THE UNIVERSITY OF QUEENSLAND

AUSTRALIA

Habitat requirements for nesting and early life-stages of the endangered Mary River turtle (*Elusor macrurus*): Insights for conservation.

Mariana A. de Micheli Campbell

B.Sc. (Hons), M.Sc.

A thesis submitted for the degree of Doctor of Philosophy at

The University of Queensland in 2012

School of Biological Sciences

Abstract

Freshwater turtles are one of the most threatened of all vertebrate groups. There is however, a distinct lack of scientific knowledge about the biology of many species. Ecological information regarding the early life-stages is important for freshwater turtle management and conservation, because creating and preserving suitable habitat for embryos, hatchlings and juveniles will increase the number of individuals attaining reproductive age. The overall aim of this thesis was to describe the nesting biology and juvenile ecology of an endangered species of freshwater turtle, which is found only in a single river system in South-East Queensland, Australia.

The Mary River turtle *Elusor macrurus* is one of the largest freshwater turtles on the Australian continent. The current population is listed as endangered and although hundreds of *E. macrurus* nests have been recorded since 2004, juvenile turtle are rarely sighted in the wild. This thesis documents three aspects of the biology during the early life-stages of this endangered species of freshwater turtle, from the nest-site selection by the females through incubation to the habitat required by the juvenile turtles once they enter the river. The first aim of the present study was to describe the species nesting biology, the second to reveal the effects of incubation temperature upon hatchlings' phenotype, and the third to characterise the habitat selected by the juveniles once they enter the river.

To understand the nesting biology, it was important to determine if the females returned to the same nesting grounds every year. To address the first aim of this study, a nesting bank was monitored by time-lapse infrared cameras over three consecutive years, and a novel methodology was developed to identify individual turtles based upon marks on their carapaces. The results demonstrated that female *E. macrurus* do exhibit nest-site fidelity. It was unclear however, which features of the river bank were important in the females' selection. The physical aspects of sandy banks selected by female *E. macrurus* for nesting were compared against sandy banks where females had never been recorded to nest upon (non-nesting banks). There were no statistical differences in soil characteristics or physical aspects between nesting and non-nesting banks. There was however, a significant difference in the bearing, with the nesting banks facing between 50º (NE) and 300º (NW), whilst the non-nesting banks faced between 90º and 240º. There was no difference in the overall mean temperature recorded between nesting and non-nesting banks, but

the mean daily temperature fluctuation was significantly higher at the nesting banks, which was likely due to these banks facing in a northerly direction.

Incubation temperature is known to be one of the most important factors influencing the phenotype of the hatchling turtles. The second aim was addressed through empirical studies, to investigate how mean and daily fluctuations in incubation temperature affected the phenotype and exercise performance of the hatchling *E. macrurus*. The overall mean temperature recorded from the *E. macrurus* nests ranged from 26 to 31ºC; and the mean daily temperature fluctuation recorded was 5.7ºC (with a variation that was as low as 2ºC and as high as 22ºC). The first study incubated freshly laid *E. macrurus* eggs at three constant thermal regimes. The treatment groups were similar to the mean nest temperature recorded in the wild (26, 29 and 32ºC). Contrary to what was hypothesised, embryos incubated at the warmest thermal regime had a higher rate of mortality. Those that hatched showed a significantly reduced body size, mass, post-hatch growth rate and poorer performance when swimming and tested for self-righting time compared to those hatchlings incubated at lower temperatures. The second study incubated freshly laid *E. macrurus* eggs at constant (28ºC) and fluctuating (28 ± 3ºC and 28 ± 6ºC) thermal regimes. These results also refuted my hypothesis and showed that a variation in daily incubation temperature between zero and 6ºC, did not alter the phenotype or performance of hatchling *E. macrurus*. Daily variations of 12ºC were, however, detrimental as the eggs incubated at 28 ± 6ºC only had a 5% hatching success (n = 1).

Similarly to most freshwater turtle species, the habitat requirements of the juvenile *E. macrurus* are unknown. The third aim was to identify the characteristics of habitat selected by hatchling and juvenile turtles, and this required the attachment of miniaturised telemetry devices to the turtles before they were released into the river. The turtles were located over the subsequent nine months, and river physical characteristics were recorded and inputted into a presence-only predictive model. The results showed that juvenile *E. macrurus* occupied a very narrow range of habitat characteristics, which occurred in relatively few areas throughout the main trunk of the river.

The overall aim of this study was to understand the biology of the early life-stages of *E. macrurus* for the purposes of management and conservation. The study showed that females were nest-site fidelic and favoured north-facing banks. The results were however, inconclusive in identifying the physio-chemical characteristics that differentiated nesting areas from other sandy

banks. Moreover, the thermal-manipulation studies did not confirm that the nest-site selection was temperature related. Therefore, the reason why specific banks are recurrently selected over others by nesting female *E. macrurus* remains a mystery. Importantly, the results illustrated that critical habitat for juvenile *E. macrurus* are limited throughout the Mary River and were not located in the impounded sections. I argue that the preservation of nesting areas and habitat for juveniles is vital for the long-term conservation of *E. macrurus*, and recommend such actions to be included in future recovery plans.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my research higher degree candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the General Award Rules of The University of Queensland, immediately made available for research and study in accordance with the *Copyright Act 1968*.

I acknowledge that copyright of all material contained in my thesis resides with the copyright holder(s) of that material. Where appropriate I have obtained copyright permission from the copyright holder to reproduce material in this thesis.

Publications during candidature

Peer-reviewed publications

Micheli-Campbell, M.A., Baumgartl, T., Campbell, H.A., Connell, M., Booth, D.T., and Franklin, C.E. (*Under Review*). Nest-site fidelity and location preferences of an endangered turtle. *Herpetologica*.

Micheli-Campbell, M.A., Campbell, H.A., Cramp, R.L., Booth, D., and Franklin, C.E. (2011). Staying cool, keeping strong: Incubation temperature affects performance in a freshwater turtle. *Journal of Zoology* **285**(4): 266-273.

Micheli-Campbell, M.A., Gordos**,** M.A., Campbell, H.A., Booth, D., and Franklin, C.E. (2012). The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle. *Journal of Zoology*. doi/10.1111/j.1469-7998.2012.00934.

Micheli-Campbell, M.A., Campbell, H.A., Connell, M., Dwyer, R.G., and Franklin, C.E. (*Under review*). Characterising and locating critical habitat for riverine animals: A case study on juvenile freshwater turtles. *Freshwater Biology*.

Conference abstracts – oral presentations

Micheli-Campbell, M.A., Booth, D., and Franklin, C.E. (2011). Effects of warmer than normal incubation temperature upon the behavioral function of the hatchling freshwater turtles. *In*: 9th Annual Symposium on Conservation and Biology of Tortoises and Freshwater Turtles: Turtle Survival Alliance (TSA) and IUCN Tortoise and Freshwater Turtle Specialist Group, Orlando, Florida, USA (winner of the Best Student Presentation Award).

Micheli-Campbell, M.A., Booth, D., and Franklin, C.E. (2011). Predicting the impact of climate change upon a freshwater turtle. *In*: 2011 Annual Main Meeting of the Society for Experimental Biology, Glasgow, Scotland, U.K.

Micheli-Campbell, M.A., Booth, D., and Franklin, C.E. (2010). Staying cool, keeping strong: incubation temperature affects performance in a freshwater turtle. *In*: 27th Meeting of the Australian New Zealand Society for Comparative Physiology and Biochemistry, The Australian National University (ANU), Canberra, ACT, Australia (3rd Prize in the student presentation).

Conference abstract – poster

Micheli-Campbell, M.A., Franklin, C.E., and Connell, M. (2011). Every one's a winner: the Turtle, the Science, the Community. *In*: 9th Annual Symposium on Conservation and Biology of Tortoises and Freshwater Turtles: Turtle Survival Alliance (TSA) and IUCN Tortoise and Freshwater Turtle Specialist Group, Orlando, Florida, USA.

Publications included in this thesis

Micheli-Campbell, M.A., Baumgartl, T., Campbell, H.A., Connell, M., Booth, D.T., and Franklin, C.E. (*Under Review*). Nest-site fidelity and location preferences of an endangered turtle. *Herpetologica* (*incorporated as Chapter 2*).

Micheli-Campbell, M.A., Campbell, H.A., Cramp, R.L., Booth, D., and Franklin, C.E. (2011). Staying cool, keeping strong: Incubation temperature affects performance in a freshwater turtle. *Journal of Zoology* **285**(4): 266-273 (*incorporated as Chapter 3*).

Micheli-Campbell, M.A., Gordos**,** M.A., Campbell, H.A., Booth, D., and Franklin, C.E. (2012). The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle. *Journal of Zoology*. doi/10.1111/j.1469-7998.2012.00934 (*incorporated as Chapter 4*).

Micheli-Campbell, M.A., Campbell, H.A., Connell, M., Dwyer, R.G., and Franklin, C.E. (*Under review*). Characterising and locating critical habitat for riverine animals: A case study on juvenile freshwater turtles. *Freshwater Biology* (*incorporated as Chapter 5*).

Contributions by others to the thesis

Chapter 2. Micheli-Campbell, M.A., Baumgartl, T., Campbell, H.A., Connell, M., Booth, D.T., Franklin, C.E. Nest-site fidelity and location preferences of an endangered turtle.

Statement of contribution: MAMC was responsible for 70 % of conception and design, 80 % of data collection, 80 % of analysis, 80 % of interpretation and 60 % writing and drafting; TB 10 % of analysis; HAC was responsible for 5 % of conception and design, 5 % of data collection and 15 % writing and drafting; MC was responsible for 15 % of data collection, 10 % of interpretation and 5% writing and drafting; DTB was responsible for 10% of conception and design, 10 % of analysis, 10 % interpretation and 5 % writing and drafting; CEF was responsible for 15% of conception and design, 10 % writing and drafting.

Chapter 3. Micheli-Campbell, M.A., Campbell, H.A., Cramp, R.L., Booth, D., Franklin, C.E. Staying cool, keeping strong: Incubation temperature affects performance in a freshwater turtle.

Statement of contribution: MAMC was responsible for 50 % of conception and design, 100 % of data collection, 90 % of analysis, 70 % of interpretation and 60 % writing and drafting; HAC was responsible for 10 % of analysis and 15 % writing and drafting; RLC was responsible for 5% writing and drafting; DTB was responsible for 20% of conception and design, 10 % writing and drafting; CEF was responsible for 25% of conception and design, 20 % of interpretation, 10 % writing and drafting.

Chapter 4. Micheli-Campbell, M.A., Gordos**,** M.A., Campbell, H.A., Booth, D., Franklin, C.E. The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle.

Statement of contribution: MAMC was responsible for 55 % of conception and design, 100 % of data collection, 80 % of analysis, 70 % of interpretation and 60 % writing and drafting; MAG was responsible for 5 % of conception and design and 5 % writing and drafting; HAC was responsible for 10 % of analysis and 15 % writing and drafting; DTB was responsible for 15 % of conception and design, 10 % of analysis, 10 % writing and drafting; CEF was responsible for 25 % of conception and design, 20 % of interpretation, 10 % writing and drafting.

Chapter 5. Micheli-Campbell, M.A., Campbell, H.A., Connell, M., Dwyer, R.G., Franklin, C.E. Characterising and locating critical habitat for riverine animals: A case study on juvenile freshwater turtles.

Statement of contribution: MAMC was responsible for 50 % of conception and design, 70 % of data collection, 60 % of analysis, 60 % of interpretation and 50 % writing and drafting; HAC was responsible for 40 % of conception and design, 20 % of data collection, 20 % of interpretation and 30 % writing and drafting; MC was responsible for 10 % of data collection and 5 % writing and drafting; RGD was responsible for 40 % of analysis, 5 % writing and drafting; CEF was responsible for 10% of conception and design, 20 % of interpretation, 15 % writing and drafting.

Statement of contributions by others to the thesis as a whole

Craig Franklin, David Booth and Matthew Gordos significantly contributed to the concept and design of this project as a whole through discussions with me. Craig Franklin, Matthew Gordos, and Hamish Campbell critically reviewed the final draft of my thesis. Their comments and suggestions resulted in improvements to the content and interpretation of the General Introduction (Chapter 1) and the General Discussion (Chapter 6).

Statement of parts of the thesis submitted to qualify for the award of another degree

None.

Acknowledgements

Throughout my Ph.D., I have had the honour and the pleasure of meeting many wonderful people, who have helped me in countless ways. I could not have done this project without them, so thank you…

Firstly, I would like to thank my main supervisor, Craig Franklin. Thank you for accepting me as your Ph.D. student, and for your support throughout my candidature. And when I mean support, I mean it all: intellectually, emotionally, and financially. You have guided me through both pleasant and difficult situations, and have always encouraged me to persevere with my ideas. I have learned so much, grown so much, and I will be forever grateful for your trust, guidance, support, and friendship.

Secondly, I would like to thank my co-supervisor, David Booth. Thank you for your guidance and valuable advice throughout my candidature. You have taught me so much, not only about turtles and egg husbandry (which was extremely important by the way), but also about experimental design, and that the water retention curve is far more complicated that it seems… Thank you!

Thirdly, thank you to my other co-supervisor, Matthew Gordos. Matt, I am honoured for have had you as part of my supervisory team. Thanks for your advice throughout, especially on my writing and development of experimental designs. I would not have had one of my experimental chapters in this thesis today if it wasn't for you! Thank you so much for that and for always having time for a chat!

Thank you to my Ph.D. readers, Anne Goldizen and Craig White. Your words of encouragement and valuable advice in each one of my milestones were very important for me, and for that I will be always grateful.

Thank you very much to the School of Biological Sciences staff, especially John Bertram, Selena Hobbs, Gail Walter, Lyn Beard, Louise Kuchel, and Anthony O'Toole. You guys have helped me so much, in so many different ways, often making my work so much easier, and for that I am deeply thankful.

To all past and present members of the ECO-lab, thank you! Firstly, my deep and special thanks go to Rebecca Cramp. Beck, I cannot thank you enough for your help throughout my candidature. You have always been there for me, for all of us. Your 'admin skills' and kind nature were essential to keeping me on track in many ways, and for that I will be forever thankful! Thank you to my office mates, Sara Loeffen, Lesley Alton, Karen Young, Rebecca

McPhee, Beau Reilly, Michel Ohmer, and Joe Fittell. It was an honour and so much fun to share an office with you guys!

Could I have done my Ph.D. without my dear volunteers? No, of course I could not. Thank you very much to my dear friends Fabio Cortesi, Kathryn Rudland, Henry and Margaret Milne, and Harry Macdermott. Your help was precious and I am deeply grateful for your friendship.

One of the most important elements of my Ph.D. was the people that I have met, the friends I have made. Thank you to Nat, Les, Fabio, Kathy, Billy, Karen, Beck, Candice, Skye, Sara, Beau, Gwen, Robbie, Amanda, Sean, Tom, Beck and Matt. Thank you so much for your time, your ears, your shoulders, and for the laughter! Natalie Schimpf, I wish you were here to share this moment with me and I can't wait for your return, so we can celebrate it all again! Les, thank you for your help at all times, and for being an example for me! Thank you for being such great friends! I also thank my friends from outside the University (Caz, Des, Hannah, Luke, Debs Loose and Patricia); and to my old friends from Brazil (Bru, Dé, Ju Lessa, Fer Schenferd, Ju Thomaz, Carks, Fer Pessoa, Gabi, Tha, Julia, Mari B., Mari D.), who are always in my heart and supported me so much when I said "I'm moving to Australia!".

I would like to thank the Australian Government and The University of Queensland for my Ph.D. scholarship (IPRS). As a foreign student, I deeply appreciate the once-in-a-life-time opportunity that this country has given to me.

Thank you to the Tiaro & District Landcare Group (TDLG). I have no words to describe how much I appreciate everything you have done for me. First, thank you for the research scholarship that you awarded to me; second, thanks for having introduced me to this incredible animal (Mary River turtle) and to this amazing place (Mary River); and third, and probably most important, thank you for your unconditional support, indispensable help and precious friendship. As I have said in our last meeting: "Sorry, you can't get rid of me now, I'll always be around!" and I can't wait for the next nesting season! I would like to especially thank: Lynn and Ray Klupfel, for you warm hospitality, for investing in our ideas, for your help in the field, and for those amazing eggs and pumpkins! Carol and Col Nielsen, Noel and Sue Dean, Adrian and Vicki Ross, Ron Black, Debbie and Garry Brischke, thank you for your hospitality, for helping in the office and in the field, for being so handy so often, and for towing us when we needed! I also would like to thank the Stilers (Chris, Peter, Emily, Abbie and Wayne). You probably didn't know how much trouble was coming when you let us access your property, did you?! Thank you for all your help and for being so welcoming.

Yes Marilyn, you totally deserve an entire paragraph! Thank you and thank you again to Marilyn Connell, for your help in the field, for each one of your questions, for your incentive, for your unconditional support, for your hospitality, and for your friendship. You were much more than the turtle project officer for TDLG, you became a great friend, and for this I am honoured. For these and for the other things that I probably forgot: thank you!

I also wish to thank a very important person, John Cann. You kindly and unexpectedly sent me your book right at the beginning of my Ph.D., because I said that I would need it then, and not at the end of my study. And I was right: I needed it at all times! You are an example for me, thank you for being so accessible, for your incentive and valuable advice.

Thank you to my family back in Brazil, especially my dear vó Zulmira, Tia Lina and Tia Lourdes. Thank you for your love, trust, help and support, which started when I was born, and for teaching me the value of knowledge. Thank you to my extended Scottish family too! Thank you for your encouragement and for your love. Thank you to Jenny and Brian Dwyer, and Simon Campbell for your help in the field. I especially thank Rebecca Jamieson and Ross Dwyer, not only for the survival kit and happy moments, but also for your help in the field and during my statistical analysis. It was a pleasure working with you!

I would like to dedicate this thesis to the two most important people in my life: my mother, Marina Freitas, and my husband, Hamish Campbell.

Thank you mum, for giving up your dreams to make mine real, for always putting me first, for always making my education your priority, even when it cost more than what you had… Thank you for your unconditional support throughout each phase of my life, for your understanding, for never saying "please don't go to the other side of the world" but instead "you can do it"… You are my example of a strong, faithful, kind and loving woman. I love and miss you very much! Thank you!

Thank you so very much to my dear husband Hamish. I can only say that you have helped me in every way a person can be helped… Thank you for being my husband, in every meaning of this word. I will be forever thankful for the opportunity of having worked and shared each moment of my Ph.D. with you. You taught me more than you can imagine, you were always there for me, and I will be forever grateful for everything you have done for me. I hope you forget the moments of my 'latin-tears' and only remember our great adventures, the times in the field, the laughter, and the successes we have achieved together. I love you amor! Eu amo você! 'Thanks' only will never be enough, but thank you very much anyway!

Keywords

Elusor macrurus, freshwater turtle, endangered, nesting biology, incubation temperature, hatchling performance, juvenile ecology, critical habitat, telemetry, conservation.

Australian and New Zealand Standard Research Classifications (ANZSRC)

060204 – Freshwater Ecology, 20 %
060806 – Animal Physiological Ecology, 40 %
050211 – Wildlife and Habitat Management, 40 %

Fields of Research (FoR) Classification

0502 – Environmental Science and Management, 20 %
0602 – Ecology, 40 %
0608 – Zoology, 40 %

Table of contents

List of Figures

List of Tables

List of Abbreviations

ANN – Artificial Neural Networks

AUC – Area Under the Curve

CL – carapace length

EGVs – Ecogeographical variables

ENFA – Ecological Niche Factor Analysis

GAM – Generalised Additive Models

GLM – Generalised Linear Models

GLMMs – General Linear Mixed Models

GR – growth rate

GzLMM – Poisson Generalised Mixed Model

HS – Habitat Suitability

MC – moisture content

NL – notch length

SCL – Straight carapace length

TDLG – Tiaro & District Landcare Group

TSD – temperature dependent sex determination

VHF – Very High Frequency

CHAPTER 1

General Introduction

The world is facing a significant decline in species biodiversity. The current rate of extinction is a thousand-times greater than the historical background level and is occurring in direct consequence of human disturbance and habitat modification (Myers and Knoll 2001; Balmford, Green and Jenkins 2003; Baillie, Hilton-Taylor and Stuart 2004). The 2004 IUCN global assessment listed approximately 15,600 species as threatened, and stated that species extinctions have become alarmingly more common in recent decades (Baillie *et al*. 2004).

In the fight to preserve Earth's biodiversity, conservation science is playing a pivotal role (Chazdon 2008; Cooke *et al.* 2008; Seebacher and Franklin 2012). Conservation biology draws upon a wide range of scientific disciplines, from genetics through to economics, social sciences and population ecology (Soulé 1985). Conservation physiology has recently emerged as a discipline of study to investigate how the abiotic environment affects an individual, and how this may impact upon the fitness of the population (Wikelski and Cooke 2006). In contrast to the natural world, the laboratory setting allows for the conditions that an organism experiences to be controlled, and this enables recording of response variables not possible in free-ranging animals. Such conditions are however, unlikely to reflect the complexity of the numerous stressors occurring in the wild. Interdisciplinary studies combining traditional physiological theory with field-based ecological monitoring not only validate or improve the understanding of laboratory results, they also allow for a more realistic picture of how organisms may respond to changing environmental conditions (Chown 2012; Cooke *et al.* 2012). Utilising data from numerous studies in fish, Metcalfe *et al.* (2012) demonstrated that the integration of physiological and telemetry data was a valuable strategy for providing insights for conservation and management.

Turtles are amongst the most threatened vertebrate groups, considered at higher risk of extinction than elasmobranches, amphibians, birds and most mammals, paralleled only by the primates (Rhodin *et al.* 2011). A well-publicised recent example was the death of Lonesome George, the last Pinta Island tortoise: a sub-species of Galapagos tortoise (*Chelonoidis nigra abingdonii*). Lonesome George was the most endangered Chelonian in the world, a position now filled by the Red River Giant Softshell turtle (*Rafetus swinhoei*), with only four known remaining

individuals. These losses are not isolated cases within the group, because Chelonians (taxon that includes all turtles and tortoises) are in considerable decline across the world. Six of the seven species of marine turtles are threatened. Out of the 58 known species of tortoises (family Testudinidae), eight are already extinct and 66 % are listed as threatened or endangered. The statistics are not much better for the 263 species of freshwater and terrestrial turtles, with 45 % of the species threatened or endangered (IUCN 2011). Overharvesting and habitat loss are cited as the primary causal factors responsible for the decline in freshwater turtle populations (Rhodin *et al*. 2011). However, effective management and conservation for many freshwater turtle species is being hampered by limited scientific information regarding their ecology and habitat requirements.

This integration between physiological and ecological studies may prove particularly useful in understanding the reasons behind the current world-wide decline in turtle populations. Overall, Chelonians have received much less scientific attention than birds, fish and mammals, and freshwater turtles have been given considerably less consideration than their marine cousins (Rhodin *et al*. 2010). At present, there is insufficient knowledge about the characteristics that constitute optimum habitats for freshwater turtles and how these species respond to changes in environmental conditions. The relative paucity of scientific information about freshwater turtle ecology is ironic, considering that continental waters are one of the most modified environments in the world, as a result of human activities. The construction of dams and weirs, for the generation of electricity and water consumption, significantly alters the physical conditions of the rivers, including: changes in stream structure and flow, water level, dissolved oxygen, turbidity, temperature, and pH (Ward and Stanford 1989; Ligon, Dietrich and Trush 1995; Bodie 2001). It is well known that such alterations in the freshwater environment can be detrimental to the inhabiting fish populations, but far less attention has been given to freshwater turtle species. Interdisciplinary studies may also be advantageous in studying the effects of changing climatic conditions upon turtle populations. Such studies are crucial for the dwindling populations of Australian freshwater turtles, because 1/ the majority of these species bury their eggs in relatively shallow nests and are therefore, vulnerable to changes in ambient conditions; and 2/ climate models predict the ambient temperature to increase in Australia at a greater rate than most of other places on Earth (Australian Greenhouse Office 2003; CSIRO 2007). Numerous studies have investigated the effects of temperature upon nest-site election and embryo development in marine turtles, but fewer studies have been undertaken upon tortoises and freshwater species. Moreover, very few of the published empirical studies considered the realised thermal conditions

experienced by the species when nesting in their natural environment. Such information is urgently required to aid the understanding and reversing the ongoing decline in freshwater turtle populations. It is particularly important to investigate the early life-stages' biology, because this period is when reptiles are most sensitive to environmental changes. Moreover, reptiles often lay a large number of eggs and have a high rate of infant mortality (Kolbe, Janzen and Gatten Jr 2002; Paterson, Steinberg and Litzgus 2012); management strategies focused at protecting habitat for these stages of the life cycle will increase the potential for juveniles attaining reproductive age and therefore, ensuring population recruitment.

Biology of the early-life stages

For all egg-laying species, nest-site selection by the adult females is a vital component influencing hatchling survivorship (Wilbur and Morin 1988). In turtles, this selection may be to reduce the potential for predation (Congdon *et al*. 1983; Marchand and Litvaitis 2004). For example, the Australian freshwater turtle *Emydura macquarii* favours alternate nest-sites in areas where foxes are present (Spencer and Thompson 2003). Nest-site selection can alter incubation conditions, and consequently, influence offspring phenotype (Wilson 1998; Deeming 2004). The environmental conditions to which the eggs are exposed during incubation may influence physiological traits such as developmental rate, sex ratio, body size of hatchlings, locomotor performance, thermoregulatory behaviour, and post-hatch growth rate (Miller, Packard and Packard 1987; Packard *et al.* 1987; Janzen *et al.* 1990; Packard 1999; Kolbe and Janzen 2001; Booth *et al.* 2004). Possible reasons why different species select specific nest-site features could be related to water availability (Ackerman 1991; Booth 2002), soil characteristics, landscape composition (Marchand and Litvaitis 2004; Bonach *et al*. 2007) or temperature (Janzen 1993; Wood and Bjomdal 2000).

