conservation of Loggerhead Turtles*. Washington, DC: Smithsonian Institution Press, pp. 93-113.
- Limpus, C.J., Limpus, D.J. and Hamann, M., 2002. Freshwater turtle populations in the area to be flooded by the Walla Weir, Burnett River, Queensland: baseline study. *Memoirs of the Queensland Museum*, 48(1), pp.155-168.

- Linderman, P.V., 2000. Resource use of five sympatric turtle species: effects of competition, phylogeny, and morphology. *Canadian Journal of Zoology*, 78, pp.992-1008.
- Litzgus, J.D. and Brooks, R.J., 1998. Testing the validity of counts of plastral scute rings in spotted turtles, *Clemmys guttata*. *Copeia*, 1, pp.222-225.
- Lovette, I.J., 2004. Mitochondrial dating and mixed support for the "2% rule" in birds. *The Auk*, 121(1), pp.1-6.
- Maginniss, L.A., Ekelund, S.A. and Ultsch, G.R., 2004. Blood oxygen transport in common map turtles during simulated hibernation. *Physiological and Biochemical Zoology*, 77(2), pp.232-241.
- Manning, B. and Grigg, G.C., 1997. Basking is not of thermoregulatory significance in the "basking" freshwater turtle *Emydura signata*. *Copeia*, 1997(3), pp.579-584.
- Martins, F.I. and Souza, F.L., 2008. Estimates of growth of the Atlantic Rain Forest Freshwater Turtle *Hydromedusa maximiliani* (Chelidae). *Journal of Herpetology*, 42(1), pp.54-60.
- Mathie, N.J. and Franklin, C.E., 2006. The influence of body size on the diving behaviour and physiology of the bimodally respiring turtle, *Elseya albagula*. *Journal of Comparative Physiology B.*, 176, pp.739-747.
- McCord, W.P. and Ouni, M.J., 2007. A new genus of Australian longneck turtle (Testudines: Chelidae) and a new species of *Macrochelodina* from the Kimberley region of Western Australia (Australia). *Reptilia*, 31, pp.56-64.
- McCord, W.P. and Thomson, S., 2002. A new species of *Chelondina* (Testudines: Pleurodira: Chelidae) from Northern Australia. *Journal of Herpetology*, 36(2), pp. 255-267.
- McDowell, S.B., 1983. The genus *Emydura* (Testudines: Chelidae) in New Guinea with notes on the penial morphology of Pleurodira. In: Rhodin A, Miyata K (eds) *Advances in herpetology and evolutionary biology: essays in honour of Ernest E. Williams*. *Museum of Comparative Zoology, Harvard University*, pp.169-189.
- Meeks, L.M. and Ultsch, G.R., 1990. Overwintering behavior of snapping turtles. *Copeia*, 1990(3), pp.880-884.
- Meine, C., Soule, M.E. and Noss, R.F., 2006. "A mission-driven discipline": the growth of conservation biology. *Conservation Biology*, 20(3), pp.631-651.
- Miller, S.A., Dykes, D.D. and Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, 16, p.1215.
- Moritz, C., 1995. Uses of molecular phylogenies for conservation. *Philosophical*

*Transactions of the Royal Society of London* 349:113–118.

Morreale, S.J. and Gibbons, J.W., 1986. *Habitat suitability index models: slider turtle*, Washington DC.

Mueller, J.M., Sharp, K.R., Zander, K.K., Rakestraw, D.L., Rautenstrauch, K.R. and Lederle, P.E., 1998. Size-specific fecundity of the Desert Tortoise (*Gopherus agassizii*). *Journal of Herpetology*, 32(3), pp.313-319.

Murphy, S.A., Joseph, L., Burbidge, A.H. and Austin, J., 2010. A cryptic and critically endangered species revealed by mitochondrial DNA analyses: the Western Ground Parrot. *Conservation Genetics*, Short Communication.

Newbound, C.N., Hisheh, S., Suanto, A., How, R.A. and Schmitt, L.H. *et al.*, 2008. Markedly discordant mitochondrial DNA and allozyme phylogenies of tube-nosed fruit bats, *Nyctimene*, at the Australian-Oriental biogeographical interface. *Biological Journal of the Linnean Society*, 93, pp.589-602.