Hydric conditions, landscape and soil characteristics during nesting

During incubation, turtle eggs exchange water with the environment. The eggshell structure is important in controlling the water exchange between the eggs and the environment. Eggshell structure can be categorised into two functional types in Chelonians: 1/ flexible-shelled eggs, which have a lightly calcified external layer that allows the eggs to easily expand or shrink; and, 2/ rigid-shelled eggs, which have a heavily calcified external coating that restricts egg

expansion (Packard, Packard and Boardman 1982). Eggs with flexible shells are highly permeable and exchange water with the environment, whereas rigid-shelled eggs are less permeable and exchange water more slowly (see Packard 1991; 1999). Nevertheless, hydric conditions may influence embryo development in both these egg types (Booth 2002).

Moist substrates ensure the clutch remains in a positive water balance, i.e. the eggs absorb water instead of losing it to the environment (Ackerman 1991; Packard 1999; Booth 2002). The rate and direction of water exchange between the eggs and the soil surrounding the chamber are influenced by the degree of contact between the eggshell and the substrate or gas spaces (Ackerman *et al*. 1985; Packard 1999; Booth 2002; Booth and Yu 2009). The type of substrate in which the eggs have been laid also influences water exchange, because the size of the pores between the particles is related to the rate of water movement through the soil (Koorevaar, Menelik and Dirksen 1983; Ackerman 1991). The results of the few studies that have investigated the relation between soil and nesting biology in freshwater turtles are dissimilar, with the soil type having no influence upon nest-site selection in some species (Vestjens 1969; Ehrenfeld 1979; Booth 2010), whilst it is important for others (Bonach *et al*. 2007).

The landscape of turtle nesting sites can be described by the amount and type of vegetation, slope aspect and physical dimensions. Some species show no preference for landscape type when nesting, whilst others clearly favour specific features, such as open non-vegetated areas (Ehrenfeld 1979; Janzen 1994; Booth 2010). It has been suggested this may be a behavioural strategy by the nesting females to increase the nest exposure to sunlight, resulting in warmer temperatures during incubation.

Incubation temperature and hatchling phenotype

The temperature to which the eggs of reptiles are exposed during incubation is one of the most important factors influencing embryo development. Nest temperature determines the rate of embryonic growth and development and thus the duration of the incubation period (Deeming and Ferguson 1991). Generally, the length of incubation decreases with rises in temperature, with this pattern being consistent for many species of freshwater turtle (Yntema 1978; Choo and Chou 1987; Gutzke *et al.* 1987; Packard *et al.* 1987; Du *et al.* 2007; Du, Shen and Wang 2009). The warmer the temperature the faster the rate that the yolk is absorbed by the embryo, and the shorter the incubation period (Gutzke *et al*. 1987; Packard *et al*. 1987). If the temperature is too high

however, malformations of the embryos may occur, such as loss or anomalies of limbs and jaw, aberrations of the central nervous system and eyes, and deformity of the vertebral column (Burger, Zappalorti and Gochfeld 1987; Deeming and Ferguson 1991). Extremely low temperatures extend incubation time and may lead to embryo death. For those hatchlings that do emerge, a large amount of unabsorbed yolk is left in the eggshell, and consequently they are of smaller body size (Burger *et al*. 1987; Deeming and Ferguson 1991).

The temperature experienced by embryos during incubation can determine sex ratio, which has been one of the most studied phenotypic traits in turtles (see Valenzuela 2004). A large number of turtle species exhibit temperature dependent sex determination (TSD; Ewert and Nelson 1991). For most species with TSD, female turtles develop when the embryos are incubated at higher temperatures, while the cooler thermal regimes induce the development of males. A lesser number of species have a different pattern, with either high or low incubation temperatures inducing the development of females and intermediate temperatures producing only males (Ewert and Nelson 1991). Not all Chelonians exhibit TSD and a number of species (mostly freshwater and terrestrial turtles) have their sex determined genetically (Georges and McInnes 1998; Ji *et al*. 2003; Booth *et al*. 2004). Such species are excellent models for examining the influence of temperature on phenotypic variation of the hatchlings, because the differences inherent of each sex will not affect the results (Ashmore and Janzen 2003).

The temperature experienced by eggs during incubation can influence a number of physiological and behavioural phenotypic characteristics of the hatchling turtles. These phonotypical alterations can be severe enough to affect the survivorship of the hatching turtles (Deeming and Ferguson 1991; Booth 2006). Phenotypic traits influenced by temperature are: pigmentation patterns, post-hatch growth rate (Ewert 1979; Janzen 1993; Booth *et al*. 2004; Delmas *et al*. 2007), strength, endurance (Janzen 1993; Rhen and Lang 1999; Du and Ji 2003; Booth *et al.* 2004; Burgess, Booth and Lanyon 2006; Delmas *et al.* 2007) and behavioural patterns (Deeming and Ferguson 1991; Booth 2006).

Once the hatchlings leave the nests, which are generally located in underground chambers, the turtles have to make their way to the water. Hatchlings may be easily destabilised by obstacles on the path, such as rocks, logs and irregularities in the soil. The ability to right itself if turned upside down is vital for the hatchling turtle, because if unable to do so quickly the hatchling increases its chance of mortality by either predation or overheating by long sun-

exposure. A number of studies have revealed that shifts in incubation temperatures have a significant effect upon the hatchling freshwater turtle's ability to righting itself (Janzen 1993; Rhen and Lang 1999; Du and Ji 2003; Delmas *et al*. 2007). Once in the river, the hatchling turtles must be able to evade predators and catch prey. Abnormally small or miscoloured hatchlings, due to suboptimal incubation temperature, may represent an increase in predation potential and may also limit the hatchling's foraging success in the wild (Heithaus and Frid 2003). Hatchlings must also be able to swim effectively through the water column, and often against water flow, in order to access the surface to breathe air, forage and successfully escape from danger. If natural incubation conditions are altered, the locomotor and swimming performances of the hatchlings may be impaired, with potential implications upon behavioural function (Janzen 1993; Du and Ji 2003; Booth *et al*. 2004; Burgess *et al*. 2006). The optimal incubation temperature and the extent to which phenotype is influenced by temperature is highly species-specific.

Amongst the studies that have investigated the influence of incubation temperature upon the phenotype of hatchling turtles, a large number were focused on species with TSD and have utilised constant thermal treatments (Brooks *et al*. 1991; Spotila *et al*. 1994; Valenzuela and Janzen 2001; Janzen and Morjan 2002; Valenzuela 2004). There is a gap in knowledge regarding the influences of daily fluctuations in incubation temperature upon the embryo development in turtles with sex determined genetically, which avoids data being biased by differences inherent of the sexes. Furthermore, from the existing literature, it is difficult to compare the effects of incubation temperature upon phenotypic traits between different species of turtle. This difficulty occurs because many of the studies conduct experimental manipulations of incubation temperature without detailed knowledge of the thermal regimes that the eggs would naturally experience in the wild.

Habitat requirements for juvenile turtles

One of the most important factors for the effective conservation of a species is ensuring that individuals attain reproductive age. For most organisms, mortality potential is highest during the early life-stages due to a 'knife-edge' energy budget and a high vulnerability to predation (Mogensen and Post 2012). For animals with no parental care, like turtles, the habitat requirements for juveniles may be very different from the adult form (Kinney and Simpfendorfer

2009; Ward, Nislow and Folt 2011). A particular habitat will be selected by juvenile turtles to provide food, shelter and protection from predators and the physical environment (Reagan 1974).

The hatchlings and juveniles of freshwater turtle species often show different dietary requirements from the adults. In many species, the diet of the hatchling turtles is carnivorous, whilst the adults are mainly herbivorous (Moll 1976; Plummer and Farrar 1981; Hart 1983). This ontogenic shift in dietary requirements has been attributed to changes in the animal's metabolic demand throughout the life cycle (Clark and Gibbons 1969; Parmenter and Avery 1990). A carnivorous diet is required by the young turtles because it provides nutrients, such as protein and calcium, vital for growth and shell hardening (Clark and Gibbons 1969; Parmenter and Avery 1990).

Predation rate of hatchlings is extremely high and in order to survive and achieve reproductive age, turtles must inhabit areas that provide effective shelter and predator avoidance (Paterson *et al.* 2012). Juveniles may seek protection in areas different from the adults due to differences in body sizes and the types of animals that may predate upon them (Haskell *et al.* 1996; Janzen, Tucker and Paukstis 2000). Because of their small size, juveniles will be more susceptible to changes in the environment than the adult form. These may be changes in the level, velocity and temperature of the water (Congdon *et al.* 1999; Elnitsky and Claussen 2006; Tamplin 2006; Clark, Gordos and Franklin 2008). Consequently, the immediate environment elected by hatchling turtles may be more particular and critical than that selected by the adults, and will likely influence their survival potential.

The loss of habitat, for both juveniles and adults, is known to be one of the main causes of population decline for many species of freshwater turtle (Rhodin *et al*. 2011). Whilst habitat selection has been moderately investigated in adult turtles, virtually nothing is known about the habitat preferences of juvenile freshwater turtles. There are a number of logistical constraints that limit such investigations upon juvenile freshwater turtles. These are: low density of individuals, low-visibility and refuge-rich environments, small body size, as well as shy and cryptic behaviour. Biological data pertaining to the critical habitat selected by juvenile turtles are urgently required to identify protection areas. Miniaturised telemetry methodology, developed for locating fish (Welch, Ward and Batten 2004; Barry *et al.* 2007; Stickler *et al.* 2008), combined with spatially explicit models, which are ecologically realistic (Hirzel *et al.* 2002; Armstrong and Nislow 2006; Basille *et al.* 2008), would be effective tools for this cause.

Study species: Mary River turtle Elusor macrurus

The present study investigated the biology of the early life-stages of the Mary River turtle, *Elusor macrurus* (Figure 1.1). This species is a bimodal respiring freshwater turtle endemic to the Mary River in Queensland, Australia (Cann and Legler 1994). *Elusor macrurus* is an excellent species for the investigation of the influences of incubation temperature upon the phenotypic traits of the hatchlings, because: 1/ the eggs are laid in shallow nests (Cann and Legler 1994) and are, therefore, more likely to be subjected to ambient temperature variation in comparison to deep nests (Booth 2006); 2/ the females lay rigid-shelled eggs (Cann and Legler 1994), which are only slightly affected by the hydric conditions surrounding the eggs and therefore, the influences of incubation temperature can be studied in isolation (Packard 1991; 1999; Booth *et al*. 2004; Booth 2006; Booth and Yu 2009); and, 3/ *E. macrurus* does not exhibit temperature dependent sex determination (Georges and McInnes 1998), which allows investigations upon phenotypic traits without data being biased by differences inherent of each sex (Congdon and Gibbons 1985; Booth *et al*. 2004).

Figure 1.1: Adult male (a) and hatchling (b) *Elusor macrurus* (Mary River turtle).

Elusor macrurus is one of the largest Australian short-necked Chelids. The females attain a straight carapace length of up to 40 cm and the males up to 48 cm (SCL; Figure 1.1a). The hatchlings are however, small (~ 33 mm SCL; Figure 1.1b) in comparison to other species of freshwater turtles of similar adult size (Cann 1998). A number of distinct morphological characters separate this turtle from its close relatives (genus *Elseya* and *Rheodytes*), resulting in *E. macrurus* being placed in its own genus (Figure 1.2; Cann and Legler 1994). The distinguishing morphological characteristics of this species include presence of a vestigial nictitating membrane and the abnormally large tail of the males. The tale of a male *E. macrurus* can attain up to 70 % of their carapace length and is unique amongst Chelids. The function of such large tail is however, not clear and more investigations upon the species behaviour and physiology are required. The lateral compression of the tail has been suggested to act as a "paddle-like" structure that may improve swimming ability, whilst the extreme large size has been proposed to have a purpose during mating.

Elusor macrurus has a reproductive cycle similar to other Australian freshwater turtles. It has a long reproductive cycle, and the species has been estimated to take approximately 25 - 30 years to attain sexual maturity (Flakus 2002). The maturation of the follicles in female *E. macrurus* has been shown to take up to twelve months. Uncommonly for Chelonians, female *E. macrurus* do not have a reproductively inactive phase immediately after the nesting season (Flakus 2002). These conclusions were based in observations of an absence of ovary regress after nesting (Johnson 1996). *Elusor macrurus* close relatives often lay two or more clutches within their respective breading seasons (Legler and Cann 1980; Georges 1983; Legler 1985), but evidences suggest that *E. macrurus* lay only one clutch per year (Flakus 2002).

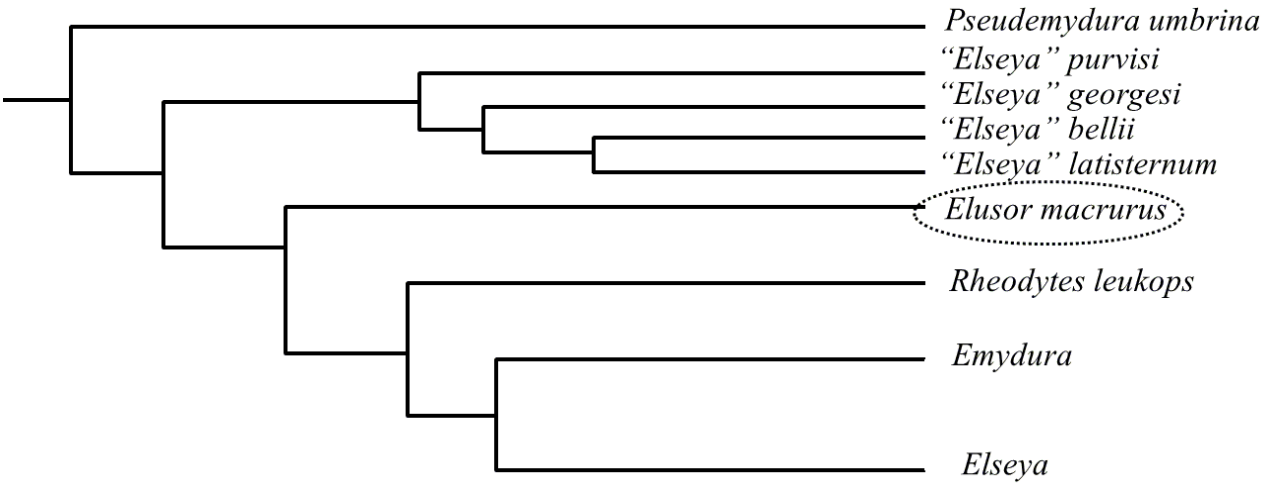


Figure 1.2: Phylogenetic tree of the relationship of short-necked Australian Chelid turtles (modified from Iverson *et al*. 2007).

Currently, *E. macrurus* is considered to be in imminent threat of extinction, being the second most endangered species of Australian freshwater turtle (IUCN, 2011). The *E. macrurus* population has been reported to have declined by ~ 95 % in the last 30 to 40 years (Flakus 2002). This reduction is largely attributed to the extensive collection of eggs during the 1960's and 70's from the nesting banks along the Mary River. These were incubated in captivity and the hatchlings sold into the pet trade (Cann and Legler 1994). Another contributing factor to the decline of the *E. macrurus* population is its limited geographical range, as the species is only found in the Mary River catchment. *Elusor macrurus* is known to inhabit the mainstream of the river from Kenilworth, in the upper reaches, to the saltwater tidal barrage downstream of Tiaro (Cann and Legler 1994; Cann 1998). The species may also occur in some of the major creeks both upstream and downstream from the tidal barrage (Tinana and Gutchy Creeks). Because the entire geographical range of the species is confined to this one river system, understanding habitat utilisation at different life-stages is critical to make effective management decisions. Changes in land and water use along the margins of this river may deeply impact upon the long-term survival of the species.

Since 2001, Tiaro & District Landcare Group has been monitoring a number of *E. macrurus* nests. This community group is mostly composed of farmers and residents from small towns situated along the margins of Mary River. The program protects the nests of *E. macrurus* throughout an approximate 20 km stretch of the river. They also monitor the weather conditions during the nesting season and the hatching success of a number of sites. The data recorded by the group has shown that at least 400 hatchlings enter this section of the river each year. Their fate once they enter the river is however, unknown, as the juveniles are rarely sighted in the wild, and virtually nothing is known about their habitat requirements. Similarly, limited data is currently available about various aspects of the biology of *E. macrurus* at different life-stages. Such information is however, urgently required in order to aid the recruitment and population status thought the improvement of current conservation projects, and also by creating effective management acts that will protect critical habitats for the species.

The habitat of Elusor macrurus: the Mary River

The geographical range of *Elusor macrurus* is restricted to one catchment: the Mary River (QLD, Australia). The river is home of six species of freshwater turtle, four short-necks: *E. macrurus*, *Elseya albagula*, *Emydura macquarii krefftii* and *Wollumbinia latisternum*, and two long-necks: *Chelodina longicollis* and *Chelodina expansa*. The catchment covers an area of approximately 7,000 km^2 and is located in the south-east portion of Queensland, Australia (Figure 1.3a). The river rises in the Sunshine Coast hinterland at Booroobin and flows north through the towns of Kenilworth, Gympie, Tiaro and Maryborough before emptying into the Great Sandy Strait, which is a passage of water between the mainland and Fraser Island (17 km south of Hervey Bay). The major tributaries of the Mary River include Tinana Creek, Munna Creek, Obi Obi Creek, Yabba Creek, Wide Bay Creek and the Susan River (Figure 1.3b). The average annual rainfall in the Mary River ranges from approximately 2,000 mm in the upper-catchment to around 1,200 mm near the river mouth. Flooding regularly occurs between the December and April wet season, a period characterised by heavy rainfall in the upstream reaches.

Currently, the Mary River is mostly surrounded by small towns and farmland. Commercial activities began in the river around 1840s, when wool and timber were transported along its length. In 1959, the town of Maryborough was declared an official Port of Entry for Australia, expanding the economic activities in the region to include cotton, cane and livestock.

Today, sugar cane and livestock are the major agricultural activities along the banks of the Mary River, with sand and gravel mining also occurring in places. The river shows signs of degradation due to these human-derived activities. Many river banks are damaged by livestock, with high concentrations of weeds present in-stream. Additionally, compared to pre-European conditions water turbidity has increased significantly in line with broad losses of riparian vegetation cover. The impacts of habitat alteration and loss upon the river and its inhabitants remain poorly understood.

An approximate 15 km stretch of the Mary River was selected as the study area due a known high concentration of *E. macrurus* nesting banks (Figure 1.3b). This section of the river, between the towns of Tiaro and Gundiah, is characterised by of a series of large deep pools with slow flowing water (Figure 1.3c) and shallow riffle zones with high water velocity.

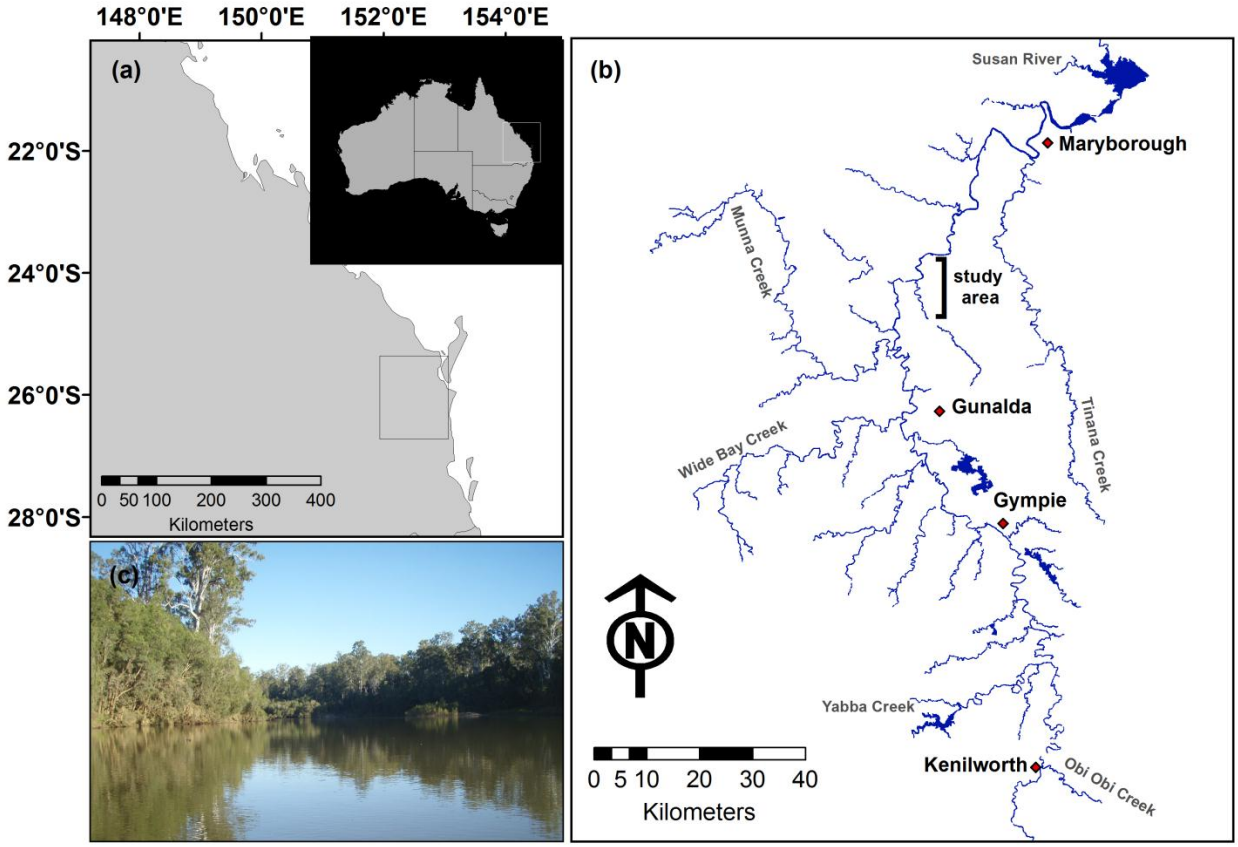


Figure 1.3: a) The geographical location of the Mary River (QLD, Australia). b) The river flows from the south to the north. Red symbols represent the location of the four main towns along the river, and the black bracket marks the study area for this investigation. c) Image of the Mary River.

Aims of research

Turtles are currently at high risk of extinction, and ecological information regarding their early life-stages is urgently required for freshwater turtle management and long-term conservation. The preservation of suitable habitat for embryos, hatchlings and juvenile turtles will improve recruitment and increase the number of individuals attaining reproductive age, thereby improving population fitness. The overall aim of this thesis was to describe the nesting biology and juvenile ecology of an endangered species of freshwater turtle, *Elusor macrurus*, which is found within a single river system in South-East Queensland, Australia. This thesis documents three aspects of the turtle's biology during early life-history stages, from the nest-site selection by the females, through incubation, to the habitat required by the juvenile turtles once they enter the river.

Monitoring changes in populations and introducing effective management are challenging for the conservation of many turtles, primarily due to a lack of information about the species' reproductive biology. The first aim of this thesis was to describe the nesting biology of the endangered *E. macrurus* (Chapter 2). Importantly, nest-site selection by adult females may influence embryo development and the survivorship of hatchlings (Wilbur and Morin 1988), thus the second aim of this thesis was to understand the nest-site selection by female *E. macrurus* (Chapter 2).

The success of an individual in the wild is highly dependent upon its physical condition. Incubation temperature is known to be one of the principal factors influencing the phenotype of hatchling turtles, hence their chance of survival. The third aim of this thesis was to investigate, through empirical studies, how mean and daily fluctuations in incubation temperature affected the phenotype and exercise performance of hatchling *E. macrurus* (Chapters 3 and 4).

Ensuring that juvenile turtles reach reproductive age is vital for population longevity, yet the habitat requirements of juvenile turtles are often unknown. Identifying juvenile turtle habitats can be challenging due to their small size and elusive behaviour. The fourth aim of this thesis was to identify the characteristics of the habitat selected by hatchling and juvenile *E. macrurus* once they enter the river (Chapter 5). The fifth aim of this thesis was to develop a methodology that would overcome the false-absences inherent to locating small, cryptic and shy animals within low-visibility and refuge-rich environments (Chapter 5).

The sixth and final aim of this thesis was to use the study findings to provide direction for management and conservation strategies for *E. macrurus* (Chapter 6).

Structure of thesis

This thesis is composed of four experimental chapters that investigate physiological and ecological aspects regarding the early-life stages of an endangered Australian freshwater turtle, *Elusor macrurus*, for the purpose of management and conservation. The first experimental chapter (Chapter 2) describes the nesting biology of *E. macrurus* and presents a novel approach for investigating nest-site selection and the behaviour of nesting females. Chapters 3 and 4 assess the temperature regimes recorded from *E. macrurus* nests in the wild and investigate how changes in incubation temperature impact upon embryo development and the phenotype of the hatchlings. Chapter 3 investigates how small rises in mean incubation temperature influence hatchling phenotype, while Chapter 4 examines how daily fluctuations in incubation temperature affect the performance of the hatchlings. The final experimental chapter (Chapter 5) develops and applies a presence-only predictive model to identify habitat characteristics selected by juvenile *E. macrurus*. Chapters 3 and 4 are published articles, and Chapters 2 and 5 are currently under peer-review. Each chapter is written as a complete work, containing an abstract, introduction, materials and methods, results and discussion sections. The final chapter of this thesis (Chapter 6) concludes by summarising the findings of the four experimental chapters for the purpose of providing insights for management and conservation of the study species, while also presenting directions and considerations for future research.

CHAPTER 2

Nest-site fidelity and location preferences of an endangered turtle.