Noonan, B.P., 2000. Does the phylogeny of pelomedusoid turtles reflect vicariance due to continental drift? *Journal of Biogeography*, 27, pp.1245-1249.

Nowak-Kemp, M. and Fritz, U., 2010. Chelonian type specimens at the Oxford University Museum. *Zootaxa*, 2604, pp.1-19.

NSW National Parks and Wildlife Service, 2001. *Bellinger River Emydura: Emydura macquarii (Bellinger River) Recovery Plan*, Hurstville, NSW.

Ogilby, J.D., 1890. Description of a new Australian tortoise. *Records of the Australian Museum*, 1, pp.56-59.

Ohmer, M.E., Robertson, J.M. and Zamudio, K.R., 2009. Discordance in body size, colour pattern, and advertisement call across genetically distinct populations in a Neotropical anuran (*Dendropsophus ebraccatus*). *Biological Journal of the Linnean Society*, 97, pp.298-313.

Parham, J.F., Macey, J.R., Papenfuss, T.J., Feldman, C.R., Tukoza, O., Polymeni, R. and Boore, J., 2006. The phylogeny of Mediterranean tortoises and their close relatives based on complete mitochondrial genome sequences from museum specimens. *Molecular Phylogenetics and Evolution*, 38(1), pp.50-64.

Parmenter, C.J., 1976. *The natural history of the Australian freshwater turtle Chelodina longicollis Shaw (Testudinata, Chelidae)*. University of New England Editor.

Parmenter, R.R. and Avery, H.W., 1990. The feeding ecology of the slider turtle. In Gibbons, J. (ed.) *Life history and ecology of the slider turtle*. Washington: Smithsonian Institution Press, pp. 257-266.

Pauly, G.B., Hillis, D.M. and Cannatella, D.C., 2009. Taxonomic freedom and the role of official lists of species names. *Herpetologica*, 65(2), pp.115-128.

- Penney, D.G., 1987. Frogs and turtles: different ectotherm overwintering strategies. *Comparative Biochemistry and Physiology Part A: Physiology*, 86(4), pp.609-615.
- Peterson, C.C. and Greenshields, D., 2001. Negative test for cloacal drinking in a semi-aquatic turtle (*Trachemys scripta*), with comments on the functions of cloacal bursae. *Journal of Experimental Zoology*, 290, pp.247-254.
- Phillimore, A.B., Owens, I.P.F., Black, R.A., Chittock, J., Burke, T. and Clegg, S.M., 2008. Complex patterns of genetic and phenotypic divergence in an island bird and the consequences for delimiting conservation units. *Molecular Ecology*, 17, pp.2839-2853.
- Posada, D. and Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics*, 14(9), pp.817-818.
- Pratt, K.L., Wilson, R.S., Blomberg, S.P. and Franklin, C.E., 2009. Diving and digestion - the effect of an elevated metabolic rate on submergence in an aquatic ectotherm. In *Integrative and Comparative Biology*. Annual meeting of the Society for Integrative and Comparative Biology. United Kingdom: Oxford University Press, pp. 3138-3138.
- Priest, T.E., 1997. *Bimodal respiration and diving behaviour of the Fitzroy river turtle Rheodytes leukops*. BSc honours thesis. Brisbane: The University of Queensland.
- Priest, T.E. and Franklin, C.E., 2002. Effect of water temperature and oxygen levels on the diving behavior of two freshwater turtles: *Rheodytes leukops* and *Emydura macquarii*. *Journal of Herpetology*, 36(4), pp.555-561.
- Reese, S.A., Jackson, D.C. and Ultsch, G.R., 2002. The physiology of overwintering in a turtle that occupies multiple habitats, the common snapping turtle (*Chelydra serpentina*). *Physiological and Biochemical Zoology*, 75(5), pp.432-438.
- Reese, S.A., Stewart, E.R., Crocker, C.E., Jackson, D.C. and Ultsch, G.R., 2004. Geographic variation of the physiological response to overwintering in the painted turtle (*Chrysemys picta*). *Physiological and Biochemical Zoology*, 77(4), pp.619-630.
- Reese, S.A., Ultsch, G.R. and Jackson, D.C., 2004. Lactate accumulation, glycogen depletion, and shell composition of hatchling turtles during simulated aquatic hibernation. *The Journal of Experimental Zoology*, 207, pp.2889-2895.
- Rhodin, A.G.J., 2006. Foreword. Turtles and humans in Florida and the world: a global perspective on diversity, threats, and economic development. *Chelonian Research Monographs*, 3, pp.18-27.
- Roe, J.H. and Georges, A., 2007. Heterogeneous wetland complexes, buffer zones and travel corridors: Landscape management for freshwater reptiles. *Biological Conservation*, 135, pp.67-76.