Abstract

Here we describe the nesting biology of an endangered species of freshwater turtle (*Elusor macrurus*) from Australia. Nesting data gathered between 2004 and 2011 demonstrated that female *E. macrurus* lay their eggs at specific localities along the banks of the Mary River (Queensland, Australia). These areas are characterised by sandy soil and are clear of riparian vegetation. Time-lapse infrared photography and image-identification analysis proved a valuable method in the identification of individual turtles and demonstrated that female *E. macrurus* exhibit nest-site fidelity. We aimed to determine differences between the favoured nesting sandy banks and other non-preferred sandy banks, within the close vicinity, by measuring five soil characteristics and six physical attributes. The only observed significant difference between preferred and non-preferred nesting banks was the slope aspect: the preferred nesting banks were northerly facing whereas the non-preferred banks were not. In the southern hemisphere, northerly facing slopes are exposed to higher levels of solar radiation, and as a consequence, these banks exhibited significantly greater daily fluctuations in temperature at *E. macrurus* nest depth. This aspect of *E. macrurus* nesting biology requires further investigations because of its significance for the protection/creation of nesting areas.

Introduction

The activities of humans are drastically reducing turtle populations. The International Union for Conservation of Nature (IUCN) documents that 45 % of the 263 identified species of freshwater and terrestrial turtles are either threatened or endangered (IUCN 2011). Chelonians are at higher risk of extinction than birds, amphibians, elasmobranches and mammals, and are paralleled amongst vertebrates only by the primates (Hoffmann *et al.* 2010). Monitoring changes in populations and introducing effective management are challenging for many species because very little is known about their general biology. Information regarding the early life-stages is crucial for the protection of freshwater turtles, because creating and preserving suitable habitat

for embryos, hatchlings and juveniles will increase the number of individuals attaining reproductive age.

For egg-laying reptiles, nest-site selection by the adult females influences the survivorship of the hatchlings (Wilbur and Morin 1988). The environmental conditions, in particular temperature and moisture, experienced by the egg during incubation has been shown to influence embryonic developmental rate, hatching success, and hatchling sex ratio, body size, locomotor performance, thermoregulation behaviour, and post-hatch growth rate (Miller *et al.* 1987; Packard *et al.* 1987; Janzen *et al.* 1990; Ackerman 1991; Janzen 1993; Packard *et al.* 1999; Booth 2002; Booth *et al.* 2004; Booth 2006; Micheli-Campbell *et al.* 2011). The temperature and moisture content within the nest will be influenced by the landscape aspect and substrate characteristics of the nesting site, as well as external ambient conditions (Ackerman 1991; Marchand and Litvaitis 2004; Bonach *et al.* 2007). In sandy soils, such as the ones present on the banks of many Australian rivers, the pores between the particles are relatively large, which facilitates water movement through the soil (Koorevaar *et al.* 1983; Ackerman 1991). Although a number of studies have investigated the influence of substrate type and landscape features on the nesting biology and embryo development of marine turtles (Mortimer 1990; Wilson 1998; Wood and Bjomdal 2000; Ficetola 2007; Afonso *et al.* 2009), little is known about such aspects for freshwater species.

Elusor macrurus (Mary River turtle) is the second most endangered species of freshwater turtle in Australia (IUCN, 2011). The species was only scientifically described in 1994, and by this date the population was estimated to be 95 % depleted (Flakus 2002). The decline of the *E. macrurus* population is largely attributed to extensive collection of the eggs from the nesting banks during the 1960's and 70's (Cann and Legler 1994); however, the population does not appear to be showing recovery even though there has been no egg collection for over 30 years (Flakus and Connell 2008). *Elusor macrurus* only inhabits the Mary River catchment in South-East Queensland (Australia), and because of this limited geographical distribution, changes in land and water use within the catchment may have ramifications for the entire population. Female *E. macrurus* appear to only nest on a limited number of nesting banks that consist of sandy soil without riparian vegetation cover along the river, despite other seemingly suitable sites being available nearby (Flakus 2002). The aim of this study was to characterise the nesting biology of *E. macrurus*, and in particular to understand the nest-site selection by the females. In order to undertake this task we: 1/ measured a range of variables from 28 nests during the nesting season

of 2009; 2/ correlated nest timing data from 2004 until 2011 with weather conditions; 3/ determined if female *E. macrurus* exhibited nest-site fidelity; and 4/compared a wide range of physical characteristics of preferred nesting banks to other non-vegetated sandy banks where *E. macrurus* has not been recorded to nest on.

Material and Methods

Study area

The present study was conducted along a 15 km stretch of the Mary River, QLD, Australia (Figure 2.1). This area was selected because a high number of *Elusor macrurus* nests are predictably laid within this section of the river. Nesting data were collected from four known *E. macrurus* nesting banks (Figure 2.2) and compared with similar data collected from four other river sandy banks within the study area that have not recorded *E. macrurus* nesting.

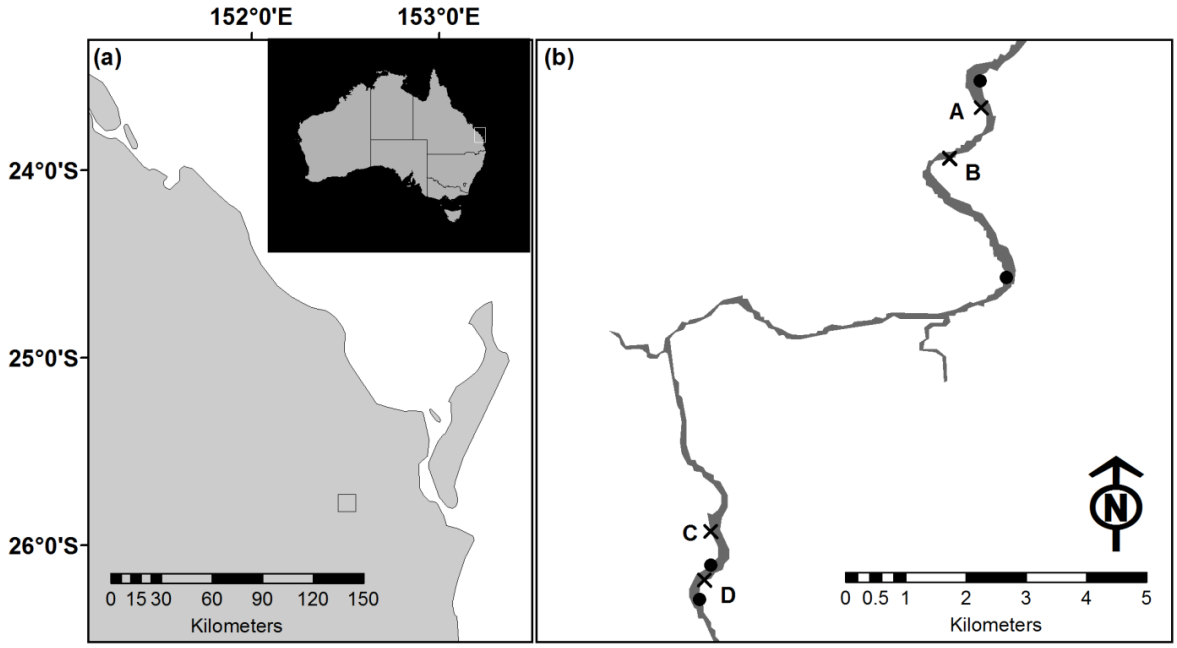


Figure 2.1: a) The geographical location of the Mary River (QLD, Australia). b) The river flows south-west to north-east. Black crosses represent the location of the nesting banks monitored during the study (nesting banks A, B, C and D). Black circles represent the location of the river banks where *Elusor macrurus* nests have never been recorded (non-preferred banks).

Figure 2.2: Sandy bank used by female *Elusor macrurus* for nesting (Mary River, QLD, Australia).

Nest data collection

Between October and December 2009, nesting activity by female *E. macrurus* was monitored at four selected river banks located within the study area. Female *E. macrurus* lay their eggs in moist sand, leaving a distinctive trace that enables easy identification of freshly laid clutches. Nests were located on the following morning after oviposition, when bank slope angle (using a clinometer), straight-line distance from the nest to the water and to the nearest vegetation edge were measured. Nest elevation was calculated as the sine of slope angle times the straight-line distance from the nest to the water (for details see Wood and Bjomdal 2000). Each nest was carefully excavated to expose the entire egg chamber and allow measurement of nest parameters. Clutch width was measured in two directions perpendicular to each other (W = widest, N = narrowest). The eggs were carefully removed from the nest and counted (clutch size), clutch depth measured (distance from the bottom of the clutch to soil surface) and soil samples collected from inside the nest chamber (see details below). The eggs were then returned to original position, a temperature logger (2 cm diameter, Hobos® TidBit®, Onset, Bourne, MA, USA),

programmed to record data every 40 min, was placed as close as possible to the eggs and the clutch was then re-buried.

This study was undertaken in collaboration with Tiaro & District Landcare Group (TDLG), a community group that undertakes a nest protection program with the aim of aiding the recruitment of hatchlings *E. macrurus* into the river. The protection program began in 2001 and continues to present. The program collects weather and nesting activity during the nesting season (October – January).

Identification of nesting females

To investigate nest-site fidelity, a known nesting bank (bank B, Figure 2.1b) was monitored for three consecutive years with three surveillance cameras (PM75 RapidFire Mono IR, Reconyx, Holmen, WI, USA) mounted in purpose built housing ~ 2 m above the ground and positioned at the water's edge. The cameras faced the slope of the bank and the females were photographed as they walked through the view of the cameras when travelling from the river up the bank's slope. Each camera view covered approximately 2 m^2 of the river bank surface. The cameras were set in time-lapse mode and took an image every 30 s. Each image displayed the date, time, air temperature and moon phase. For each camera deployment, an image of the ground covered with a plastic mesh composed of 5 cm^2 squares was taken. These pictures were used to provide real-distance measurements and enable the calibration of the image-analysis software.

The images containing females were identified and catalogued. These images were then analysed with image-analysis software (SigmaScan®, Systat Software Inc., San Jose, CA USA). Identification of individual females was only possible for the turtles which had indentations, naturally or man-made, on the marginal scutes (notches). Measurements taken from each turtle image were: carapace length (CL), notch length (NL; for each notch present), perpendicular distance between the lower edge of the notch (closer to tail) to the CL line, perpendicular distance between the upper edge of the notch (further away from tail) to the CL line, and distance between the upper edge of the notch and the beginning of the CL line (by head; see Figure 2.3a). A set of these measurements was taken for each notch present on the females' carapaces.

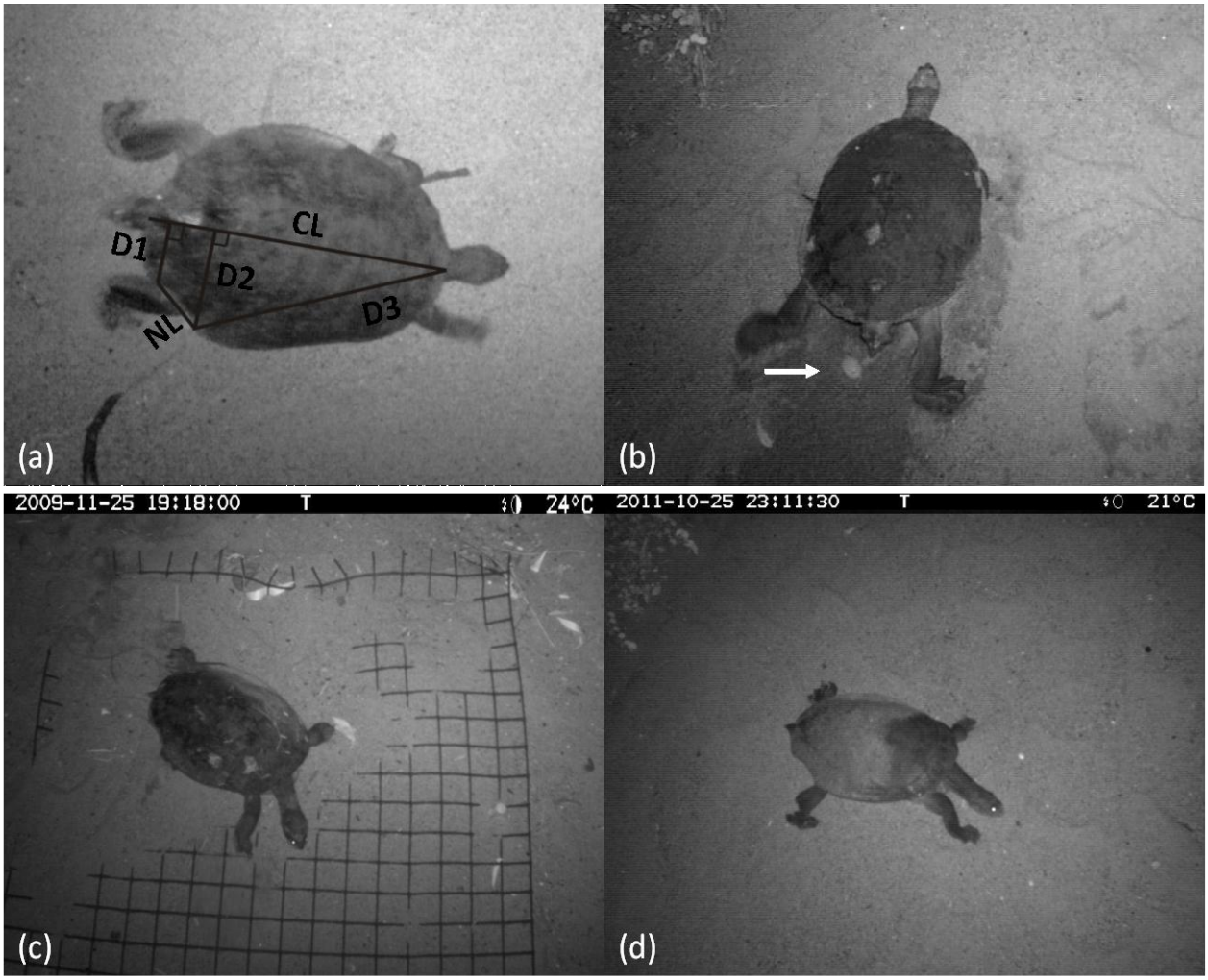


Figure 2.3: a) Set of measurements used to identify different females. They were obtained by the image-analysis software and were taken for each mark/notch present on the carapace. b) Female *Elusor macrurus* laying a clutch in the view of the camera (white arrow indicates freshly laid egg). c) and d) One of the individually identified female *E. macrurus* photographed when accessing nesting bank B in two different years (c – 2009; d – 2011).

River bank characterisation

Physical characteristics were recorded for both preferred and non-preferred nesting banks (Figure 2.1b). The river banks not used by *E. macrurus* for nesting had the soil samples collected

and temperature loggers (n = 18; Hobos® TidBit®, Onset®) placed into three random locations (~ 20 cm depth – the mean *E. macrurus* nest depth) across the bank.

Measurements taken for each bank were: width of the bank (straight distance between the water's edge and the top of the bank), bank slope angle, bank elevation (to the highest point of the bank), length at the top and at the bottom of the bank (by the water's edge), and bank slope aspect (i.e. the compass bearing perpendicular to the water's edge).

Soil analysis

Soil samples were collected from both preferred (egg chambers) and non-preferred nesting banks. First, a small sampling tube (10 cm^3) was filled with sand collected from the bottom of the chamber for quantifying soil organic matter (Wt %) by dry-combustion (CNS-2000 analyser, LECO Corporation, St Joseph, MI, USA). These samples were kept refrigerated until laboratory analysis.

Second, a metal cylinder (95 cm^3) was carefully inserted into the soil surrounding the clutch, and a spatula was then used to help remove the cylinder filled with soil from the ground, aiming to minimise soil structure disturbance. Petri dishes were placed on both sides of the cylinder secured by adhesive tape and then the cylinder was placed into a zipper-storage bag. In the laboratory, these soil samples were analysed to determine: soil moisture content (MC %), bulk density (g cm^{-3}), hydraulic conductivity (cm s^{-1}) and soil water retention curve. MC was determined by weighting the fresh soil sample and then placing it in the oven at 105ºC for 24 h and then re-weighed. The difference in mass between the wet and dry soil sample was then divided by the wet sample mass and multiplied by 100. The bulk density was calculated as the mass of the oven dried soil sample divided by the total soil sample volume (95 cm^3).

A laboratory permeameter was used to measure the permeability of the soil samples by creating a difference in water pressure on both ends of a saturated soil sample and measuring the resulting flow of water. The permeameter is a closed system where water is pumped up from a storage cistern to an adjustable level regulator through a filter. A complete saturated cylinder filled with soil sample was placed in a cylinder-holder and a sieve disc placed on the top of it. The cylinder was then turned upside down (so the sieve was under the sample) and then placed inside the container. A plastic siphon channelled the water oozing from the sample to a burette,

which released the water into a leak basin connected to the storage cistern. The siphon created a difference in water level inside and outside the cylinder-holder, inducing a continuous flow of water through the sample. The permeability coefficient (K-factor) of the samples was established by applying the following formula (derivate from Darcy's Law) using the volume of drained water through the burette during a fixed period of time:

$$K = \frac{V.L}{A.t.h}$$

Where: K = permeability coefficient or "K-factor" (cm d^{-1}); V = volume measured in the burette (cm^3); L = length of the soil sample (cm); A = cross-section surface of the sample (cm^2); t = time used for flow through of water volume V (d); d = time dimension day; h = water level difference inside and outside sample cylinder (cm).

The pressure plate technique was used to obtain a soil water retention curve (for details see Otto and Alcaide 2001; Lucas *et al.* 2011; Moret-Fernandez *et al.* 2012). Undisturbed water-saturated soil samples were exposed to 1, 2, 3, 10, 50 and 1000 kPa and the pressure versus moisture points were adjusted by the van Genuchten model analysed by RETC software (PC-Progress, Prague, Czech Republic).

Statistical analysis

Multiple analyses of variance (ANOVAs) were used to analyse the physical characteristics of the river banks: slope width, slope angle, elevation, length at the top and at the bottom of the bank, and temperature data. A t-test was performed to analyse data obtained from the soil samples: organic matter, bulk density, moisture content and hydraulic conductivity. The F-test was used to denote a significant difference between the means. All data are presented as mean ± S.E., and a difference between groups was deemed significant if $P < 0.05$ (Statistica10, StatSoft Inc., Tulsa, OK, USA). Circular analysis of Variance (high concentration F-test) was performed to analyse the slope aspect data in the R programming language (R Development Core Team 2011) using the 'circular' library of functions (Jammalamadaka and SenGupta 2001).

Results

Nesting and archival data

Data from twenty-eight freshly laid *Elusor macrurus* nests were collected throughout the 2009 nesting season from four nesting banks (Table 2.1). There was a large variation in the distance from the water chosen by the females to lay their eggs; some nests were as close as 1.3 m and others as far as 43.6 m from the water. Similarly, nests were laid both close (~ 1 m) and far away (~ 15 m) from vegetation, but they were not under the shade of any plants between 8:00 and 16:00 h. The elevation and distance to the water that a nest was laid above the river level was influenced by the river bank height and size and water level at the time of laying. The highest nest was found ~ 7 m above the water level, while the lowest was 0.5 m.

Table 2.1: Biological data collected from *Elusor macrurus* clutches laid in four different nesting banks located along a15 km stretch of the Mary River.

Nests (n = 28)	**Mean ± S.E.**	**Min – Max**
Distance to vegetation (m)	6.1 ± 0.9	0.5 – 15.1
Distance to water (m)	13.1 ± 1.9	1.3 – 43.6
Elevation (m)	3.6 ± 0.5	0.5 – 6.9
Clutch size	14.9 ± 0.9	2 – 22
Nest depth (cm)	21.0 ± 0.6	15.5 – 27.0
Nest width N (cm)	7.6 ± 0.3	4.1 – 9.6
Nest with W (cm)	9.1 ± 0.3	5.5 – 10.9

The *E. macrurus* nest protection program undertaken by TDLG provided long-term data (2004 – 2011) for one of the nesting banks investigated in the present study (nesting bank B). Females commenced nesting mid-October and the last clutch was laid towards the end of December. The number of clutches laid per season on nesting bank B was fairly consistent across the eight years, except for a dramatic decline in 2009 and 2010 (Table 2.2). Nest construction was clustered around a few nights over the nesting season, and these events were correlated with rainfall (Figure 2.4). On a few occasions however, nests were constructed independent of rainfall.

Table 2.2: *Elusor macrurus* nesting data, collected by TDLG between 2004 and 2011during the nesting seasons (October to December) at the nesting bank B.

Year	Date of 1st nest	Date of last nest	Total number of nests
2004 - 2005	19/10/2004	29/11/2004	23
2005 - 2006	14/10/2005	17/11/2005	21
2006 - 2007	06/11/2006	02/01/2007	26
2007 - 2008	12/10/2007	12/12/2007	22
2008 - 2009	13/10/2008	31/12/2008	18
2009 - 2010	14/10/2009	22/12/2009	9
2010 - 2011	09/10/2010	27/10/2010	5
2011 - 2012	17/10/2011	29/12/2011	20

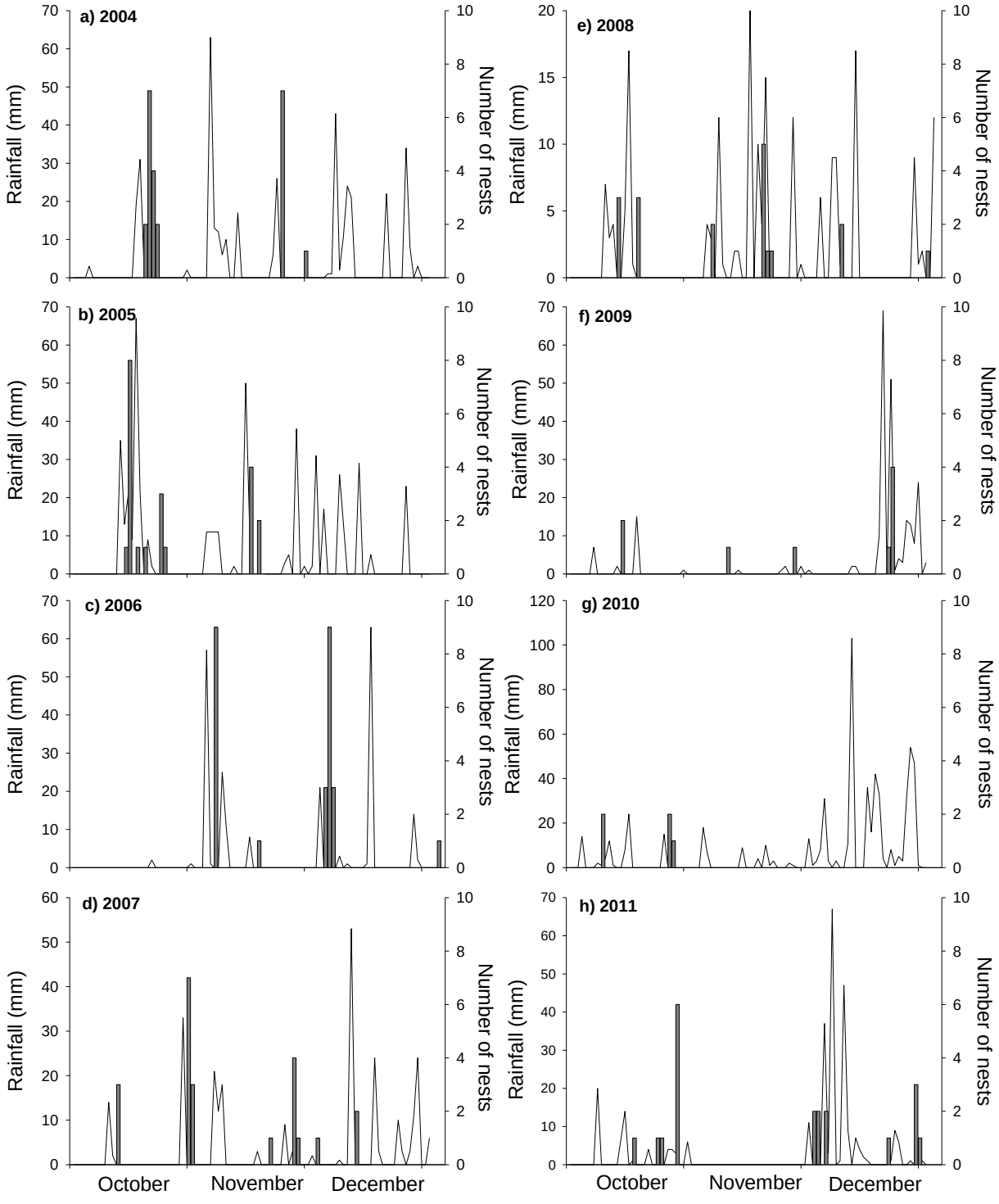


Figure 2.4: Number of *Elusor macrurus* nests constructed at the nesting bank B (bars) and local rainfall (mm; line) recorded between October and December from 2004 to 2011.

Nesting females

Nesting bank B was monitored by infrared cameras during the nesting season of 2009, 2010 and 2011. Female *E. macrurus* accessed the bank from sunset until sunrise, with an increase in the number of recorded females between 23:00 - 03:00 h (Figure 2.5). Moon phase and ambient temperature had no significant effect upon the frequency of activity on the bank or number of nests constructed, and females were photographed nesting in air temperatures between 12ºC and 28ºC (18.9 ± 0.1ºC, mean ± S.E.). Analysis of sequential images showed that females inspected the bank before finally completing nest construction, walking throughout the slope in all directions and digging sample holes in the sand multiple times. Six females were recorded laying their clutches within camera view (example Figure 2.3b), so it was possible to calculate nesting time (the time from when digging the nest started until the female left the nest site), which varied between 34 and 57.5 min (36.9 ± 6.3 min, mean ± S.E.).