- Roe, J.H. and Georges, A., 2008. Maintenance of variable responses for coping with wetland drying in freshwater turtles. *Ecology*, 89(2), pp.485-494.
- Roman, J., Santhuff, S.D., Moler, P.E. and Bown, B.W., 1999. Population Structure and Cryptic Evolutionary Units in the Alligator Snapping Turtle. *Conservation Biology*, 13(1), pp.135-142.
- Rougier, G.W., Fuente, M.S.D.I. and Arcucci, A.B., 1995. Late Triassic turtles from South America. *Science*, 268, pp.855-858.
- Rubinoff, D., 2006. Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology*.
- Salzman, J., 1998. Ecosystem Services and the Law. *Conservation Biology*, 12(3), pp.497-498.
- Saunders, D.K., Roberts, A.C. and Ultsch, G.R., 2000. Blood viscosity and hematological changes during prolonged submergence in normoxic water of northern and southern musk turtles (*Sternotherus odoratus*). *Journal of Experimental Zoology*, 287, pp.459-466.
- Saunders, D.L., Meeuwig, J.J. and Vincent, C.J., 2002. Freshwater protected areas: strategies for conservation. *Conservation Biology*, 16, pp.30-41.
- Schofield, G., Bishop, C.M., Katselidis, K.A., Dimopoulos, P., Pantis, J.D. and Hays, G.C., 2009. Microhabitat selection by sea turtles in a dynamic thermal marine environment. *Journal of Animal Ecology*, 78(1), pp.14-21.
- Seber, G.A.F., 1965. A note on the multiple recapture census. *Biometrika*, 52, pp.249-259.
- Seddon, J.M., Georges, A., Baverstock, P.R. and McCord, W.P., 1997. Phylogenetic relationships of chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA gene sequence variation. *Molecular Phylogenetics and Evolution*, 7(1), pp.55-61.
- Shapiro, L.J., Seiffert, C.V.M, Godfrey, L.R., Jungers, W.L., Simons, E.L. and Randria, G.F.N., 2005. Morphometric analysis of lumbar vertebrae in extinct Malagasy Strepsirrhines. *American Journal of Physical Anthropology*, 128, pp. 823-839.
- Shine, R. and Iverson, J.B., 1995. Patterns of survival, growth and maturation in turtles. *Oikos*, 72, pp.343-348.
- Siebenrock, F., 1901. Beschreibung einer neuen Schildkrotengattung aus der Familie Chelydidae aus Australien: Pseudemydura. *Anzeiger der Akademischen Wissenschaften Wien*, 38, pp.248-251.
- Simberloff, D.S., 1974. Equilibrium theory of island biogeography. *Annual Review of Ecology and Systematics*, 5, pp.161-182.