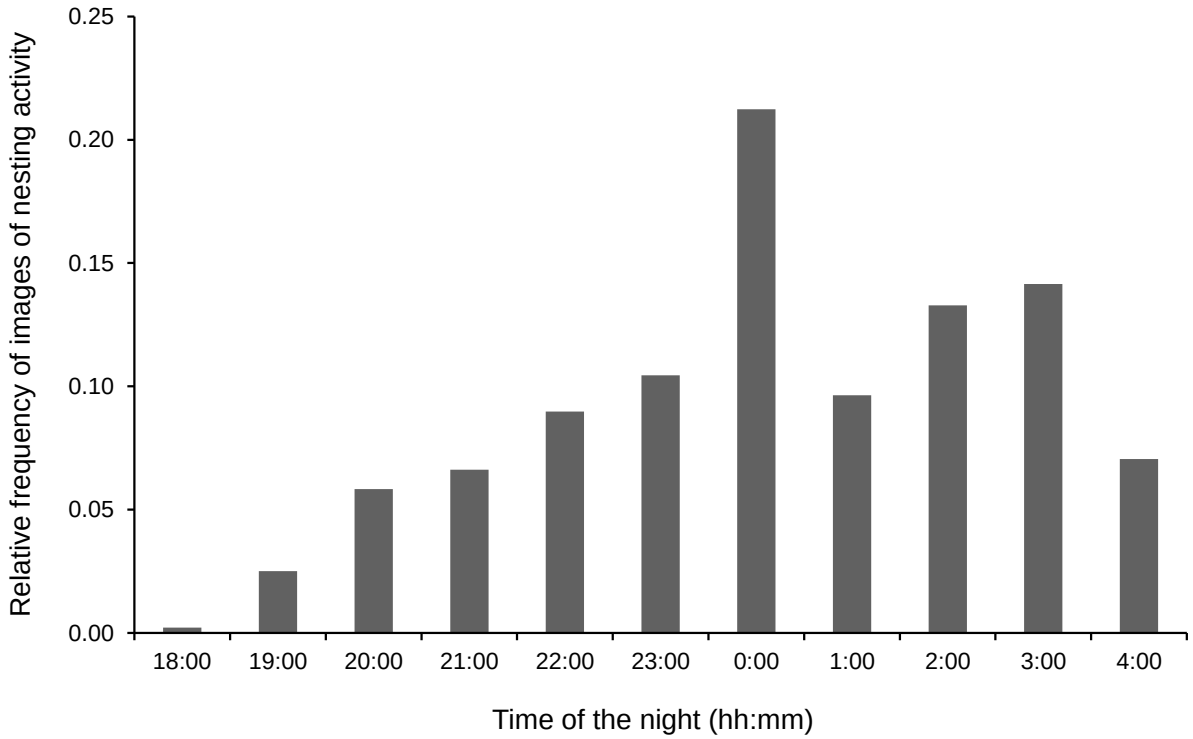


Figure 2.5: Frequency distribution of the number of images taken of female *Elusor macrurus* that accessed the river bank B throughout the night over three subsequent nesting seasons (2009 – 2011).

Image analysis of the infrared photographs revealed that female *E. macrurus* exhibit nest-site fidelity. A total of 22 females were individually identified and nine of those were recorded accessing the bank over three years, with two individuals photographed over three consecutive years (example Figure 2.3c - d). The second year of the study was hampered by an unusually early and large flood event, and therefore, the data collection time was reduced as the cameras had to be removed from the river bank after only a few weeks of recording.

Nesting banks characterisation

We aimed to record temperature data from all four nesting banks. Due to an unexpected flood event, however, five loggers were lost and temperature was only recorded from three banks. Mean daily temperature fluctuations between these three nesting banks were not statistically different ($F_{(2,13)} = 2.96$, $P > 0.05$; Figure 2.6a). Nesting bank C showed the largest variation and the highest mean temperature, but these were not significantly different from the other two banks ($F_{(2,13)} = 3.25$, $P > 0.05$; Figure 2.6b). There was no statistical difference between the mean temperature recorded for preferred and non-preferred nesting banks ($F_{(1,24)} = 2.34$, $P > 0.05$; Table 2.3), but the mean daily temperature fluctuation was significantly higher at the banks selected by female *E. macrurus* for nesting ($F_{(1,24)} = 1.19$, $P < 0.05$; Table 2.3).

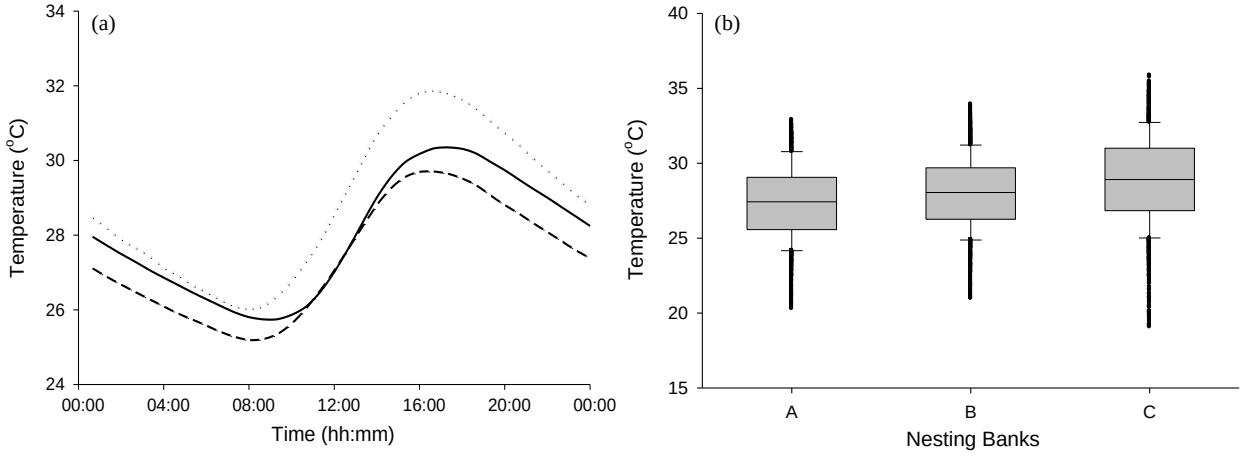
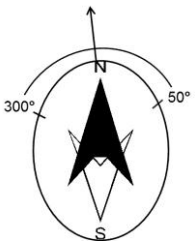
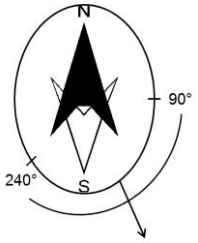


Figure 2.6: a) Daily temperature fluctuation recorded from 16 *Elusor macrurus* nests laid in three different nesting areas (dashed line = nesting bank A; solid line = nesting bank B; dotted line = nesting bank C). b) Box-and-Whisker plot showing the temperature range recorded throughout the 2009 nesting season from nests laid in three distinct nesting areas (A - n = 6; B - n = 4; C - n = 6; $P > 0.05$).

Table 2.3: Physical features and soil characteristics recorded for eight river banks distributed along a 15 km stretch of the Mary River used (nesting banks, n = 4) and not used (non-preferred banks, n = 4) by female *Elusor macrurus* for nesting. Data are mean ± S.E. and symbol (*) indicates statistical differences ($P < 0.05$).

Physical	**Nesting banks**	**Non-preferred banks**
Mean temperature (ºC)	28.5 ± 0.02	28.0 ± 0.04
Mean daily temperature fluctuation (ºC)	5.7 ± 0.1*	5.3 ± 0.2*
Bank width (m)	6.9 ± 1.4	9.7 ± 2.0
Bank angle (degrees)	25.1 ± 3.3	13.6 ± 4.0
Bank Elevation (m)	2.3 ± 0.6	1.9 ± 0.7
Bank length at top (m)	22.2 ± 7.1	7.5 ± 2.2
Bank length at water's edge (m)	19.3 ± 5.8	10.6 ± 3.2
Bank slope aspect (degrees from N)	50 – 300*	90 – 240*
		
Soil		
Organic matter (Wt %)	0.16 ± 0.03	0.16 ± 0.03
Bulk density ($g.cm^{-3}$)	1.2 ± 0.02	1.2 ± 0.04
Moisture content (%)	6.3 ± 0.3	10.3 ± 0.9
Hydraulic conductivity ($cm.s^{-1}$)	0.07 ± 0.01	0.10 ± 0.02

Most of the physical characteristics recorded from the nesting banks (Figure 2.2) were similar to the ones measured at the river banks that were not used by female *E. macrurus* for nesting (Table 2.3). The slope aspect of the banks was however, significantly different between preferred and non-preferred nesting banks. The four nesting banks preferred by female *E. macrurus* for nesting were northerly facing, whilst the aspect of the non-preferred banks varied between East and South-West ($F_{(1,18)}$ = 53.47, P < 0.01; Table 2.3). There was no statistical difference in organic matter, bulk density, water content and hydraulic conductivity between soil collected from river banks selected by female *E. macrurus* for nesting and that collected from the non-preferred banks (P < 0.05; Table 2.3). A water retention curve was generated from the soil samples collected from *E. macrurus* nests (Figure 2.7), and indicates that at the time of nest construction nest water potential ranged between -1 and -40 kPa.

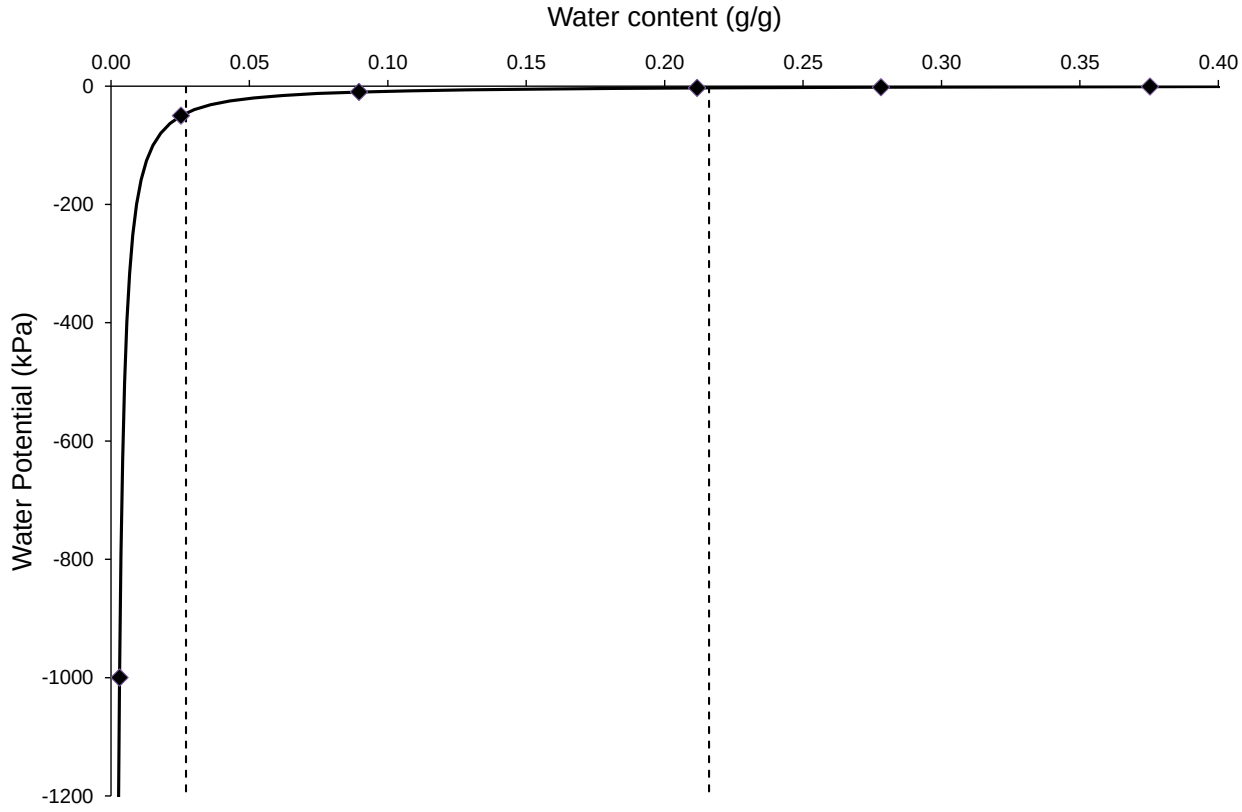


Figure 2.7: Soil water retention curve (solid line) generated from soil samples collected from 28 *Elusor macrurus* nests laid in 2009. Dashed lines represent minimum and maximum values of water content present in the sand samples collected from the *E. macrurus* nests. Symbols represent the actual measured values used to generate the curve.

Discussion

The site selected by female turtles in which to lay their eggs is vital for the survivorship of the resultant hatchlings (Wilbur and Morin 1988). Female *Elusor macrurus* selected particular banks along the Mary River in which to lay their eggs, with a large number of nests occurring within the same discrete area every year. Here we demonstrate for the first time that female *E. macrurus* exhibit nest-site fidelity to these particular sandy banks. Visually, the banks selected each year by the nesting *E. macrurus* are characterised by sandy soils, an absence of riparian vegetation and are of a sloping aspect, which makes them significantly higher than the river level within a short-distance. There are however, numerous other areas along the river that show these same general characteristics, but *E. macrurus* nests have not been recorded at these localities (Connell, pers. comm.). In an attempt to identify which features of the nesting areas were relevant for *E. macrurus*, we assessed eleven physio-chemical parameters from river banks that were preferred for nesting by *E. macrurus* and compared them against river banks where nests of this species have never been recorded, but shared similar general features, to the human visual observer. The study found that banks selected by female *E. macrurus* for nesting were all northerly facing, and exhibited a higher daily temperature fluctuation that the non-preferred nesting banks. The study demonstrates that *E. macrurus* are selective in the locality in which to lay their eggs and provides a reasonable rational for the nest-site selection.

General nesting biology

Typically, *E. macrurus* laid their eggs away from the edge of the river and generally several meters above water level. These features are shared by other freshwater turtles, and are likely to occur in order to reduce the chances of the nests being flooded (Booth 2010). The females nested after periods of rainfall during the night. Other Australian freshwater turtles also nest during darkness, which reduces the risk of predation by visual predators and exposure to high daytime temperatures (Booth 2010). Nesting after periods of rainfall is a common strategy of freshwater turtles and probably makes nest construction easier because moist sand gives better purchase for the female and reduces the chance of egg chamber collapse during the construction. Moreover, moist sand ensures the eggs will be in a positive water balance when first laid, which means gaining water from the environment. This signifies that they are able to take up water from the environment and form a water reserve that the embryos can call upon if the nest dries out later during the incubation period (Vestjens 1969; Booth 2010).

The nesting bank that was monitored in this study exhibited an approximate 60 % reduction in the number of nests laid in 2009 and 2010, compared to the previous 5 years. In 2009 very little rainfall occurred during the nesting season. Although only nine nests were constructed in this season, the infrared surveillance cameras photographed numerous females throughout the season searching for suitable places to nest (including the digging of "test-holes"). The females that did nest, laid their eggs within a positive water balance environment, as shown by the water retention curve. We cannot be certain that these females did not lay their eggs in another bank, but female freshwater turtles are known to have the capacity to store mature eggs inside their uterus for up to three months (Goode and Russell 1968), and the high number of searching females recorded during this time highlights their site-fidelity to these particular areas. This evidence suggests that female *E. macrurus* are site-selective when deciding upon a nesting location and the low number of nests recorded on this bank in 2009 was likely due to the females not finding suitable places to lay their eggs because of a low moisture content in the soil.

In 2010, the Mary River was exposed to a "once in 30-year" extreme flood event that extremely disrupted *E. macrurus* nesting season. The low number of nests in both 2009 and 2010 indicate that *E. macrurus* reproductive success is highly dependent on rainfall, with low hatching success due to low nesting activity in drought years, and drowned nests in flood years. In 2011, the rainfall during the nesting season was average for this time of the year and the number of *E. macrurus* nests was comparable to the archival data from 2004 to 2008.

Nest-site fidelity

Inter-year nesting site fidelity was observed in several individuals. We never captured or marked any of the turtles in this study, but we were able to confidently identify 22 females from visual markings on their carapaces. Less than half of the females identified were recorded to return to the same bank to nest on subsequent years, which suggested that not all females were photographed when they came to nest. This seems reasonable because the cameras only monitored a small section of the nesting area (~ 5 %). Other possibilities are that not all females are nest-site fidelic, or that not all females nest every year. Nest-site fidelity may be a behavioural strategy for maximising reproductive success and has been observed in other species of freshwater turtle (Loncke and Obbarde 1977; Congdon *et al.* 1983; Jackson and Walker 1997; Valenzuela and Janzen 2001). We are uncertain if *E. macrurus* has natal homing fidelity, i.e. they return to nest at the nesting bank from where they were hatched. This phenomenon has been

previously shown for both marine and freshwater turtle species (Freedberg *et al.* 2005; Lohmann *et al.* 2006; Bowen and Karl 2007; Sheridan *et al.* 2010), and would be an incredibly important observation for the long-term management of *E. macrurus*. Further investigations involving marking hatchlings and genetics tests are required to discover if natal homing fidelity occurs in this species.

The challenge for this study was to record the nesting activities of a freshwater turtle that is extremely shy and easily disturbed by human presence. The females only nest during the hours of darkness and therefore required infrared photography for monitoring their behaviour. Standard motion-detection cameras are developed primarily for photographing warm-blooded creatures and were not appropriate for this study because turtles do not generate sufficient external heat to trigger the infra-red sensor within off-the-shelf motion detection cameras. Instead, we utilised time-lapse photography and mounted the cameras above the nesting banks, at an orientation to take overhead images of the turtles as they passed underneath. To identify different individuals, we relied upon measurements that were taken in relation to carapace length and naturally occurring marks or notches on the carapace. This methodology was non-invasive and did not require capture or handling of the turtles. Unlike sea turtles, a number of freshwater species will stop laying their eggs and flee back to the river if approached when egg laying (Cann 1998). Therefore, we argue that the methodology developed in this study is a reliable and valuable strategy for observing nesting behaviour in fresh-water turtles, and necessary in order that the nesting activities of endangered species are not disrupted. The limitation of this technique however, was that we could only recognise females that had obvious marks or notches on their carapaces, and therefore some females could not be individually identified.

Nesting and river banks landscape characteristics

In this study, we monitored four nesting banks that consistently have a high number of *E. macrurus* nests each nesting season. We measured a number of soil characteristics and physical features of these banks, and compared them against four river banks that have shown similar general characteristics to the nesting areas. These were composed of sandy soil and were free of overhanging or riparian vegetation. The only physical and micro-environment differences found between preferred and non-preferred river banks for nesting *E. macrurus* were that favoured nesting banks faced north and experienced greater daily temperature fluctuation at nest depth. North-facing banks are probably preferred because they receive more direct solar radiation in the

southern hemisphere and therefore, are generally warmer than south-facing ones (Geiger 1965). A similar preference for north-facing banks has also been observed for nesting estuarine crocodiles (*Crocodylus porosus*) in Australia (Magnusson 1980). In the northern hemisphere, the midland painted turtle (*Chysemys picta marginata*) lays its eggs in south-westerly facing slopes (Hughes and Brooks 2006). Although there is scarce documentation of this reproductive tactic, it may be a common reproductive strategy in oviparous reptiles, and it requires further investigations because of its significance for the protection/creation of protection areas. The preference of *E. macrurus* for northerly facing banks with no vegetation cover indicates that the females may attempt to provide warmer conditions for the developing embryos through nest-site selection. We failed however, to find a significant difference in mean temperature at nest depth between preferred and non-preferred banks. We expected to find a significantly greater mean temperate in north-facing banks compared to those facing other directions, and although there was a trend towards this, we suspect the lack of significance was due to a complex interaction between the soil characteristics and changes in ambient thermal conditions. We argue that a greater number of intra and inter-banks samples may be required to generate statistical significance.

The hatchlings of *E. macrurus* have their sex determined genetically (Georges and McInnes 1998), and not by incubation temperature, so sex determination is not a driving force for nest site selection in this species. However, nest temperature is known to influence incubation time in oviparous reptiles (Deeming and Ferguson 1991). Thus, selecting river banks with higher temperature to nest in would reduce incubation time (Micheli-Campbell *et al.* 2011; Micheli-Campbell *et al.* 2012), and therefore, limit the time the eggs would be in the nest and vulnerable to predation (infrared cameras photographed lace monitors, plovers and European red foxes predating upon *E. macrurus* nests throughout the study). It may also be a conditioned strategy to improve the phenotype of the hatchlings (Janzen 1993; Janzen and Morjan 2002; Ji *et al.* 2003; Booth *et al.* 2004; Micheli-Campbell *et al.* 2011; Micheli-Campbell *et al.* 2012).

Implications for management and conservation

The results of the present study showed that female *E. macrurus* exhibit nest-site fidelity to specific areas along the Mary River to nest. These areas were of a northerly facing aspect, and although we found no significant difference in the other measurements undertaken, we accept that there may be important features that were not recorded in this study. Nevertheless, the study

demonstrates critical nesting areas for *E. macrurus* which should be considered for protection in future management strategies. Furthermore, the non-vegetated banks are a consequence of river flow patterns and deposition during natural flood events. Reduction in the intensity or frequency of these floods could result in the loss of these areas, and therefore, we recommend that flow regimes should also be considered when developing management strategies for *E .macrurus.*

CHAPTER 3

Staying cool, keeping strong: Incubation temperature affects performance in a freshwater turtle

Abstract

It is unclear how predicted rises in ambient temperature associated with climate change will impact upon the survivorship of oviparous reptiles. Given that incubation temperature can influence hatchling phenotype, understanding how elevated temperatures during development can affect the ability of hatchlings to undertake routine behaviours is important, especially for threatened species. Here we tested if raising mean incubation temperature above natural levels altered the physiology of hatchlings to an extent that behavioural function was impaired. Firstly, incubation temperatures were recorded from nests of the freshwater turtle (*Elusor macrurus*) in the wild, and the observed thermal range (26ºC to 31ºC) used to define the experimental protocol. Then, freshly laid *E. macrurus* eggs were collected and incubated at three constant temperatures (26ºC, 29ºC and 32ºC). Embryos incubated at 32ºC had the lowest hatching success. Those that did hatch were smaller than the other groups and had a reduced post-hatch growth rate. On land, the ability of hatchling turtles to right themselves is critical, and the turtles incubated at 32ºC took 30-times longer to do this than those incubated at 26ºC. Once in the water, hatchling turtles must be able to swim effectively to evade predation and obtain food items. During swimming trials the 32ºC group exhibited a lower mean stroke force (10.5 ± 0.3 mN) and spent less time swimming (133.7 ± 17.7 s) compared to hatchlings incubated at 29ºC (13.4 ± 0.4 mN, 281.3 ± 25.7 s) and 26ºC (15.7 ± 0.5 mN, 270.8 ± 28.5 s). The results of the present study illustrate that even slight rises in the mean incubation temperature, over that observed in the wild, can impact upon a hatchling's performance.

Introduction

Incubation temperature affects embryonic growth and development of oviparous animals (Deeming and Ferguson 1991). If the incubation temperature falls outside the embryo's optimum

thermal range for significant periods of time (low or high) embryonic malformation may occur, such as bone deformity, organ failure, and dysfunction of the central nervous system (see Deeming and Ferguson 1991; Burgess, Booth and Lanyon 2006). Thermal conditions during incubation are therefore an important determinant of the embryos fitness, and birds generally incubate their eggs at relative constant temperatures with little variation among species (Deeming and Ferguson 1991). Most reptiles do not provide parental care and their eggs are exposed to far more variation in incubation temperature than birds (Deeming and Ferguson 1991). Many species of turtle do however, bury their eggs in an underground chamber, which not only provides protection from predation but also buffers the eggs from diel variations in temperature and prevents desiccation (Miller and Dinkelacker 2008).

The thermal buffering effect of chamber depth on egg incubation temperature is substantial, with one study reporting diel variations of 40ºC at the surface are nullified at 50 cm depth (Booth 2006). Generally, there is a linear relationship between the body size of the nesting female and the depth of the egg chamber (Booth and Astill 2001), and because of this freshwater turtles generally lay shallower nests compared to marine turtles. Shallower nests will be more susceptible to variations in the environmental conditions, and therefore the effects of localised shifts in ambient temperature may first become apparent within freshwater turtle species.

Varying the temperature of incubation by only a few degrees centigrade has been shown to alter both the morphological and physiological traits of hatchling turtles, such as body size, amount of residual yolk upon hatching, growth rate, diving ability, and locomotor performance (Janzen 1993; Bobyn and Brooks 1994; Roosenburg 1996; Booth 2000; Steyermark and Spotila 2001; Du and Ji 2003; Booth *et al.* 2004; Burgess *et al.* 2006; Du, Zheng and Shu 2006; Delmas *et al.* 2007; Du *et al.* 2007; Mickelson and Downie 2010). However, it is difficult to draw solid conclusions from the literature due to large inter-study variation to the response of varying incubation temperature. This may be due for two main reasons: 1. the sex of many of the species examined is determined by incubation temperature with the threshold temperature varying between species; and 2. the range of experimental temperatures are disparate in their relationship to the incubation temperature experienced in the wild nests. We argue that experimental trials need to be undertaken on freshwater turtle species where the influence of temperature sex determination can be removed, and secondly, that experimental results need to be relevant to what may be expected in the natural conditions.

If environmental conditions are unfavourable for a species local extinctions may occur, and species with a limited geographic distribution will be more susceptible. Freshwater turtles are often confined by suitable habitats and many have small geographical ranges (Cann 1998). *Elusor macrurus* (Mary River turtle) is a freshwater turtle, which is only found within a single river system in Queensland, Australia. The population has dramatically decreased over the past decades and the species is currently listed as endangered (IUCN 2011). Females lay rigid-shelled eggs in shallow nests (< 20 cm depth) on open sandy banks with no vegetation cover (Cann and Legler 1994). Climate records for the local area have shown a gradual increase in ambient temperature over the past three decades, which are particularly prevalent during the spring and summer months (~ 1ºC, QLD Government, 2009). This time correlates with the egg incubation period for *E. macrurus* and we hypothesised that alterations in hatchling physiology due to changes in the incubation temperature would be deleterious to the survival of the hatchling turtles.

The aims of the present study were to assess the range of mean incubation temperatures experienced by the eggs in the wild, and then to determine how different incubation temperatures may influence hatchling phenotype. We also wanted to assess how predicted rises in ambient temperature for the local area may impact upon the physiology and performance of the hatchlings.

Materials and Methods

Study site and nest temperatures

Between October and December, the Mary River turtle *Elusor macrurus* nests on non-vegetated sandy banks along the Mary River, QLD, Australia. Nesting occurs at night after a period of heavy rain (Cann and Legler 1994), and for this study the nests were located the following morning during spring 2009. Upon locating nests, the egg chamber was exposed by hand, and a temperature data logger (2 cm diameter, Hobos® TidBit®, USA) that logged temperature every 40 min was placed adjacent to the clutch at a depth which corresponded to the middle of the nest chamber. The sand was then replaced and the clutch covered to the original depth. The nests were monitored once a week for signs of hatching, and the temperature data

logger was then removed once the hatchlings had exited the nest. Data were obtained for 16 nests that were laid on four river banks along a 30 km stretch of river.

Egg incubation and hatchling morphology

Fifty-six *Elusor macrurus* eggs (from three different clutches) were collected 48 h after being laid in nests along the Mary River, QLD, Australia. The eggs were weighed and randomly distributed into three containers (one per experimental treatment) filled with wet sandy soil collected from the nesting site. Each container was placed into a controlled temperature incubator (I-36VLC9 Intellus Ultra, Percival Scientific Inc., USA) set at 26ºC, 29ºC or 32ºC. The sand surrounding the eggs were sprinkled with water every 48 h throughout the entire incubation period, in order to maintain near constant water potential. The eggs were weighed every 14 days. Twenty-four hours after hatching, the turtles were removed from the incubators and each group placed into a separate tank containing gravel, shelters, basking platforms and water at 20 cm depth. The holding tanks were kept in an outdoor facility with a climate similar to the turtle's geographic location. Water and air temperatures varied with ambient conditions (between 20ºC and 28ºC) and were similar for all holding tanks. The hatchlings were fed three times a week on commercial turtle pellets and pre-frozen bloodworms.

Straight carapace length was measured with electronic callipers and body mass recorded with electronic scales upon hatching, 10 days after hatching (once they have completely absorbed the yolk sacs) and then every 14 d, and the growth rate (GR, g/d), which is linear and it was calculated using the following equation:

$$GR = \frac{\left[mass_t\right] - \left[mass_{t_0}\right]}{t - t_0}$$

where t is time difference between measurements (t = 10 days old; t_0 = 105 days old).

Righting Response

The "time to right" of the hatchlings is defined as the time from when the turtle started to move, after being placed upside down, to the moment it righted itself (Delmas *et al.* 2007). A paper lined 5 l plastic container was partitioned into quarters and a 10 d old hatchling was placed

into each quarter (4 hatchlings per trial). Hatchlings (26ºC and 29ºC groups, n = 17; 32ºC group, n = 10) were placed inside the cells 45 min prior to the trial to allow them to acclimate to the experimental environment. The ambient temperature was maintained at 26 ± 0.5°C for the duration of the experimental period. Hatchlings were fasted for 24 h prior to experimentation. Each hatchling performed three righting events per replicate. The trials were recorded by a digital video camera (Sony DCR-HC52) and the time to right data were collected and analysed from the images. In some instances the turtle did not right itself after 30 min and due to ethical concerns the trial was aborted and the data were not used in the study. The study was repeated two weeks later, resulting in each turtle being tested six times.

Swimming performance

Swimming performance was assessed by examination of stroke frequency and force, and the proportion of time spent swimming. At four weeks of age ten hatchlings were randomly selected from each treatment group and swum individually in a glass aquarium (41 x 26 x 35 cm) filled with 30 cm of freshwater at constant temperature (26 ± 0.5ºC). A Velcro patch (1 cm^2) was glued to the carapace and a monofilament nylon line attached to the Velcro (Burgess *et al.* 2006). The nylon tether was attached to a force transducer (MLT010, ADInstruments) in the perpendicular plane, resulting in the measured force on the vertical plane regardless of the direction the turtle swam (for details see Burgess *et al.* 2006). The force transducer was calibrated before each trial by suspending a known mass in the vertical plane. The force transducer was connected to a data acquisition system (Power Lab 2/20 connected to a ML110 Bridge amplifier) and the force sampled at a frequency of 100 Hz. Each hatchling swimming trial was replicated 14 days apart, and hatchlings were not fed for 18 h prior to swimming. Force recordings (Figure 3.1) were analysed for the following variables: (i) mean stroke force: mean force recorded for each consecutive 30 s period over an 8 min recording period; (ii) total time spent swimming during the 8 min recording period; (iii) stroke frequency: determined by averaging the number of force peaks per second within each swimming event.

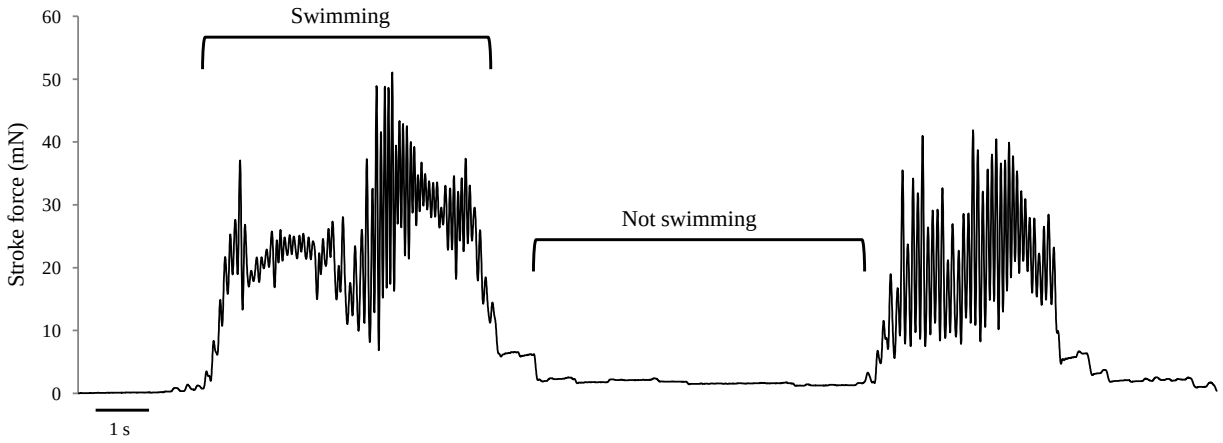


Figure 3.1: Force trace generated by a hatchling *E. macrurus* during a trial of the swimming performance experiment.

Statistical analysis

Straight carapace length, body mass and growth rate data from the three experimental groups were tested using multiple sample analyses of variance (MANOVA). The F-test was used to denote a significant difference between the means. If significant differences existed, Multiple Range Tests were used to show which means were significantly different from each other. Kruskal-Wallis tests were used if the presence of outliers was detected. All data are presented as mean ± S.E, and a difference between groups was deemed significant if $P < 0.05$ (StatGraphics Plus 5.1).

The data for the righting response and mean stroke force were investigated using General Linear Mixed Models (GLMMs; Zuur *et al.* 2008). These data were log-transformed to standardise and homogenise residuals. Body mass was incorporated as a covariate and because measurements were taken repeatedly from the same individuals, turtle ID was included as a random effect. For the swimming performance data the interaction between incubation temperature and time was included in the model. All mixed models were performed in the R programming language (R Development Core Team 2010) using the nlme library of functions (Pinheiro *et al*. 2010).

The effects of incubation temperature upon time swimming and mean stroke frequency were tested using a Multifactor ANOVA, accounting for body mass as a covariate (StatGraphics Plus 5.1).

Results

Thermal profiles of nests

The average incubation temperature of *Elusor macrurus* eggs in the wild ranged from 26ºC to 31ºC (Figure 3.2). Out of the 16 nests measured, 25% had a mean temperature of 29ºC, with one nest having a mean temperature of 26ºC and two nests a mean of 31ºC. Collectively the mean temperature for all nests measured over the entire incubation period was 28.5 ± 3.2ºC (mean ± S.D.).

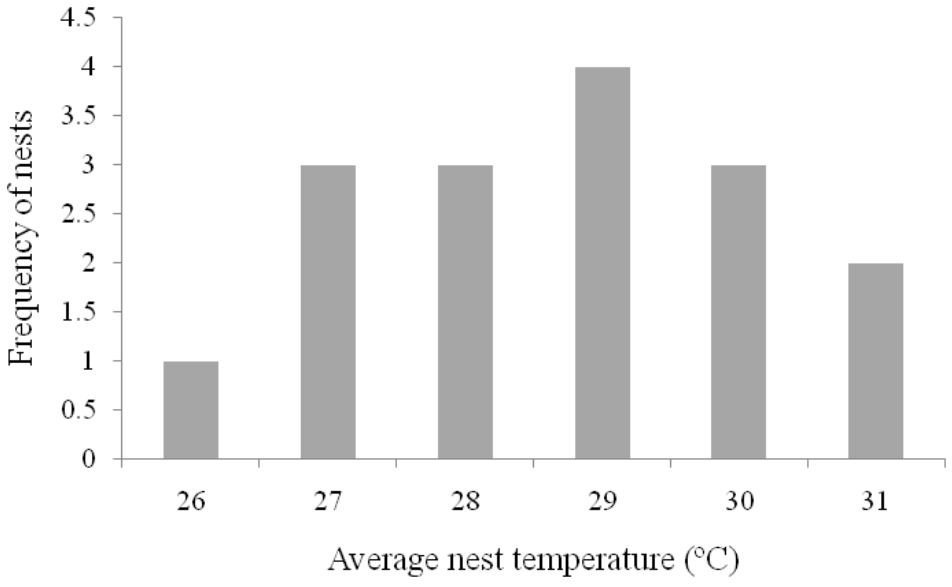


Figure 3.2: The distribution of mean nest temperature for 16 *E. macrurus* nests during the 2009 nesting season. Data were collected from four nesting banks along a 30 Km stretch of the river.

Egg incubation and hatchling morphology

There was no difference in the initial egg mass between the temperature treatments prior to incubation ($F_{(2,53)}$ = 0.29, P = 0.75). The incubation period was significantly different for each treatment group with eggs incubated at higher temperatures hatching earlier (Table 3.1, $F_{(2,41)}$ = 73.68, P < 0.001). Eggs incubated at 32ºC had a lower hatching success compared to 26ºC and 29ºC (Table 3.1). Furthermore, 10 d after hatching, hatchlings incubated at 32ºC were smaller than those from 26ºC and 29ºC ($F_{(2,41)}$ = 7.24 and 6.81, respectively, P < 0.05; Table 3.1). Hatchlings from the 32ºC group remained smaller than their siblings incubated at lower temperatures for the remaining experimental period (~ 100 days), because of a slower post-hatch growth rate ($F_{(2,41)}$ = 6.54, P = 0.003; Table 3.1).

Table 3.1: Summary data from eggs and hatchling *E. macrurus* incubated in captivity at three constant temperatures: 26ºC, 29 ºC and 32ºC. Symbols (*) indicate statistical differences (P < 0.05) and numbers indicate the mean ± S.E.

Incubation temperature	**26ºC (n=17)**	**29ºC (n=17)**	**32ºC (n=10)**
Incubation length (days)	70 ± 0.7 (68 – 72)*	52 ± 0.7 (50 – 54)*	42 ± 0.5 (41 – 43)*
Hatching success	89%	89%	56%
SCL (mm) upon hatching	34.9 ± 0.5	34.9 ± 0.3	32.7 ± 0.4*
Body mass (g) – upon hatching	7.38 ± 0.2	7.31 ± 0.1	6.91 ± 0.1*
Growth rate (g)/day - 105 days old	0.047 ± 0.003	0.041 ± 0.003	0.029 ± 0.004*

Righting Response

Righting time for 10 d old hatchlings was influenced by incubation temperature (Figure 3.3). Hatchlings incubated at 26ºC group righted themselves 10-times faster than hatchlings

incubated at 29ºC, and 34-times faster than the 32ºC group (L = 30.57, df = 2, P < 0.01; Figure 3.3). A repeat of the same experiment with the same individuals 14 d later showed the same response between groups (P < 0.05). Body mass (g) was not significantly correlated with the time to right (L = 1.24, df = 1, P = 0.26).

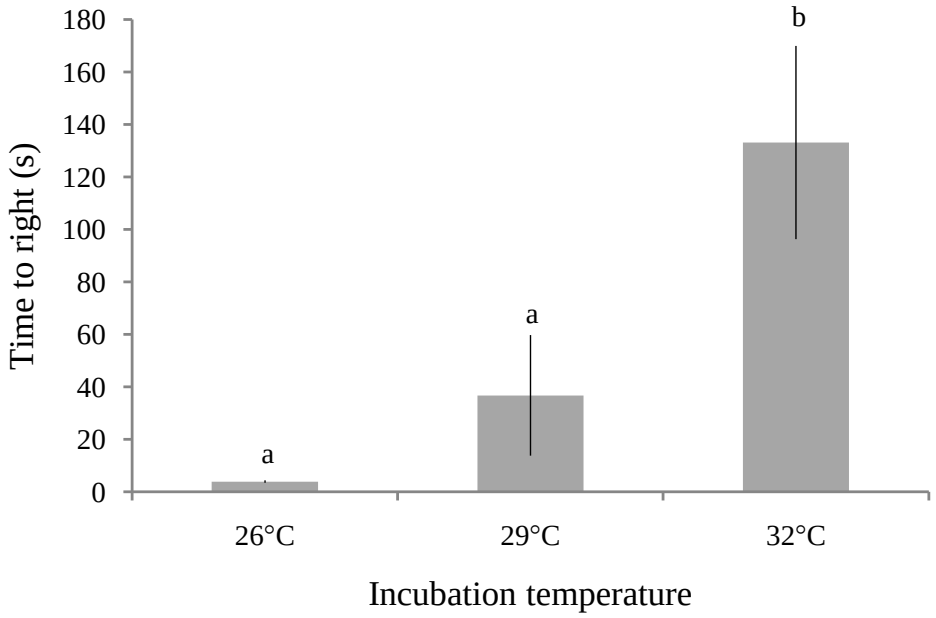


Figure 3.3: Time for *E. macrurus* hatchlings (10 d old) to right themselves since they started to move after being place upside down (time to right). Hatchlings were incubated at three constant temperatures (26ºC and 29ºC groups: n = 17; 32ºC group: n = 10). Bars height and error bars indicate the mean ± S.E. Different letters indicate significant differences (P < 0.001).

Swimming performance

In general, the behaviour of the hatchlings during the swimming trials was similar with the highest stroke force exerted during the first few seconds after entrance into the water after which the mean stroke force declined over approximately 2 min, and then remained relatively constant throughout the remainder of the trial (Figure 3.4). The interaction between time and

incubation temperature was significant for the model (L = 10.17, df = 2, P < 0.01). The mean stroke force during the first 30 s of the trial was significantly different between all three treatments (L = 6.43, df = 2, P < 0.05). Hatchlings incubated at 32ºC had a significantly lower mean stroke force than those incubated at 26ºC and 29ºC (ANCOVA LS adjusted means: 26ºC group = 29.0 ± 1.2 mN, 29ºC group = 29.7 ± 1.0 mN, 32ºC group = 25.4 ± 1.3 mN; $F_{(2,27)}$ = 71.43, P < 0.01; Figure 3.4). This pattern was continued with the 32ºC group maintaining a significantly lower mean stroke force for the 8 min duration of the trial (L = 7.37, df = 1, P < 0.01; Figure 3.4). Hatchlings incubated at 26ºC maintained a greater mean stroke force for the remaining trial period when compared to the other two groups (L = 4.27, df = 1, P < 0.05; Figure 3.4). Body mass (g) was significantly correlated with mean stroke force during swimming performance trials (L = 14.41, df = 1, P < 0.001), but even when adjusted for differences in body mass, the hatchlings incubated at 32ºC still had a significantly reduced mean stroke force during the first 30 s of the swimming trial (Figure 3.4) relative to the other two groups.

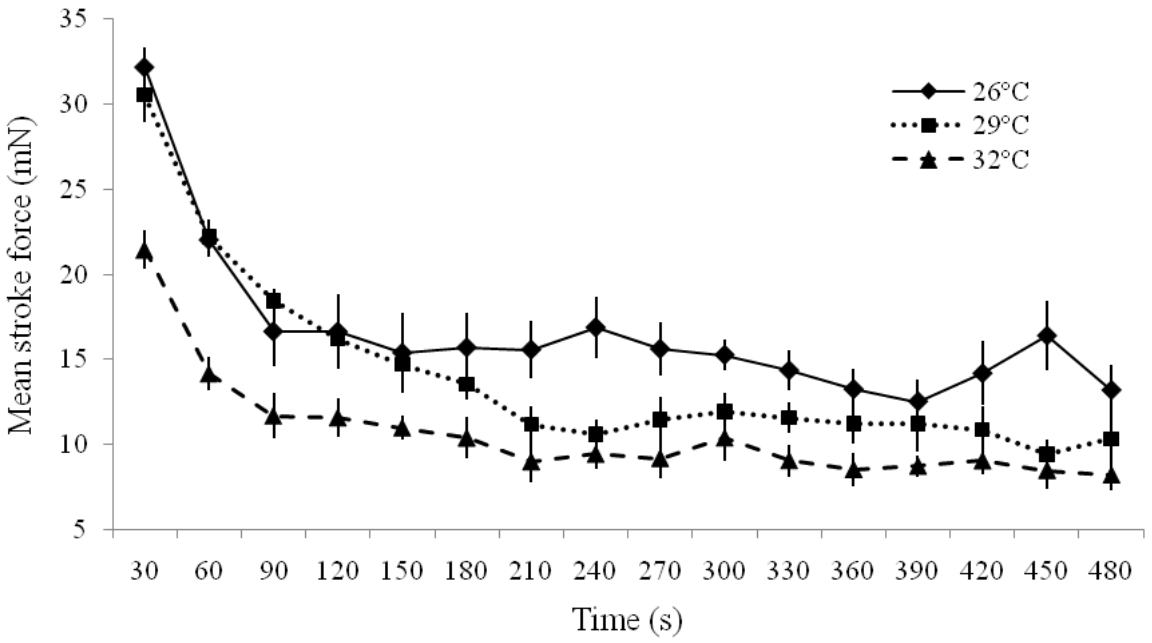


Figure 3.4: Mean stroke force (mN) generated every 30 s over an 8 min trial period by *E. macrurus* hatchlings (n = 10) incubated at 26ºC, 29ºC and 32ºC. Symbols indicate the mean ± S.E.

Hatchlings incubated at 32ºC spent significantly less time swimming during the 8 min trial (26ºC group = 270.8 ± 28.5 s, 29ºC group = 281.3 ± 25.7 s, 32ºC group = 133.7 ± 17.7 s; $F_{(2,27)}$ = 11.38, P < 0.01; Figure 3.5a), but had a significantly greater stroke frequency per swimming event when compared to the other two groups (26ºC group = 4.7 ± 0.1 Hz, 29ºC group = 4.4 ± 0.1 Hz, 32ºC group = 6.1 ± 0.2 Hz; $F_{(2,27)}$ = 43.78, P < 0.01; Figure 3.5b).

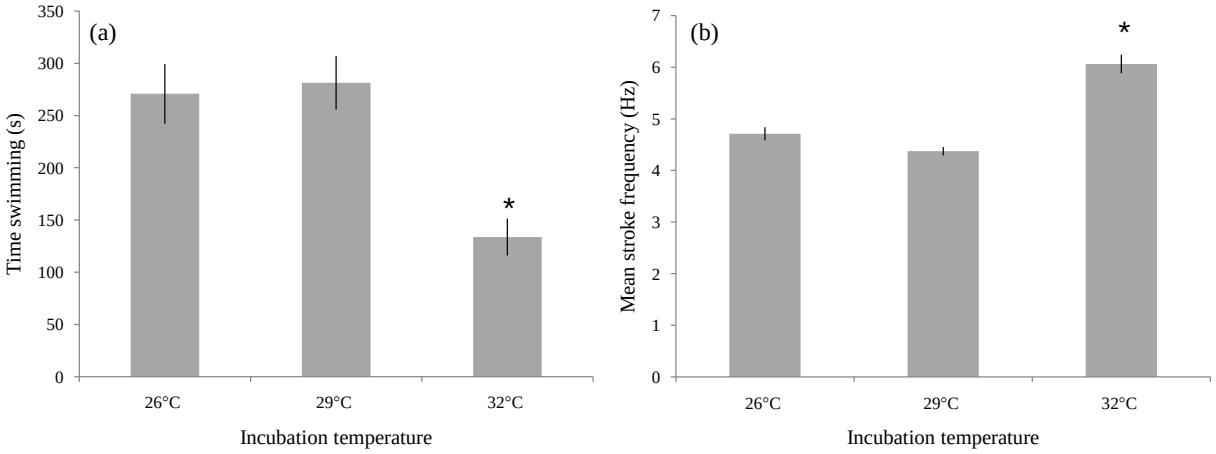


Figure 3.5: Total time spent swimming (a) and stroke frequency per power stroking output (b), of *E. macrurus* hatchlings (n = 10) incubated at 26ºC, 29ºC and 32ºC. Bars height and error bars indicate the mean ± S.E. Symbol (*) indicates statistical difference (P < 0.01).

Discussion

The present study showed conclusively that differences in incubation temperature of only a few degrees altered the phenotype and performance of hatchling Mary River turtles (*Elusor macrurus*). The eggs were incubated at three experimental temperatures, two were within the range of natural nest temperatures, and one treatment was 1ºC warmer than the highest mean nest temperature recorded in the wild. Those hatchlings incubated at this higher than average nest temperature had a significantly poorer performance throughout experimental trails, and this physiological limitation upon behavioural function may have detrimental ecological implications for this species.

The *E. macrurus* embryos hatched earlier when incubated at warmer temperatures. This is a typical response in oviparous reptiles, resulting from an increased embryonic rate of development (Deeming and Ferguson 1991). A consequence of a shorter period for incubation can be less yolk material being converted into tissue (Booth 2000), and accordingly, *E. macrurus* hatchlings incubated at 32ºC had a significantly smaller body size upon hatching than those from the 29ºC group. Body size was similar between the 26ºC and 29ºC groups, suggesting that the thermal effects upon yolk conversion were only significant at the higher thermal range of the study. This result is opposite to that observed for the smooth softshell turtle *Apalone mutica* which hatched with a larger body size when incubated at 30ºC, compared to 28ºC and 26ºC (Janzen 1993). Similar discrepancies can be observed throughout the freshwater turtle literature (Brooks *et al.* 1991; Rhen and Lang 1995; Roosenburg and Kelley 1996; Janzen and Morjan 2002; Ji *et al.* 2003; Booth *et al.* 2004) and may have arisen due to disparity in the relationship between the experimental thermal range and the optimum ambient temperature for the embryos. For example, the average thermal range of *Apalone mutica* nests in the wild is between 28ºC and 36ºC (Ewert 1979), and therefore the experimental study undertaken by Janzen (1993) was within the lower portion of the nest thermal range.

After hatching, the growth rate of *E. macrurus* incubated at 32ºC remained significantly lower than those incubated at 26ºC and 29ºC. Turtles with high post-hatch growth rates would attain a larger size quicker, which may decrease predation risk, increase digestive breadth (i.e. handle and consume a greater range of prey items) and reach sexual maturity earlier (Perez, Collado and Ramo 1979). Post-hatch growth rates have been used as an indicator of post-hatch fitness in Chelonians, but again the influence of incubation temperature shows contrasting inter-study results; with increased post-hatch growth occurring at the highest (Bobyn and Brooks 1994; Booth *et al.* 2004), the intermediate (Spotila *et al.* 1994), and the lowest experimental incubation temperatures (Roosenburg and Kelley 1996), and some studies finding that incubation temperature had no effect on post-hatch growth rate (Ji *et al.* 2003).

As in most turtles, female *E. macrurus* lay their eggs in a nest buried on land, and the hatchlings must make their own way to water. On land, hatchling turtles are destabilized easily and frequently find themselves tipped upside down (Burger 1976). This may increase mortality through predation or dehydration (Finkler 1999; Steyermark and Spotila 2001; Kolbe and Janzen 2002). Experimental manipulation of *E. macrurus* hatchlings in the laboratory showed that the ability of a hatchling to right itself was substantially reduced when incubated at 32ºC. This effect

was maintained over three weeks post-hatching, thus covering the period when they would be leaving the nest and moving by their own accord to the river. Despite the importance of righting behaviour to the survival of hatchling turtles, only a few studies have examined how it is influenced by incubation temperature. Hatchlings of the freshwater turtle *Graptemys ouachitensis* and *Trachemys scripta elegans* took a longer time to right themselves when incubated at 25ºC compared to 30ºC (Freedberg *et al.* 2004). The sex of these species of freshwater turtle is however, determined by incubation temperature with incubation at 25ºC producing all males and at 30ºC producing all females. Therefore, the extent by which temperature influences the hatchlings ability to right itself was potentially confounded by differences in sex. *E. macrurus* do not show temperature-dependent sex determination (Georges and McInnes 1998), and therefore the effects of incubation temperature could be examined without the complicating influence of sex.

Once they have entered water, hatchling turtles need to move through the aquatic environment, acquire food and evade predators. These behaviours are likely to be influenced by the strength and stamina of swimming, and performance may influence survival. Hatchlings incubated at 26ºC maintained mean stroke force at a significantly higher level than hatchlings from 29ºC, and 29ºC were significantly higher than 32ºC throughout the 8 minute swimming trial. The lower absolute force of the 32ºC group was in part caused by their smaller body size, but the reduction in mean stroke force remained significant even when differences in body mass were accounted for by using body mass as a covariate. Although the 32ºC group had a significantly higher stroke frequency per swimming bout than the other groups, this did not compensate for their lower mean stroke force and shorter length of the swimming bout. Comparing all previous studies that have examined the effects of incubation temperature upon swimming performance in turtles, there is general disagreement about its effect, with warmer incubation temperature increasing performance in some species but decreasing in others (Janzen, 1993; Du and Ji, 2003; Booth *et al.*, 2004; Burgess *et al.*, 2006).