- Sites, J.J.W. and Marshall, J.C., 2004. Operational criteria for delimiting species. *Annual Review of Ecology and Systematics*, 35, pp.199-227.
- Soule, M.E. and Simberloff, D.S., 1986. What do genetics and ecology have to say about reserve design? *Biological Conservation*, 35, pp.19-40.
- Spencer, R.J., 2002a. Experimentally testing nest site selection: Fitness trade-offs and predation risk in turtles. *Ecology*, 83, pp.2136-2144.
- Spencer, R.J., 2002b. Growth patterns of two widely distributed freshwater turtles and a comparison of common methods used to estimate age. *Australian Journal of Zoology*, 50, pp.477-490.
- Spencer, R.J., 1998. The diet and digestive energetics of an Australian short-necked turtle, *Emydura macquarii*. *Comparative Biochemistry and Physiology - Part A: Molecular and Integrative Physiology*, 121(4), pp.341-349.
- Spencer, R.J. and Thompson, M.B., 2005. Experimental analysis of the impact of foxes on freshwater turtle populations. *Conservation Biology*, 19, pp.845-854.
- Spinks, P.Q. and Shaffer, H.B., 2005. Range-wide molecular analysis of the western pond turtle (*Emys marmorata*): cryptic variation, isolation by distance, and their conservation implications. *Molecular Ecology*, 14, pp.2047-2064.
- Stone, P.A., Dobie, J.L. and Henry, R.P., 1992. Cutaneous surface area and bimodal respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternum subrubrum*). *Physiological Zoology*, 65(2), pp.311-330.
- Storey, E.M., Kayes, S.M., De Vries, I. and Franklin, C.E., 2008. Effect of water depth, velocity and temperature on the surfacing frequency of the bimodally respiring turtle *Elseya albagula*. *Functional Ecology*, 22, pp.840-846.
- Stuart, B.L. and Fritz, U., 2008. Historical DNA from museum type specimens clarifies diversity of Asian leaf turtles (*Cyclemys*). *Biological Journal of the Linnean Society*, 94, pp.131-141.
- Templeton, A.R., 1981. Mechanisms of speciation - A population genetic approach. *Annual Review of Ecology and Systematics*, 12, pp.23-48.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. and Higgins, D.G., 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 24, pp.4876-4882.
- Thompson, M.B., 1985. Functional significance of the opaque white patch in eggs of *Emydura macquarii*. In G. Grigg, R. Shine, and H. Ehmann, eds. *Biology of Australasian Frogs and Reptiles*. Sydney: Australian Zoological Society of New South Wales, pp. 387-395.

- Thompson, M.B., 1983. Populations of the Murray River tortoise, *Emydura* (*Chelodina* [sic]): the effect of egg predation by the red fox *Vulpes vulpes*. *Australian Wildlife Research*, 10, pp.363-371.
- Thomson, S., 2000a. A revision of the fossil chelid turtles (Pleurodira) described by C.W. de Vis, 1897. *Memoirs of the Queensland Museum*, 45(2), pp.593-598.
- Thomson, S., 2000b. The identification of the Holotype of *Chelodina oblonga* (Testudines: Chelidae) with a discussion of taxonomic implications. *Chelonian Conservation and Biology*, 3(4), pp.745-749.
- Thomson, S. and Georges, A., 2009. *Myuchelys* gen. nov. - a new genus for *Elseya latisternum* and related forms of Australian freshwater turtle (Testudines: Pleurodira: Chelidae). *Zootaxa*, 2053, pp.32-42.
- Thomson, S. and Georges, A., 1996. Neural bones in Australian chelid turtles. *Chelonian Conservation and Biology*, 2(1), pp.82-86.
- Thomson, S. and Mackness, B.S., 1999. Fossil turtles from the early Pliocene Bluff Downs Local Fauna, with a description of a new species of *Elseya*. *Transactions of the Royal Society of South Australia*, 123(3), pp.101-105.
- Thomson, S., White, A. and Georges, A., 1997. Re-evaluation of *Emydura lavarackorum*: Identification of a Living Fossil. *Memoirs of the Queensland Museum*, 42(1), pp.327-336.
- Tucker, A.D., 2000. *Cumulative effects of dams and weirs on freshwater turtles: Fitzroy, Kolan, Burnett, and Mary Catchments*. Queensland Parks and Wildlife Service Report, Brisbane.
- Tucker, A.D., Limpus, C.J., Priest, T.E., Cay, J., Glen, C. and Guarino, E., 2001. Home ranges of Fitzroy River turtles (*Rheodytes leukops*) overlap riffle zones: potential concerns related to river regulation. *Biological Conservation*, 102, pp.171-181.
- Turtle Taxonomy Working Group [Rhodin, A.G.J., Parham, J.F., van Dijk, P.P. and Iverson, J.B.], 2010. Turtles of the world, 2010 Update annotated checklist of taxonomy, synonymy, distribution, and conservation status. In Rhodin, A.G.J., Pritchard, P.C.H., van Dijk, P.P., Saumure, R.A., Buhlmann, K.A., Iverson, J.B., and Mittermeier, R.A. (Eds). *Conservation Biology of Freshwater Turtles and Tortoises: A Compliation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group*. *Chelonian Research Monographs*. pp. 000.85-000.164.
- Ultsch, G.R., 1988. Blood gases, hematocrit, plasma ion concentrations, and acid-base status of musk turtles (*Sternotherus odoratus*) during simulated hibernation. *Physiological Zoology*, 61(1), pp.78-94.
- Ultsch, G.R., Carwile, M.E., Crocker, C.E. and Jackson, D.C., 1999. The physiology of hibernation among painted turtles: the eastern painted turtle *Chrysemys picta*