The underlying physiological processes responsible for the significantly reduced performance in hatchling *E. macrurus* incubated at 32ºC are presently unclear. It has been proposed in alligators that one of the organs largely affected by incubation temperature is the hypothalamus, directly affecting the levels of different hormones released by this organ during embryogenesis (Deeming and Ferguson 1988; Deeming and Ferguson 1989). This may lead to a number of physiological impairments to muscle physiology, tissue aerobic capacity, bone

morphology, mitochondrial oxidation, metabolic efficiency or cardio-respiratory regulation (Kinney, Matsuura and White 1977; Burggren and Shelton 1979; White, Hicks and Ishimatsu 1989; Wang and Hicks 1996; Hicks and Wang 2004; Guderley and Seebacher 2011; Reed *et al.* 2011). Further studies are required to identify which of these are the most significant in disrupting hatchling performance.

Experimental studies can never exactly replicate the wild situation and it should be noted that the thermal regimes of the eggs in the laboratory were kept constant whereas the temperature of nests in the wild fluctuated both daily and over longer time periods. It was not possible however, to standardize the level of variability that the wild nests were exposed to because they varied widely between nests. This occurred due to nest depth, the soil composition, slope angle and nesting bank slope bearing and orientation. Here we simplified the protocol by incubating the eggs at constant thermal regimes and further comparative studies are required to explore the significance of fluctuating and constant incubation temperatures upon hatchling performance.

In the case of *E. macrurus*, the outlook does not appear good. Our study findings show that a mean incubation temperature of 1ºC above the warmest nest recorded during the 2009 nesting season reduced hatching success, hatchling growth rate, and compromised righting and swimming abilities. The mean air temperature throughout the 2009 nesting season was not significantly different from that recorded over the past decade for the area, but these years had a mean ambient temperature between 0.5 and 1.2ºC warmer when averaged over previous decades (CSIRO and BoM 2007). Although we do not illustrate a direct connection between warming of the environment and the decline in the *E. macrurus* population, our findings suggest that predicted increases in ambient temperate for the Mary River catchment (1.6ºC to 3.0ºC by 2070, QLD Government, 2009) may be detrimental to this species.

CHAPTER 4

The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle.

Abstract

Incubation temperature influences the phenotype of the hatchling turtles. The aim of the present study was to investigate the daily fluctuations in temperature to which eggs of the freshwater turtle *Elusor macrurus* are exposed to in the wild, and examine how these fluctuations may affect the phenotype and performance of the hatchlings. Eggs in the wild experienced an overall mean daily fluctuation of 5.7ºC throughout incubation period, but on particular days the variation was as low as 2ºC and as high as 22ºC. Fifty-four eggs were collected from the wild and incubated in the laboratory at one constant (28ºC) and two fluctuating (28 ± 3ºC and 28 ± 6ºC) thermal regimes. Egg mass, incubation length and hatching success (89%) were similar for the 28ºC and 28 ± 3ºC groups, whereas the 28 ± 6ºC group only had a 5% hatching success, and the incubation length was 10 d longer. Upon hatching, there was no significant difference in body mass or straight carapace length between the 28ºC and 28 ± 3ºC groups, and within the first eight weeks of hatching there was no significant difference in growth rate, self-righting time, crawling speed and swimming performance. A single survivor from the 28 ± 6ºC had a body mass that was 27% less compared to the other two groups and it did considerably poorer in all the performance tests. The study findings illustrated that daily fluctuations in incubation temperature up to 6ºC had no effect upon hatchling *E. macrurus* phenotype, but there was a limit (12ºC) by which the extent and recurrence of these fluctuations became detrimental. These thermal regimes are not yet apparent in the wild, but will occur within the geographical range of this species according to climate change predictions.

Introduction

Most oviparous reptiles bury their eggs in underground chambers for incubation (Booth 2006; Deeming 2004; Deeming and Ferguson 1991). This is an evolved behavioural strategy,

which not only protects the eggs from predation but also buffers the temperatures and hydric regime that the clutch experiences during incubation (Miller and Dinkelacker 2008). In freshwater turtles, research has demonstrated that shifts in mean constant incubation temperature of only a few degrees can significantly affect the phenotype of the hatchlings, by altering morphology, physiology and locomotor performance (Bobyn and Brooks 1994a; Bobyn and Brooks 1994b; Booth 2000; Booth *et al.* 2004; Delmas *et al.* 2007; Du and Ji 2003; Janzen 1993; Micheli-Campbell *et al.* 2011; Roosenburg and Kelley 1996; Steyermark and Spotila 2001). In the wild however, the eggs are rarely exposed to constant temperatures, as the females of most freshwater species lay shallow nests where the variation in daily temperature is increased by the proximity of the clutch to the substrate surface (Booth 2006). To date, little is known about the effects of such thermal regimes upon the phenotype of hatchling turtles.

The majority of empirical thermal incubation studies in freshwater turtles have focused upon species with temperature dependent sex determination (Demuth 2001; Du, Shen and Wang 2009; Les, Paitz and Bowden 2007; Schwarzkopf and Brooks 1985). These studies have shown that daily fluctuations in incubation temperature produced a larger proportion of females when compared to the constant treatments. Alterations in other morphological and physiological traits were also observed and the extent of these responses varied widely between species. For example, fluctuating incubation temperatures resulted in a smaller body mass of hatchling *Chinemys reevesii* compared to those incubated at a constant temperature (Du *et al.* 2009). Swimming ability and immune response were improved in *Chrysemys picta* and *Trachemys scripta* when eggs were incubated under the fluctuating thermal regimes (Ashmore and Janzen 2003; Les *et al.* 2007; Les, Paitz and Bowden 2009), whereas other species showed no significant alterations in hatchling performance between constant and fluctuating incubation temperatures (Du *et al.* 2009). Consequently, in species with temperature dependent sex determination it is inconclusive to determine whether the differences in hatching phenotype occur as a direct consequence of the thermal fluctuation, or whether these differences result from inherent variation between the sexes.

The aim of the present study was to compare the effects of constant and fluctuating incubation temperatures upon the morphology, locomotor performance and growth rate of a species of freshwater turtle (*Elusor macrurus*) whose sex is not determined by temperature (Georges and McInnes 1998). *Elusor macrurus* (Mary River turtle) lays rigid-shelled eggs in shallow nests (~ 20 cm depth) on steep sandy banks with no vegetation cover, and nesting events

typically occur between late-spring (October) and early-summer (December; Cann and Legler 1994). Because of these characteristics we predicted that the *E. macrurus* eggs would experience large daily temperature fluctuations in the wild. We assessed the extent of these daily temperature fluctuations, and then through controlled laboratory manipulations, determined the influence of fluctuating incubation temperatures upon the hatchling phenotype. We hypothesised that hatchlings incubated under thermal regimes most similar to conditions experienced by the eggs in the wild would show improved performance over hatchlings that were not.

Material and Methods

Nest temperatures in the wild

Elusor macrurus nests were located the morning following a night of heavy rainfall. The newly formed eggs were exposed by hand and a temperature data-logger (2 cm diameter, Hobos® TidBit®, USA) was placed adjacent to the clutch at a depth corresponding to the middle of the nest chamber. Each data-logger was programmed to record the temperature every 40 min. The sand was then replaced and the clutch covered to the original depth. The temperature logger was recovered once the hatchlings had exited the nest. Data were obtained for 16 nests laid in four separate river banks within an approximately 30 km stretch of river during the 2009/2010 nesting season.

Egg incubation and hatchling morphology

Fifty-four *Elusor macrurus* eggs, collected from four different clutches, were transported to The University of Queensland. In the laboratory they were randomly distributed into three containers (one per experimental treatment, each containing 4-5 eggs from each clutch), which were filled with wet sandy soil collected from the nesting site. Each container was placed into a controlled temperature incubator (I-36VLC9 Intellus Ultra, Percival Scientific Inc., USA), these were programmed to a constant (28ºC) and two fluctuating temperature treatments (28 ± 3ºC and 28 ± 6ºC; 24 h cycles). A temperature logger (Hobos® TidBit®, USA) was placed inside each incubator (beside the eggs) to record and monitor incubation temperature regime (Figure 4.1). In order to maintain near constant water potential, the sand surrounding the eggs were sprinkled

with 3 ml of water every 48 h throughout the entire incubation period. The eggs were checked in a weekly basis (for the presence of blood vessels) and weighed every 14 days.

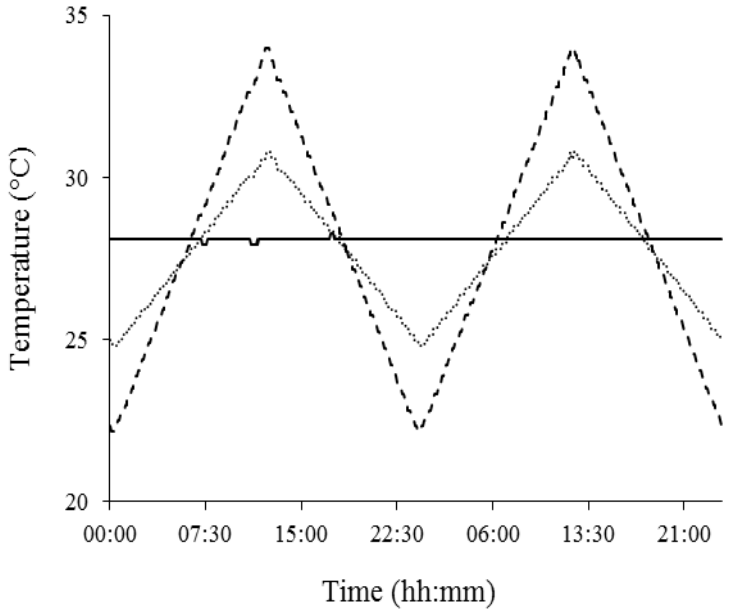


Figure 4.1: Programmed temperature profile (recorded by data loggers) experienced by *E. macrurus* eggs (n = 18) in the laboratory after being collected from the wild (solid lines = 28ºC; dotted lines = 28 ± 3ºC; dashed lines = 28 ± 6ºC).

Twenty-four hours after hatching the turtles were removed from the incubators and each group placed into a separate tank containing gravel, shelters, basking platforms and water at 20 cm depth. The holding tanks were kept in an outdoor facility under ambient conditions similar to that experienced in the wild. Temperature loggers placed inside each holding tank (Hobos® TidBit®, USA) showed that water and air temperatures varied between 20ºC and 28ºC but were similar across all holding tanks. The hatchlings were fed three times a week a commercial turtle pellet diet and pre-frozen bloodworms. The same amount of food was delivered to each tank and it was ensured that all the food was consumed.

Straight carapace length (SCL) was measured with electronic callipers and body mass recorded with electronic scales upon hatching (t_0) and every 14 d thereafter until day 56 (t). Growth rate (GR, g/d) was the same between measurement periods, so a single growth rate was calculated for the 56 d growth period using the equation:

$$GR = \frac{\lceil mass_t \rceil - \lfloor mass_{t_0} \rfloor}{t - t_0}$$

Crawling speed

After hatching, turtles were kept in the incubators for 24 h and after this period they had small spots painted on different areas of their carapaces with non-toxic whiteout for identification. Prior to being placed into their respective holding tanks, hatchlings were placed onto the centre of a hard surface, 50 cm diameter arena covered with sand. Hatchlings (28ºC; 28 ± 3ºC; 28 ± 6ºC) were placed in the arena in groups of two and covered with a dark box for 5 min. The box was then removed, and locomotion time was assessed as the time that the hatchlings started to move to the moment that they reached the edge of the arena. Each hatchling was tested twice, with an hour interval between trials, ambient temperature was constant (26 ± 0.5ºC). Crawling speed was determined by the distance covered by the hatchling divided by time.

Righting Response

The "self-righting time" is defined as the time from when the hatchling first moved until the moment it righted itself after being placed upside down on its carapace (Delmas *et al.* 2007). A 10 l plastic container was fabric lined (covering asymmetries on the surface), partitioned into eight cells of equal size (L 11 x W 11 x H 10.3 cm) and at seven days of age a single hatchling was placed into each cell (eight hatchlings per trial). Hatchlings were positioned in the centre of the cells, which were ~ 13-times larger than the hatchlings in order to avoid their contact with the walls. The ambient temperature was maintained constant (26 ± 0.5ºC) for the entire duration of the experiment. Hatchlings (28ºC; 28 ± 3ºC; 28 ± 6ºC) were fasted for 24 h prior to experimentation and placed inside the cells 45 min prior to each trial. Each hatchling performed three self-righting events per trial. The righting events were recorded by a digital video camera (Sony DCR-HC52, North Ryde, NSW, Australia) and the self-righting time determined by

analysis of recorded images. When turtles did not attempt to self-right within 30 min of being placed on their carapaces, the trail was abandoned and accounted as not-attempted.

Swimming performance

Swimming performance was assessed when the hatchlings were four weeks old (28ºC; 28 ± 3ºC; 28 ± 6ºC). Hatchlings were swum individually in a glass aquarium (41 x 26 x 35 cm) at constant temperature (26 ± 0.5ºC). Swimming force was measured using a force transducer (MLT010, ADInstruments) attached to the turtle with a monofilament nylon line and Velcro patch (see details Burgess, Booth and Lanyon 2006). Before each trial the force transducer was calibrated by suspending a known mass in the vertical plane and force was sampled at a frequency of 100 Hz (Power Lab 2/20 connected to a ML110 Bridge amplifier; see Micheli-Campbell *et al.* 2011). To ensure that the post-feeding duration was equal for all turtles, swimming trials were always undertaken 18 - 21 h after a feeding event. Swimming trials were performed between 9:00 am and 12:00 pm. The following variables were measured: (i) mean stroke force: mean force recorded for each consecutive 30 s period over a 10 min recording period; (ii) total time spent swimming during the 10 min recording period; (iii) stroke frequency: determined by averaging the number of force peaks per second within each swimming event.

Statistical analysis

Multiple analyses of variance (MANOVA) were used to analyse egg mass, while a t-test was performed to analyse straight carapace length, body mass and growth rate data. The *F*-test was used to denote a significant difference between the means. Data obtained from the one individual incubated at 28 ± 6ºC were not included into the analysis. All data are presented as mean ± SE, and a difference between groups was deemed significant if $P < 0.05$ (Statistica10).

Mixed model ANOVA was used for the analyses of the self-righting time, crawling speed, and swimming stroke frequency and force. Body mass was accounted as covariate for the tests, and time was incorporated as covariate for the stroke force data. Turtle ID was included as a random factor because the measurements were taken repeatedly from the same individuals. The effects of incubation temperature upon time swimming were tested using general linear model (ANCOVA) incorporating body mass as covariate.

Results

Thermal profiles of **Elusor macrurus** *nests in the wild*

The temperature data collected from sixteen *Elusor macrurus* nests showed considerable temperature variation within nests throughout the three month nesting season (October to December, Figure 4.2a). Throughout the incubation period the daily nest temperature showed a gradual increase from ~ 26ºC to ~ 30ºC (Figure 4.2a), with an overall average temperature for all the nests during this period of 28.5ºC. The fluctuation in daily temperature experienced by the eggs in the wild ranged from 2ºC to 22ºC (Figure 4.2b); 40% of the measurement days exhibited a daily fluctuation between 4ºC and 6ºC, 30% between 7ºC and 11ºC, and 4% between 12ºC and 22ºC. Over a 24 h daily cycle the temperature profile was similar for all nests — with the coolest time of the day occurring around 08:00 and the warmest around 16:00. The 90th percentile showed that for each hour over the 24 h period temperature varied by ~ 6ºC throughout incubation (Figure 4.2c). The inter-nest median temperature throughout the incubation period ranged between 26ºC and 30ºC, and the overall incubation temperature range also varied between nests (Figure 4.2d).

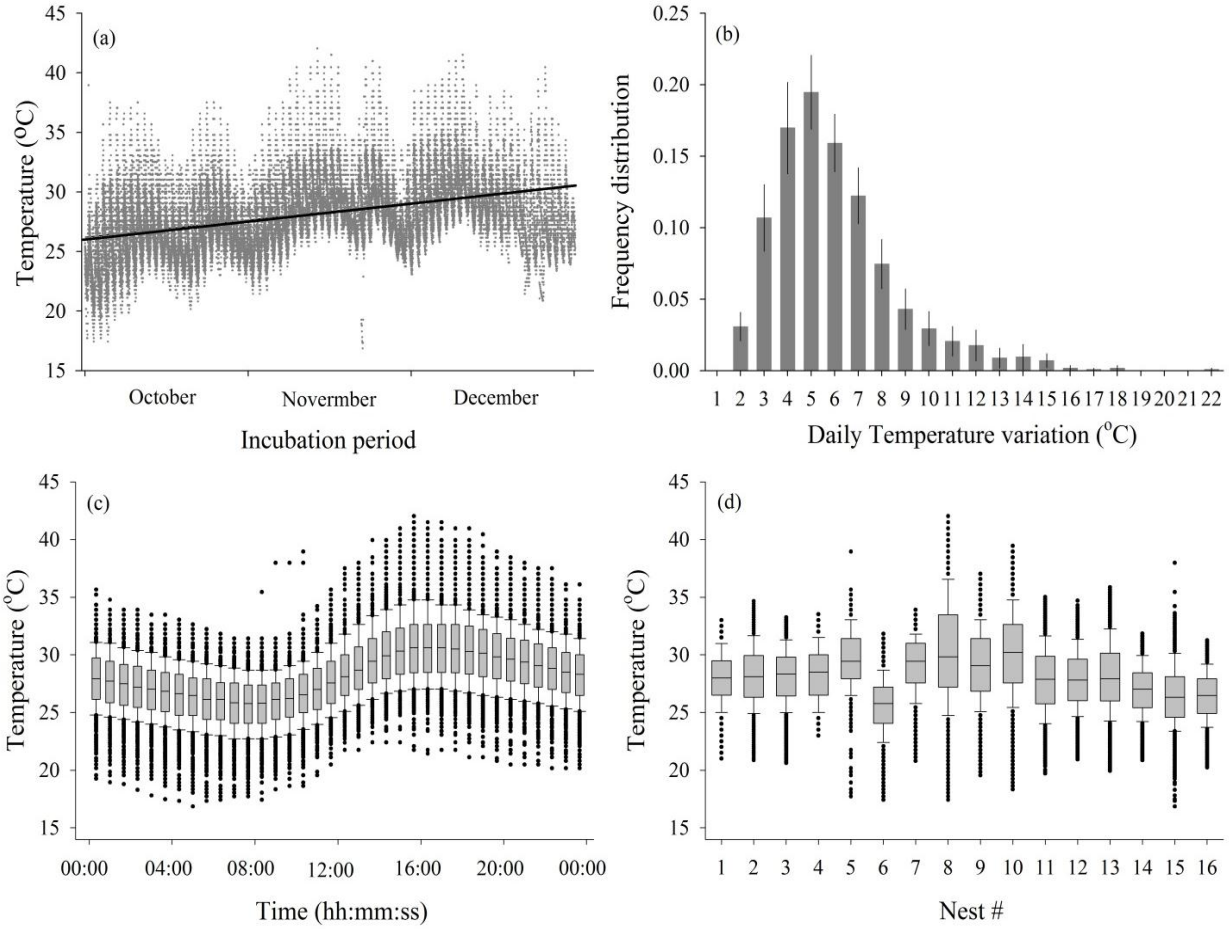


Figure 4.2: Thermal profile of *E. macrurus* nests in the wild (n = 16). (a) Overall data collected from October to December during nesting season showing the temperatures experienced by 16 *E. macrurus* nests in the wild (16 data points for each 40 minute interval throughout the incubation period; solid line represents the trend line); (b) Frequency distribution of the daily variation experienced by the nests (bars height and error bars indicate the mean ± SE); (c) Box-and-whisker plot of the daily temperature profile data experienced by the nests; (d) Box-and-whisker plot of temperature data recorded from each nest during the incubation period.

Egg incubation and hatchling morphology

Elusor macrurus eggs initially placed into the different incubation regimes were of similar mass (n = 54; 8.82 ± 0.09 g, $F_{(2,51)} = 1.05$, $P = 0.36$), with egg mass remaining similar throughout the entire incubation period for eggs incubated at 28ºC and 28 ± 3ºC. The eggs incubated at 28 ± 6ºC however, had a significantly reduced mass from the second week of development (Figure 4.3, $F_{(2,\ 51)} > 6.24$, $P < 0.01$). In this group, the embryos died at different developmental stages throughout the incubation period, with only 4 hatchlings breaking the egg shell and only one hatchling (5%) survived beyond 24 h (Table 4.1). Both constant (28ºC) and lower fluctuating (28 ± 3ºC) thermal regimes had the same hatching success (89 %), and the hatchlings had a similar mean body mass ($F_{(1,\ 30)} = 2.25$, $P = 0.12$), straight carapace length ($F_{(1,\ 30)} = 2.60$, $P = 0.07$) and post-hatch growth rate ($F_{(1,\ 30)} = 2.24$, $P = 0.12$, Table 4.1). Because only one individual hatched from the 28 ± 6ºC treatment group, it was not possible to perform statistical analyses with these data, however this hatchling had a smaller body mass, straight carapace length and post-hatch growth rate in relation to the other hatchlings incubated at the other two thermal regimes (Table 4.1).

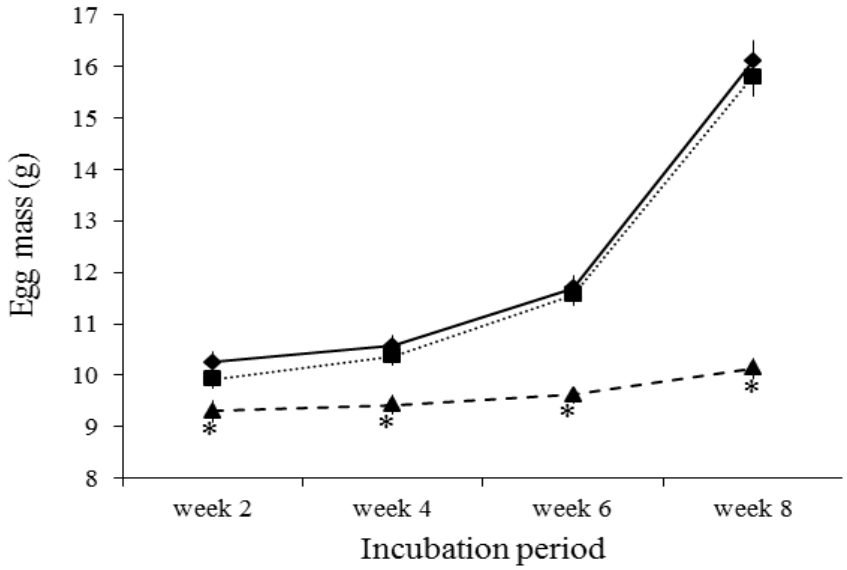


Figure 4.3: Mass during development of *E. macrurus* eggs (n = 18) during incubation in the laboratory. Symbols and error bars indicate the mean ± SE (solid lines = 28ºC; dotted lines = 28 ± 3ºC; dashed lines = 28 ± 6ºC), and (*) indicates statistical differences ($P < 0.05$).

Table 4.1: Summary data from eggs (n = 18) and hatchling *Elusor macrurus* incubated in captivity at three thermal regimes: 28ºC constant, 28 ± 3ºC and 28 ± 6ºC. The straight carapace length (SCL) and body mass data are upon hatching.

Incubation temperature	**28ºC (n = 16)**	**28 ± 3ºC (n = 16)**	**28 ± 6ºC (= 1)**
Incubation length (days)	56 ± 0.2 (55 – 58)	57 ± 0.2 (55 – 59)	66
Hatching success	89%	89%	5%
SCL (mm)	32.4 ± 0.2 (31.1 – 33.3)	32.1 ± 0.3 (30.1 – 33.8)	27.6
Body mass (g)	7.2 ± 0.1 (6.7 – 8.1)	7.3 ± 0.1 (6.4 – 8.1)	5.3
Growth rate (g/day; over 56 days)	0.15 ± 0.01	0.15 ± 0.1	0.19

Crawling speed

After removal of the darkened box the turtles typically stayed stationary for ~ 3-4 s and then started to crawl. Fortunately for measurement, all turtle movements occurred in a straight line towards the edges of the experimental arena. There was no difference ($F_{(1,30)}$ = 1.18, P = 0.84) in the crawling speed exhibited by 1-day-old hatchlings from constant (28ºC) and fluctuating (28 ± 3ºC) temperature treatment groups (Table 4.2). The one hatchling that survived from the group incubated at 28 ± 6ºC failed to crawl when tested.

Righting Response

There was no statistical difference in self-righting time between the constant (28ºC) and fluctuating (28 ± 3ºC) experimental groups (Table 4.2, $F_{(1,30)}$ = 2.51, P = 0.12). The only survivor from the 28 ± 6ºC group was the only individual that did not attempt to right itself within the maximum trial time (30 min).

Swimming performance

During the first few seconds after being placed in the water, the hatchlings exerted their highest stroke force, which declined sharply over the first 120 s, followed by a gradual decline throughout the rest of the trial period (Figure 4.4). Both time ($F_{(1,638)}$ = 713.2, P < 0.01) and body mass ($F_{(1,30)}$ = 11.24, P < 0.01) had significant effects upon the stroke force exerted by the hatchlings, which was not however, influenced by incubation thermal regime (Figure 4.4, $F_{(1,30)}$ = 0.08, P = 0.77). Both groups achieved similar maximum stroke force during the first 30 s (28ºC group = 121.0 mN; 28 ± 3ºC group = 123.4 mN), and both groups had a similar decline in swimming force, with stroke force decreasing to approximately 50 mN after 4.5 min (Figure 4.4). Stroke frequency during swimming was not affected by incubation thermal regime (Table 4.2, $F_{(1,30)}$ = 0.44, P = 0.51), but was significantly affected by body mass ($F_{(1,30)}$ = 11.10, P < 0.01). The length of time that the hatchlings spent swimming during trials was not affected by body mass (Table 4.2, $F_{(1,30)}$ = 1.94, P = 0.17) or incubation thermal regime (Table 4.2, $F_{(1,30)}$ = 0.95, P = 0.34).

The one survivor from the 28 ± 6ºC group had a similar swimming pattern; exerting its maximum stroke force during the first 30 s of the trial, followed by a period when such stroke force decreases substantially, until the last phase when the stroke force was maintained at a constant level (Figure 4.4). However, the hatchling maximum stroke force was only 69.9 mN, and its constant stroke force was approximately 30 mN (Figure 4.4). This hatchling spent 268.3 s swimming, which was about half the time spent swimming in comparison to the hatchlings from the other incubation regimes, but its stroke frequency of 31.1 Hz was greater than that exhibited by the hatchlings from the other two experimental groups (Table 4.2).

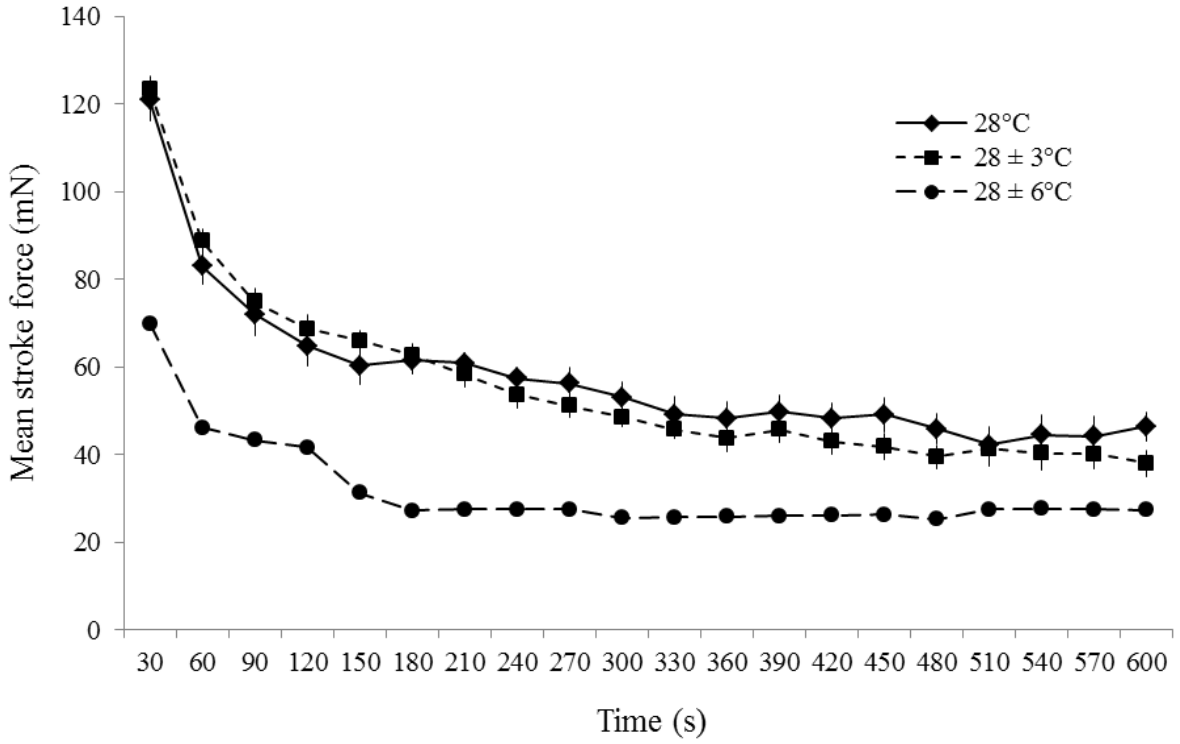


Figure 4.4: Mean stroke force (mN) generated every 30 s over a 10 min trial period by hatchling *E. macrurus* incubated at three thermal regimes: one constant (28ºC, n = 16) and two fluctuating (28 ± 3ºC, n = 16; 28 ± 6ºC, n = 1). Symbols and error bars indicate the mean ± SE ($P > 0.05$).

Table 4.2: Summary data from performance experiments upon hatchlings *E. macrurus*. Data are mean ± SE ($P > 0.05$).

Incubation temperature	**28ºC (n = 16)**	**28 ± 3ºC (n = 16)**	**28 ± 6ºC (n = 1)**
Crawling speed (cm/s)	2.3 ± 0.2	2.2 ± 0.2	--
Righting response (self-righting time; s)	27.8 ± 6.5	38.0 ± 9.1	--
Swimming performance (time swimming; s)	448.1 ± 32.8	470.7 ± 15.5	268.3
Swimming performance (stroke frequency; Hz)	23.9 ± 0.5	26.5 ± 0.5	31.1

Discussion

The present study examined the temperature profiles of *Elusor macrurus* nests in the wild, and experimentally incubated eggs in thermal regimes that mimicked those experienced in natural nests. The extent of the daily temperature fluctuations varied across the entire nesting season and between nests, with mean temperature for all the nests varying by approximately 4ºC throughout the nesting season. Approximately 66% of measured days showed a daily fluctuation in temperature of between 2ºC and 6ºC with higher daily temperature fluctuations only being experienced occasionally. Under controlled laboratory conditions, *E. macrurus* eggs incubated at a constant mean temperature of 28ºC showed no significant difference in hatchling phenotype from those incubated at the same mean temperature but under a daily fluctuation of 6ºC. A previous study showed that an increase in the mean constant incubation temperature from 29ºC to 32ºC was detrimental to hatchlings of this species of turtle (Micheli-Campbell *et al.* 2011). A limitation of this study was however, that the eggs were incubated at constant thermal regimes that did not reflect the natural thermal variability experienced by eggs incubating in natural nests. The present study addresses this limitation by demonstrating that environmentally realistic fluctuations in daily nest temperatures are not as significant in influencing hatchling phenotype as absolute differences in the mean temperature (Micheli-Campbell *et al.* 2011).

A constant thermal regime of 32ºC during incubation significantly reduced the growth rate, self-righting time and swimming performance of hatchling *E. macrurus*, as well as detrimentally affecting their morphology (smaller individuals; Micheli-Campbell *et al.* 2011). The results of the present study have shown that a fluctuating incubation thermal regime, which attained temperatures of up to 31ºC for a short-period each day throughout the entire incubation period, did not influence the phenotype of hatchling *E. macrurus*. However, this absence of effect only occurred within a certain temperature range and the incubation of hatchling *E. macrurus* under a 12ºC daily fluctuation thermal regime, with temperatures reaching 34ºC for a short period each day, was lethal for 95% of the embryos, and phenotypically detrimental for those that hatched.

The eggs that were exposed to a 12ºC daily temperature fluctuation did not gain weight to the same magnitude as the eggs from the other experimental groups. This pattern occurred from early stages of the developmental period and illustrated that the embryos exposed to a 12ºC daily fluctuating temperature were converting the yolk material into tissue at a lower rate in

comparison to the other experimental groups. Yolk consumption was not directly measured, but the eggs were checked on a weekly basis for the presence of blood vessels and weighed fortnightly. These measurements confirmed that the eggs were not dead, although they were not gaining weight at the same rate as the groups exposed to a reduced fluctuation in daily temperatures. Only four hatchlings from this group broke through the egg shell under their own volition, but only one was sufficiently strong to complete the process and survive. The timeline of embryo development has not been described for *E. macrurus*. However for the freshwater turtle *Chelydra serpentina*, after two weeks of development the embryos have a notochord and at least fourteen pairs of somites, and the tails is beginning to take shape. The neural folds, forming the cranial structure, are completely fused by the 12th day of the development, and the embryo has a partially developed heart (Yntema 1968). This phase in the development of the nervous and circulatory systems is likely to result in the embryos being highly sensitive to external thermal conditions (see Deeming and Ferguson 1991). Studies of oviparous reptile embryogenesis have shown that excessively elevated or reduced incubation temperatures during embryo development cause abnormalities of the central nervous system and vertebral column, disrupt development of the hypothalamus, and alter yolk absorption. In most cases, high embryo mortality results (Birchard and Reiber 1996; Burger, Zappalorti and Gochfeld 1987; Deeming and Ferguson 1988; Deeming and Ferguson 1989; Ferguson 1985; Vinegar 1973; Vinegar 1974; Webb *et al.* 1983; Yntema 1960). Therefore, a large number of developmental variables may be detrimentally affected by temperature during the egg incubation period in reptiles. In the present study, defining which embryogenesis stage and/or morphological structures were responsible for the high mortality in the 28 ± 6ºC group was inconclusive. The embryos died across a range of developmental stages and it was not possible to define which mechanisms were responsible for the malformations and developmental abnormalities observed for *E. macrurus* embryos exposed to 12ºC daily temperature fluctuation.

Data collected from nests in the wild showed that *E. macrurus* eggs were exposed to daily fluctuations in temperature of 12ºC and above for ~ 4% of the time. However, such variations were not experienced by the embryos on a regular basis during the developmental period. The median nest temperatures recorded here combined with findings from a previous study (Micheli-Campbell *et al.* 2011) strongly suggest that *E. macrurus* eggs in the wild are being exposed to median temperatures approaching the upper thermal threshold for the species. Occasionally, *E. macrurus* nests have been found where the whole clutch has not developed, and more regularly where a few eggs from the clutch do not develop (Micheli-Campbell, personal observation).

Although this study suggests that elevated temperatures during incubation may be responsible for some of the mortality of embryos, monitoring of nests temperature and hatching success over multiple years is required to confirm this hypothesis. The urgency for this data is highlighted by the 23 Global Climate Models (IPCC 2007), which predicted a twelve-fold increase in the number of days reaching temperatures above 35ºC over the next 60 years in the Mary River catchment (Queensland Government, 2009). This scenario may result in increases of both mean and daily fluctuations in ambient temperature. The *E. macrurus* population has crashed from that of only a few decades ago (Flakus 2002) and the species is currently listed as endangered (IUCN 2011). As a consequence of the low population the gene pool of the species has a limited capacity to respond to environmental shifts. Moreover, the population is geographically restricted to the Mary River catchment and therefore unable to relocate to a more optimal environment if conditions become unfavourable. Based on the findings of the present study, we argue that the recruitment of hatchling *E. macrurus* into the wild may be compromised due to greater fluctuations in the daily thermal regime during incubation, as well as increases in the average temperature (as shown by Micheli-Campbell *et al.* 2011). These effects may be especially pronounced if the embryos experience these conditions at the beginning of the developmental period.

CHAPTER 5

Characterising and locating critical habitat for riverine animals: A case study on juvenile freshwater turtles.

Abstract

Tropical rivers are complex and dynamic ecosystems. The identification and characterisation of critical habitat for animals inhabiting these ecosystems is challenging, and this is augmented for threatened species existing at low population densities. Here we used miniaturised telemetry devices and a presence-only predictive model to identify the characteristics of habitat selected by juveniles of the endangered Mary River turtle (*Elusor macrurus*). Due to the difficulties in capturing juvenile turtles from the wild, it was necessary to collect the eggs, incubate and head-start the young turtles. Juveniles were fitted with either a miniaturised VHF-radio (5 months of age, n = 6) or an acoustic transmitter (16 months of age, n = 10), and each was located weekly for a 21 day period. Each turtle was located to within less than one meter and depth, surface water flow measurements taken. The data populated a presence-only predictive model (Ecological Niche Factor Analysis with Mahalanobis distances) to compare the localities selected by the turtles over the mean background environment. The model revealed that the juvenile *E. macrurus* selected a very narrow range of habitat characteristics, significantly different from the mean background environment. These were discrete areas of shallow lotic water immediately upstream or downstream from riffle zones. In order to assess that the model identified critical habitat locality over extended periods of time we released a further twelve juveniles with acoustic transmitters programmed on a duty cycle to extend transmitter longevity to nine months. These transmitters were detected by eleven underwater static receivers placed at intervals throughout the study area. Turtle detection time by each receiver demonstrated that there was a significant positive relationship (X^2_1 = 7961.2; P < 0.01) between the model generated habitat-suitability score for a locality and turtle occupancy. Extrapolation of the model habitat suitability scores throughout the main trunk of the Mary River revealed the location of 49 discrete areas with habitat suitable for juvenile *E. macrurus*; none existed in impounded sections of the river. The study demonstrates the value of integrating

acoustic telemetry data with a presence-only predictive model to identify and characterise critical habitat for riverine animals.

Introduction

River systems are among the most seriously threatened and modified environments on the planet. One of the primary reasons attributed to declining stocks of riverine animals has been the loss/alteration of critical habitat (Allan & Flecker 1993; March *et al.* 2003; Stickler *et al.* 2008; Welch *et al.* 2008). In conserving habitat, resource managers often rely upon spatial explicit models to qualify the link between a species and its environment (Huck *et al.*, 2010; Donovan *et al*., 2011). These models, such as generalised linear models (GLM), generalised additive models (GAM), classification and regression tree analysis and artificial neural networks (ANN), use locational records to assess the importance of particular environmental variables to a species and can be further used to allow predictions of potentially suitable habitat throughout a species' range (see review Guisan & Zimmerman, 2000). Many of these models require good quality presence/absence data in order to generate statistical functions that allow habitat suitability to be assessed (Guisan & Zimmerman, 2000). However in situations where individuals of a species are difficult to locate, collecting presence data of sufficiently high resolution, accuracy and abundance is logistically challenging, and accounting for true absences is often inadequate or unavailable (Hirzel *et al*., 2002).This is a common issue in freshwater systems due to water turbidity, underwater refuges and because habitat type can change over a relatively fine-spatial scale.

Ecological Niche Factor Analysis (ENFA) is a multivariate approach that uses presence only data to examine habitat preferences without the requirement of absence data (Hirzel *et al*., 2002). The model searches for directions in ecological space to: 1) extract the difference between the habitat characteristics used by the species and those available throughout the study area, and 2) determine the ratio between the variance of available habitats within the study area and those occupied by the species. ENFA is frequently employed to identify critical habitat for a range of terrestrial and marine animals (insects: e.g., Gallego *et al*., 2004; corals: e.g., Tittensor *et al*., 2009; crustacean: e.g., Galparsoro *et al.*, 2009; birds: e.g., Hirzel *et al*., 2004; fish: e.g., Monk *et al.*, 2011; mammals: e.g., Dettki, Lofstrand & Edenius, 2003), but, as far as we are aware, has never been used to identify critical habitat for riverine species. This seems incongruous because

of the urgent need for such information, but is likely due to the challenges associated with surveying riverine species at a sufficiently fine spatial scale to populate the ENFA model adequately.

Recent advancements in underwater acoustic telemetry have enabled continual monitoring of the presence of aquatic animals (Heupel *et al*., 2008, Greene *et al*., 2009). This technology consists of implantable sonic transmitters that can be detected by either mobile boat-mounted hydrophones (active acoustic telemetry) or static underwater listening stations (passive acoustic telemetry). The method of active tracking enables animal location within a meter of resolution, whilst fixed underwater receivers can locate the tagged animals continuously over long periods of time but a broader spatial scale. The miniaturisation of the transmitters, to only a few grams, now offers the opportunity to record site occupancy of small-bodied animals over extended periods (Welch, Ward & Batten, 2004; Barry *et al.*, 2007; Stickler *et al.*, 2008). While acoustic telemetry has been routinely used to measure patterns in animal movement (Campbell *et al*., 2010; 2012), far less attention has been given to the incorporation of these data into studies of habitat assessment and habitat prediction. We argue that active acoustic telemetry offers the opportunity to collect animal presence data at a sufficiently fine-sale to populate predictive distribution models for riverine species. Moreover, by integrating the model findings with passive acoustic telemetry data, the location of critical habitat within the river can be identified over the annual scale.

There are over 200 species of freshwater turtle in the world and 45% of these are considered threatened (Hoffmann *et al.*, 2010). Juvenile mortality is very high for most species of turtle, with very few of the hatchlings attaining reproductive age (Cann, 1998). Current declines in turtle populations have been attributed to habitat loss (Rhodin *et al.*, 2011), and therefore, protection or generation of areas with suitable habitat characteristics for juvenile turtles represents an effective conservation strategy for many species of turtle. The problem however, is that virtually nothing is known about the habitat requirements of juvenile freshwater turtles, and we argue that biological data pertaining to the critical habitat selected by juvenile turtles is urgently required to identify protection areas.

The aims of the present study were to develop a methodology that would enable the identification of critical habitat at a sufficiently fine-scale and over an adequate duration to be ecologically relevant for riverine animals. We used five steps to identify, quantify, and locate the

habitat characteristics utilised by juveniles of the endangered freshwater turtle (*Elusor macrurus*): 1/ collection of eggs and head-starting in order to obtain a sufficiently large sample size; 2/ accurate assessment of selected habitat through active tracking; 3/ statistically compare the physical characteristics of the selected localities against the background environment; 4/ assess the relationship between the model generated habitat-suitability score and turtle occupancy over an extended period; 5/ prediction of important localities at a sufficiently high resolution (10 m^2) to guide management practise.

Material and Methods

Study area

The study was undertaken within a 4.7 km stretch of the Mary River, QLD, Australia (Figure 5.1). The locality was selected because a high number of *Elusor macrurus* nests are predictably laid within this short-section of the river. The section was composed of a sequence of large pools (deep areas with slow water flow) and riffle zones (shallow areas of high water velocity).

Animals

Eggs of the freshwater turtle *E. macrurus* were collected from four clutches within the study area in 2009 and 2010. The eggs were returned to The University of Queensland (Brisbane, Australia) and incubated at simulated natural conditions. After hatching, the turtles were placed into holding tanks containing gravel, shelters, basking platforms and water at 40 cm depth. The tanks were kept in an outdoor facility, and because the laboratory was close to the Mary River, climatic conditions were similar to the turtle's geographic distribution. Water and air temperatures were monitored hourly and were similar for all holding tanks (Hobos TidBit, Onset, Massachusetts, U.S.A.). The juvenile turtles were fed a commercial diet (Nutrafin Max, Hagen, Montreal, Canada), blood worms and spinach.

In April 2010, miniaturised telemetry devices were attached to the juvenile *E. macrurus*. These formed three cohorts; two hatched from eggs collected in 2009 (sixteen-month old, n = 22)

and the other from 2010 (five-month old, n = 6), the formers were affixed with acoustic transmitters, and the latter with VHF-radio transmitters.

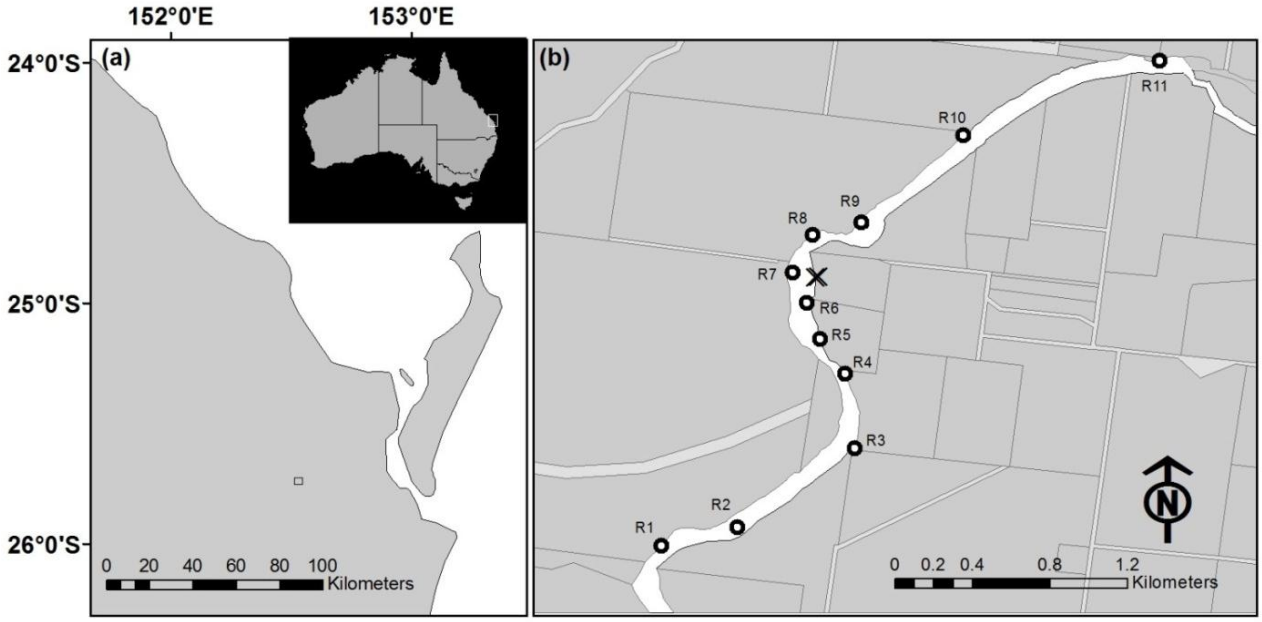


Figure 5.1: a) The geographical location of the study undertaken on the Mary River (QLD, Australia). b) The river flows from the south-west to the north-east. Black circles represent the location of the underwater listening stations (VR2Ws), and the black cross marks the release site of the juvenile *Elusor macrurus*. There are four riffle zones within the study area and they are situated between the following receivers: R1 - R2; R4 – R5; R8 – R9; and downstream from R11.

Radio telemetry

The VHF-radio transmitters (LT5, Titley Scientific, Queensland, Australia; 0.6 g in air, 0.4 g in water, with the dimensions 16 x 7 x 4 mm and a 100 x 0.4 mm antenna) and were required to enable tracking of turtles of only five-month of age. In water, the mass of these transmitters represented less than 2 % of the turtles' body mass (23.9 ± 0.3 g; mean ± S.E.; n = 6). Each transmitter emitted VHF waves at a frequency unique to each individual and had a projected battery life of 21 days. The transmitters were attached on the posterior left side of the

turtles' carapace, using a quick drying contact epoxy (UltraFix Plus, Ramset, Victoria, Australia). The overall tagging procedure time was less than 5 min. To ensure they were swimming, feeding, and able to right themselves, the juveniles were returned to the holding tanks and observed for two weeks prior to release. The turtles were released within close proximity of the nest location (Figure 5.1). Once in the river, each turtle was located three times at weekly intervals using a directional Yagi antenna and VHF receiver (Regal 2000, Titley Scientific). Once the signal was located, the boat was manoeuvred to be directly on top of the VHF signal and the geographic position (Oregon 550, Garmin, Kansas, U.S.A.), surface water velocity (Marsh-McBirney) and water depth (Eagle Marine Electronics) were recorded.

Acoustic telemetry

Twenty-two acoustic transmitters (V7, VEMCO, Halifax, Nova Scotia, Canada) were attached to the marginal scutes of sixteen-month old *E. macrurus*. In water, these transmitters (L 20 x D 7 mm; 1.6 g in air; 0.7 g in water) weighed less than 1 % of the turtle's body mass at this age (86.8 ± 5.1 g; mean ± S.E.; n = 22). To attach the transmitters, a 2.5 mm diameter hole was drilled vertically through one of the posterior marginal scutes of the carapace. The transmitter was secured onto the carapace with a sterilised plastic nut and bolt (1.5 mm). The turtles were returned to the holding tank for at least 14 days observation prior to release into the river, in close proximity to the nesting banks.

The transmitters emitted a sonic pulse every 60 s encoded with a unique ID number and had a projected battery life of up to 9 months. These animals were divided in two cohorts: 1/ ten turtles were attached with transmitters programmed to emit the acoustic pulses continuously, and the location of each of these was determined three times at weekly intervals. Their location was pinpointed to less than one meter resolution using a directional hydrophone and receiver (VR 100, VEMCO), and the river physical characteristics recorded. 2/ The second cohort of twelve turtles were attached with transmitters duty-cycled to transmit for only 20 min every 6 h. This extended the battery life of the transmitters to nine months. The acoustic transmissions from these animals were detected by an array of static underwater receivers (n = 11, VR2W, VEMCO). This later cohort was released into the river posteriorly to the 21 days of active radio and acoustic tracking. Receivers were deployed throughout 2.5 km both upstream and downstream from the release site, which provided long-term and continuous monitoring of these turtles over 9 months from their release (Figure 5.1b). The receivers were deployed in a formation so that the middle

section of each pool, plus the areas upstream and downstream from riffle zones, were within detection fields. Each receiver was secured to a concrete anchor (15 kg) and moored to a tree on the river bank by a 6 mm multi-strand stainless steel cable. The detection range of each receiver was determined before the release of the animals, by towing an activated transmitter in a pre-determined pattern away from each receiver, and then comparing the received and missing detections with the boat's location. The detection radius of each receiver was approximately 50 (100) m. The passive acoustic telemetry lacked the precision of active tracking in determining turtle location, but provided the advantage of continuous monitoring over long-periods of time. The outer most receivers of the array effectively gated the river, and demonstrated that none of the tagged turtles past up or downstream of the study area during the nine months.

Data analysis

To characterise the study area, water depth (Eagle Marine Electronics) and surface water velocity (Hach) were recorded from a 100 randomised locations on the river. The selection of these geographical locations were randomly generated using the 'splancs' library in R (Rowlingson & Diggle, 1993; R Development Core Team 2011). These data were combined with the water depth and velocity data for each turtle selected location. The Kriging function in ArcGIS 10 (ESRI) was then used to create smoothed surface maps for these variables throughout the study area (raster resolution: 10 m^2). The centre point of each riffle zone was recorded at its narrowest width and smoothed surface maps were also created for distance to riffle zones and river margins.

The smooth surface maps were used to calculate the ecogeographical variables (EGVs), which quantitatively described the water depth, surface water velocity, distance to riffle zones and distance to river margins for each 10 m^2 raster cell of the study area. These environmental raster datasets and the environmental data from the localities selected by the turtles were uploaded into an Ecological Niche Factor Analysis (ENFA), by utilising the "adehabitatHS" library of functions (Calenge, 2006) in the R programming language. ENFA described the environmental niche of a species by utilising the marginality (the absolute difference between the mean EGVs of the study area and that of the turtles' selected locations) and the specialisation indices (the variability of turtles selected EGVs relative to the overall range of EGVs within the study area; Hirzel *et al.* 2002). Marginality and specialisation values close to zero indicated little or no preference, whilst values close to -1 or 1 indicated a high preference for particular habitats

within the study area. Essentially, ENFA compares the distribution of the animal's presence data (i.e. preference) with the distribution of values in the whole study area. The Monte-Carlo test was used to assess the significance of the marginality and specialisation results from the ENFA (Basille *et al.*, 2008). This analysis minimises multicollinearity and redundancy by extracting the relevant ecological information from a set of environmental variables (Hirzel *et al.*, 2002). The variable 'depth' was not normally distributed, as showed by the standard kurtosis test. The variable data were therefore normalized through the 'box-cox' algorithm (Sokal & Rohlf 1981). The habitat suitability map was computed by utilising the squared Mahalanobis distances (i.e. the multivariate distances between the mean niche of the study species and the habitat components at each mapped location; Calenge *et al.*, 2008).