- picta*. *Physiological and Biochemical Zoology*, 72(4), pp.493-501.
- Ultsch, G.R., Hanley, R.W. and Bauman, T.R., 1985. Responses to anoxia during simulated hibernation in northern and southern painted turtles. *Ecology*, 66(2), pp.388-395.
- Ultsch, G.R., Herbert, C.V. and Jackson, D.C., 1984. The comparative physiology of diving in North American freshwater turtles. I. Submergence tolerance, gas exchange, and acid-base balance. *Physiological Zoology*, 57(6), pp.620-631.
- Ultsch, G.R. and Jackson, D.C., 1982. Long-term submergence at 3°C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. I. Survival, gas exchange and acid base status. *Journal of Experimental Biology*, 96, pp.11-28.
- Van Burskirk, J. and Crowder, L.B., 1994. Life history variation in marine turtles. *Copeia*, 1994(1), pp.66-81.
- Webb, G.J.W., 1978. Observations on basking in some Australian turtles (Reptilia: Testudines: Chelidae). *Herpetologica*, 34, pp.39-42.
- Wells, R.W., 2007. Some taxonomic and nomenclatural considerations on the class Reptilia in Australia. A new genus of the family Chelidae from eastern Australia. *Australian Biodiversity Record*, 2007(3), pp.1-13.
- Wells, R.W., 2009. Some taxonomic and nomenclatural considerations on the class Reptilia in Australia. A new Species of freshwater turtle in the genus *Wollumbinia* Wells 2007 (Reptilia: Chelidae) from eastern Australia. *Australian Biodiversity Record*, 2009(1), pp.1-12.
- Wells, R.W., 2007. Some taxonomic and nomenclatural considerations on the class Reptilia in Australia. Some comments on the *Elseya dentata* (Gray, 1863) complex with redescriptions of the Johnstone River snapping turtle, *Elseya stirlingi* Wells and Wellington, 1985 and the Alligator Rivers snapping turtle, *Elseya jukesi* Wells 2002. *Australian Biodiversity Record*, 2007(2), pp.1-12.
- Wells, R.W., 2002. Taxonomic notes on some Australian freshwater turtles of the genera *Chelodina* and *Elseya* (Reptilia: Chelidae). *Australian Biodiversity Record*, 2002(2), pp.1-30.
- Wells, R.W. and Wellington, C.R., 1985. A classification of the Amphibia and Reptilia of Australia. *Australian Journal of Herpetology, Supplementary Series*, 1, pp.1-61.
- White, A. and Archer, M., 1994. *Emydura lavarackorum*, a new Pleistocene turtle (Pleurodira: Chelidae) from fluvatile deposits at Riversleigh, northwestern Queensland. *Records of the South Australian Museum*, 27, pp.159-167.
- White, G.C. and Burnham, K.P., 1999. Program MARK: survival estimation from populations of marked animals. *Bird Study*, 46(Supplement), pp.120-138.

- Wilson, D.S., Tracy, C.R. and Tracy, C.R., 2003. Estimating age of turtles from growth rings: a critical evaluation of the technique. *Herpetologica*, 59(2), pp.178-194.
- Zangerl, R., 1969. The turtle shell. In *Biology of the Reptilia*. New York: Academic Press, pp. 311-339.
- Zuffi, M.A.L., Odetti, F., Batistoni, R. and Mancino, G., 2006. Geographic variation of sexual size dimorphism and genetics in the European pond turtle, *Emys orbicularis* and *Emys trinacris*, of Italy. *Italian Journal of Zoology*, 73(4), pp.363-372.
- Zuffi, M.A.L., Odetti, F. and Meozzi, P., 1999. Body size and clutch size in the European pond turtle (*Emys orbicularis*) from central Italy. *Journal of Zoology, London*, 247, pp.139-143.