Acoustic detection data collected by static receivers were used to determine model accuracy over an extended period of time (9 months). A total of 1,670 detections were logged from twelve juvenile *E. macrurus*. The data downloaded from the eleven static underwater receivers were arranged into a single data matrix. This matrix was then subjected to a procedural event log analysis, in order to extract and summarize turtle residence time within the detection range of each receiver, executed in the V-Track R package (Dwyer *et al.*, 2012). A turtle was considered to reside at a single receiver if detected was detected on consecutive transmitter 6 h duty-cycles.

The habitat suitability model performance generated using the short-term active tracking data was tested for an extended period of time using the data obtained by the static underwater receivers. Performance was assessed using a Poisson generalised mixed model (GzLMM), with habitat suitability score implemented as a fixed effect, residence time as the response variable, and turtle ID as a random effect. In addition, turtle occupancy time within the detection range of each receiver was compared against a randomised distribution within these detection areas using the Area Under the Curve (AUC) metric, in the 'ROC' library of functions in R (Sing *et al.*, 2009). The AUC score ranges from 0.5 to 1.0. Values close to 0.5 indicate an assignment no better than random, whilst the closer the values to the theoretical maximum of 1.0 the better the predictive model.

The models habitat suitability score was then applied to the entire main trunk of the Mary River based upon the variables 'water depth' and 'surface water velocity'(data collected by

kayaking the river and taking depth and water flow measurements) to reveal areas that contain suitable environmental variables to possess critical habitat for the juvenile *E. macrurus*.

Results

The study area was composed of three pools of varying size (400 - 1800 m length) and four riffle zones (Figure 5.2). The pools were characterised by deep (3 - 6 m) slow flowing (~ 0.2 $m.s^{-1}$) water, whilst the water within the riffle zones was shallow (0.5 - 1 m depth), and attained up to 1.5 $m.s^{-1}$. In the immediate upstream and downstream areas of the riffle zones there were patches of "slack" or "back" water (0 to -0.5 $m.s^{-1}$). These areas were shallower (0.5 - 2 m depth) than the rest of the pool, but were characterised by very slow moving water (< 0.2 $m.s^{-1}$). The river width was never greater than 120 m throughout the study area, with the narrowest points located within the riffle zones (15 - 20 m length).

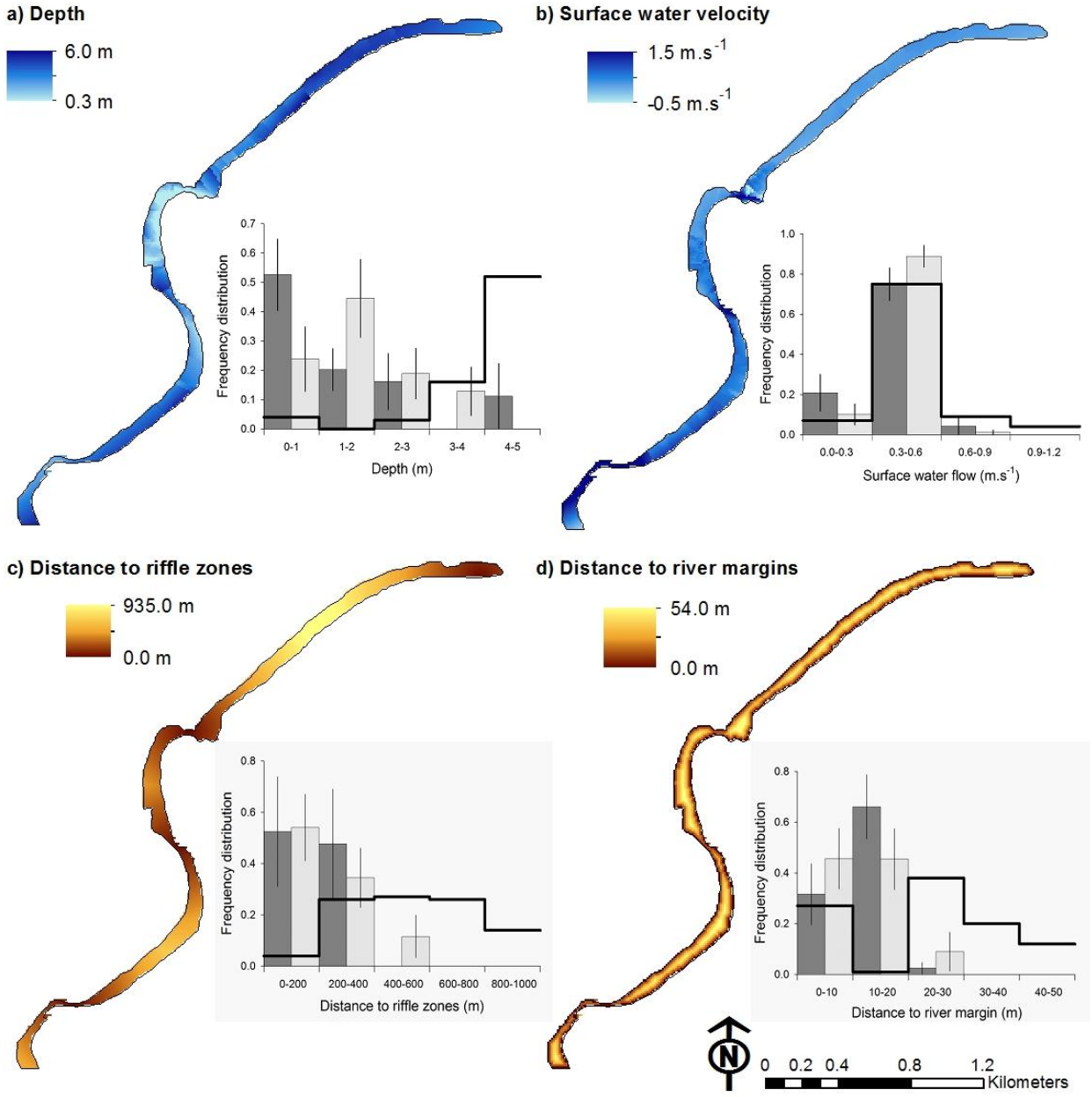


Figure 5.2: Study area profile and environmental data obtained from the tracking of juvenile *Elusor macrurus*. The four maps display the ecogeographical variables (EVGs) throughout study area (a - depth; b - surface water velocity; c - distance to riffle zones; d - distance to river margin). Inset graphs show the frequency distribution of the respective EVG recorded by radio (dark bars; n = 6; mean ± S.E.) and acoustic tracking (white bars; n = 10; mean ± S.E.) of juvenile *E. macrurus*. The black lines in the graphs represent the respective mean EVG for the entire study area.

The six hatchling and ten juvenile *Elusor macrurus* attached with VHF-radio and acoustic transmitters, respectively, were released into the Mary River, each was located three times at weekly intervals. After this time, all turtles were present and moving, and therefore presumed to alive. Out of the twelve individuals released with acoustic transmitters programmed to duty cycle to prolong tag longevity, only five individuals were still located within the study area after 9 months. No transmitters were detected by active tracking beyond the limits of the study area. Additionally, there were no detections recorded by the listening station R1, which was deployed at the upstream end of the study area. Within a few days of release, most turtles moved to the vicinities of the two riffle zones closest to the release site. Six turtles were consistently detected at these localities throughout the 9-month passive acoustic monitoring period. The further six juveniles crossed the riffle zones immediately upstream and downstream from the release site two to four months post-release. One of those turtles travelled 2.5 km to the upstream area of the subsequent downstream riffle zone, remaining there for the duration of the study. One juvenile was detected 1.8 km upstream from the release site six months post-release, and remained within close proximity of the furthest upstream riffle zone.

Ecological Niche Factor Analysis

A total of 18 location fixes were identified by radio tracking and 30 fixes were obtained by active acoustic telemetry. ENFA was performed utilising the four measured variables collected randomly throughout the study area and the environmental data gathered every time a turtle was located (water depth, surface water flow, distance to the riffle zones, distance to river margins; Table 5.1). The analysis produced a global marginality score of 3.2, demonstrating that the EGVs at the locations selected by the juvenile *E. macrurus* were significantly different from those that would be selected by random; and a specialisation eigenvalue of 10.3, illustrating that the EGV variance within the available habitat was 10-times higher than the variance within the EGVs selected by the turtles. Additional Monte-Carlo test confirmed that the EGV's selected by the turtles were significantly different from computer generated random distributions ($P = 0.014$).

Table 5.1: Contribution of the four ecogeographical variables (EGVs) to the Marginality and Specialisation factors calculated used Environmental Niche Factor Analysis (ENFA) for the actively tracked juvenile *Elusor macrurus*. Marginality ranges from -1 to 1; negative values indicate lower values of EGVs to those found throughout the study area. Specialisation ranges from 0 to 1; the higher the specialisation coefficient the narrower the EGV range in which the juvenile turtles were located.

EGVs	Marginality factor (3.2)	Specialisation factor (10.3)
Depth	-0.790	0.135
Surface water velocity	-0.212	0.801
Distance to riffle zone	-0.427	0.577
Distance to river margin	-0.383	0.078

The five-month old *E. macrurus* were primarily located in water less than 1 m depth, and the sixteen-month old *E. macrurus* were primarily located in water between 1 and 2 m depth (Figure 5.2a). The ENFA marginality factor for depth (Table 5.1) confirmed that juvenile *E. macrurus* selected shallower locations than the mean depth found throughout the river channel. The specialisation score for water depth was close to zero, showing the level of variability within the depths selected by the turtles was high compared to the variability within the environment. The tagged turtles selected locations with a narrow range for surface water velocity (between 0 - 0.6 $m.s^{-1}$) and this was similar to the mean surface water flow throughout the study area (Figure 5.2b). This resulted in a marginality factor close to zero and a high specialisation factor for surface water velocity (Table 5.1). The maximum distance that could be attained from a riffle zone within the study area was 900 m, but turtles were not ever located further than 400 m from a riffle zone (Figure 5.2c). ENFA results confirmed that juvenile *E. macrurus* significantly selected areas close to riffle zones (Table 5.1). The river within the vicinity of riffle zones is narrow in breadth, and as a consequence, turtles were rarely further than 20 m from the river bank (Figure 5.2d). ENFA results confirmed that the distance to river margin had a significant effect upon the niche selection of juvenile *E. macrurus* (Table 5.1).

Habitat modelling

A habitat suitability map was created to illustrate the locations throughout the study area where juvenile *E. macrurus* are likely to inhabit (Figure 5.3a). These predictions are based upon the occurrence of the four EVGs (water depth, surface water velocity, distance to riffle zones and to river margins) at each location throughout the study area. The map illustrated that the most suitable habitat for juvenile *E. macrurus* were marginal areas within the immediate upstream or downstream vicinities from the riffle zones. These areas were shallow and had a low surface water velocity. The riffle zones themselves, which are characterised by fast flowing water, were not selected as suitable habitat for juvenile *E. macrurus* by the model. The mid-regions of the river trunk and localities far from riffle zones were not deemed suitable habitat, presumably because the water depth was greater than 3 m in these areas. The model significantly predict turtle location, based upon the acoustic telemetry data (AUC = 0.66).

An array of static underwater acoustic receivers continually listened for the signal from twelve tagged turtles throughout the study area for nine months post-release. This cohort of acoustic-tagged turtles spent a disproportionate amount of time between the detection ranges of individual receivers (Figure 5.3b). A habitat suitability score was calculated for the detection area of each receiver, and there was a significant relationship between suitable habitat and turtle residence time ($X^2_1 = 7961.2$; $P < 0.01$). The passive acoustic telemetry data also showed that juvenile *E. macrurus* exhibited a high site-fidelity to the areas where they were located by active tracking and they did not travel beyond the limits of the study area (Figure 5.4).

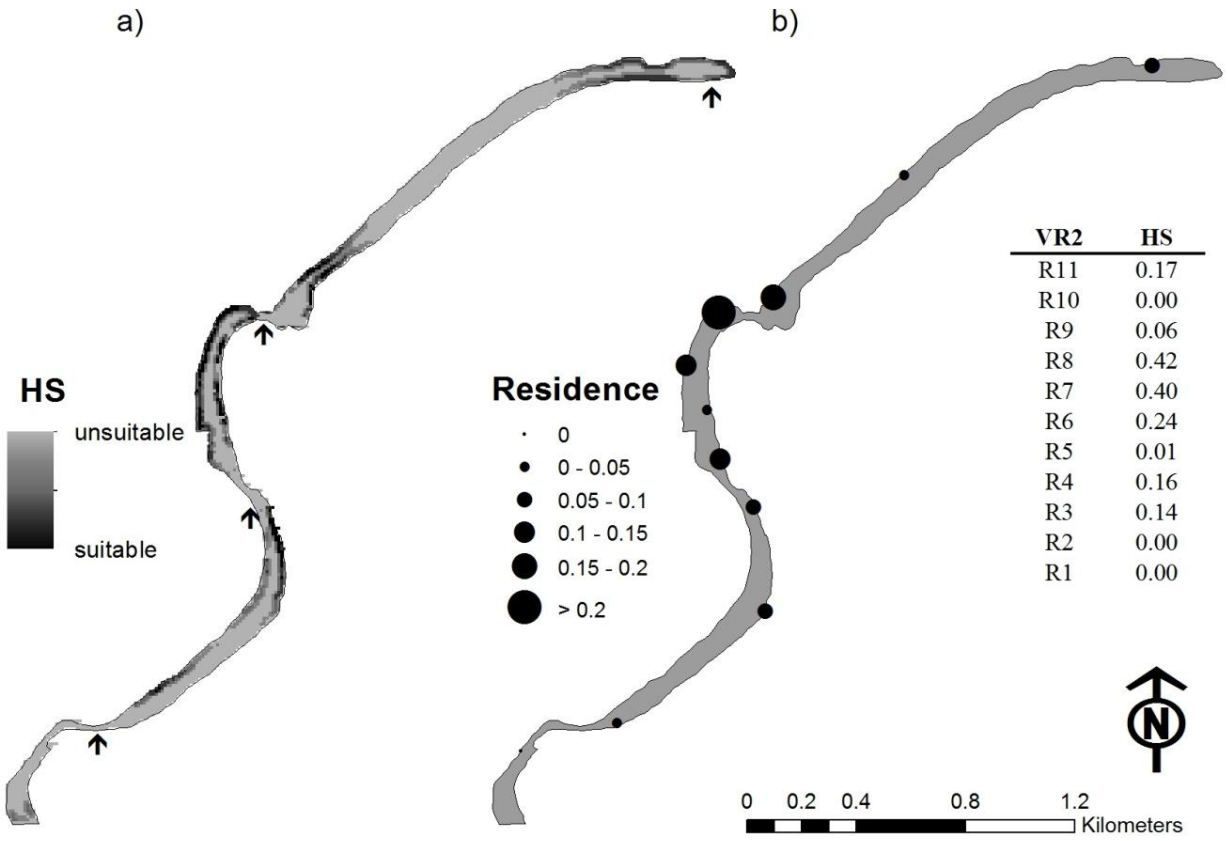


Figure 5.3: a) Habitat suitability (HS) map based upon the squared Mahalanobis distances between the habitat selected by actively tracked hatchling and juvenile *Elusor macrurus* (n = 6 with radio transmitters; n = 10 with acoustic transmitters) and the available habitats within the study area. The map is a spatial representation of habitat suitability values (0 – 1) for every 10 m^2 cell in the study area. The darker shading areas in the map represent the most suitable habitat for juvenile *E. macrurus*. Riffle zone locations are indicated by black arrows; b) Frequency distribution of the residence time spent by juvenile *E. macrurus* (n = 12) within the reception range of each passive acoustic listening station. Inset table shows the mean habitat suitability score within the detection range of each acoustic receiver.

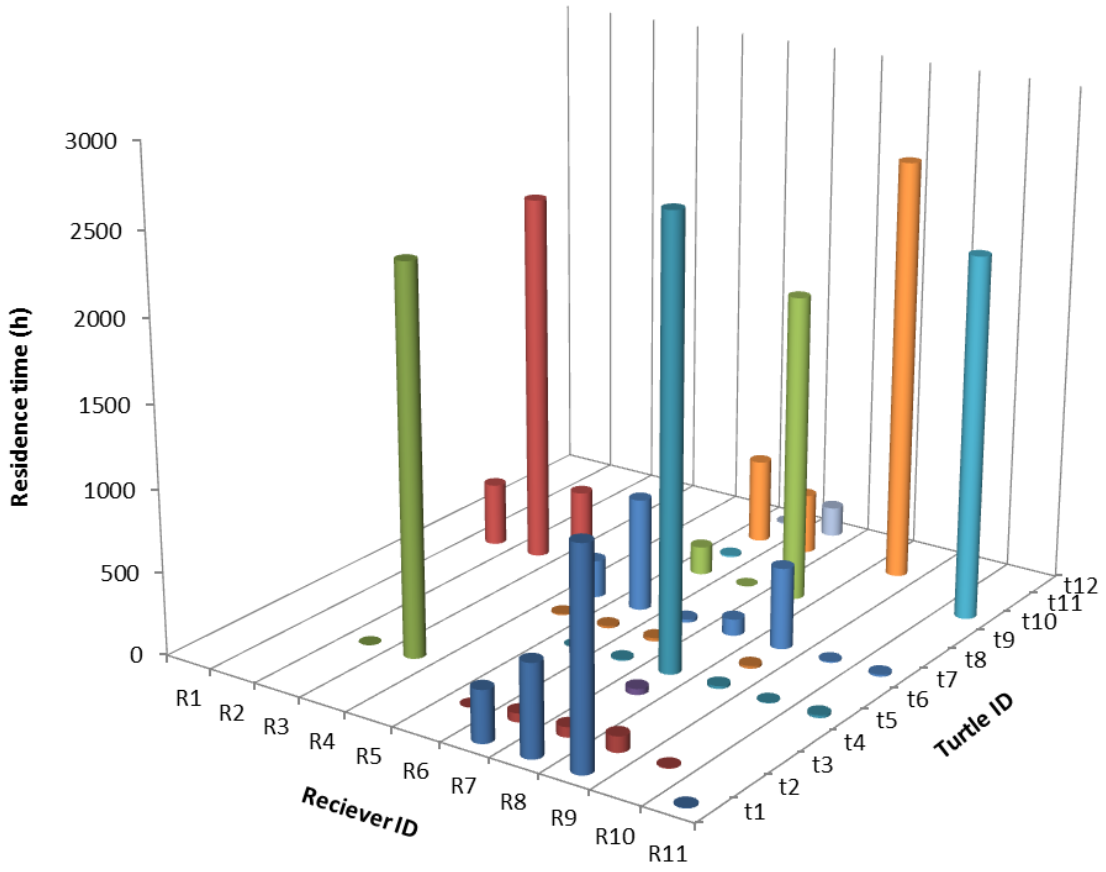


Figure 5.4: The residence time of juvenile *Elusor macrurus* (n = 12) within the detection range of each passive acoustic listening station (VR2W, n = 11). The four riffle zones are situated between R1 – R2; R4 – R5; R8 – R9; and downstream from R11.

Extrapolation of suitable habitat for juvenile *E. macrurus* throughout the main trunk of the Mary River, based upon surface water velocity and water depth, revealed 49 discrete locations (Figure 5.5). These areas were distributed throughout the length of the river, but two stretches lacked suitable habitat: a ~ 15 km section near the town of Gympie, and a ~ 24 km area upstream from the river barrage. These areas were not selected because there was not shallow slow flowing water in the immediate proximity of riffle zones.

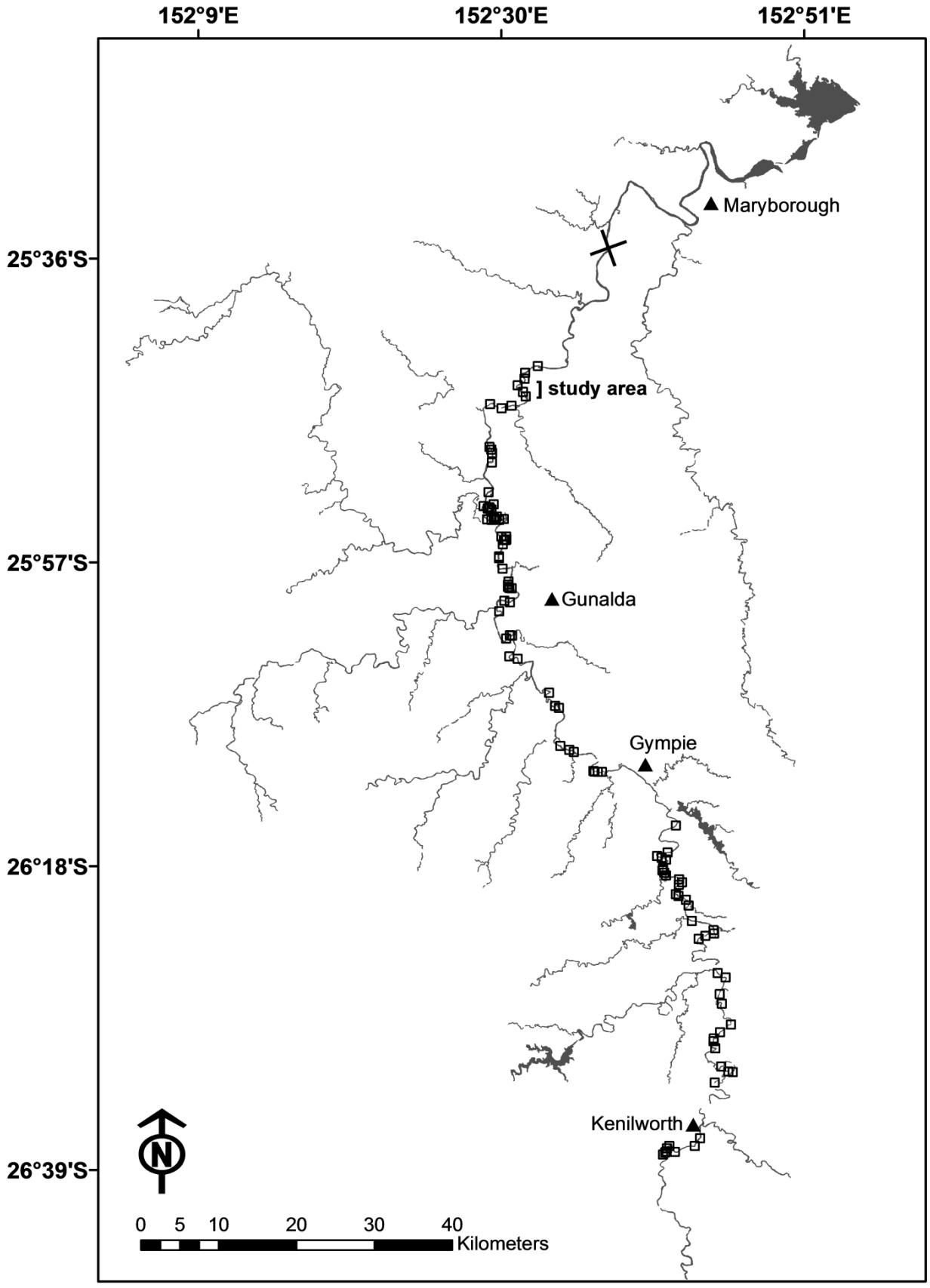


Figure 5.5: Mary River catchment (QLD, Australia). Black squares indicate proposed suitable habitat for juvenile *Elusor macrurus*, based upon 'water depth' and 'surface water velocity'. The black cross indicates the location of the tidal barrage.

Discussion

A common issue when identifying critical habitat for hard to detect species is obtaining sufficient data to populate the model and account for false-absences (McArdle, 1990). This is all too apparent for animals inhabiting river systems, particularly in the tropics, where waters are typically very turbid and flow rates vary dramatically with season. Here we were obtained accurate recordings of habitat selected juveniles of an extremely shy and cryptic species of freshwater turtle, which exists at a very low population density. The data were used develop a predictive distribution model and map to illustrate the locations of critical habitat for the juvenile turtles. Static underwater acoustic receivers demonstrated that the model's habitat suitability scores matched with turtle occupancy over an extended period of time. The models habitat suitability scores were then extrapolated throughout the geographical range of the species to demonstrate discrete areas that contain critical habitat for the juvenile *Elusor macrurus*.

Virtually nothing is known about the habitat requirements of juvenile *E. macrurus* and they are rarely sighted in the wild. Collection of data upon the type of habitat selected by the juvenile turtles was only possible by collecting eggs and head-starting the turtles, and then tagging them with miniaturised telemetry devices. This generated sufficient data for the predictive model which showed that juvenile *E. macrurus* selected a very narrow range of habitat characteristics relative to the background environment. Furthermore, these habitat characteristics were not present in human modified and impounded sections of the river. These findings demonstrate the *E. macrurus* juveniles are highly specialised in their habitat requirements, and identifies which riverine characteristics need to be conserved for the long-term viability of the population.

We selected four ecogeographical variables (EGVs) to characterise the differences in habitat conditions between pools and riffle zones. The nesting banks of *E. macrurus* are located along the pools within the study area, and once they entered the river, hatchlings would be faced with a choice of remaining in the pools or migrating to the riffle zones. Once released into the pools, the turtles migrated towards the riffle zones. They did not however, inhabit the fast flowing water, which comprised the majority of the riffle zones, but instead favoured discrete areas of shallow slow flowing water. These areas comprised only a fraction of the riverine habitat and were generally located along the river margins or immediately upstream or downstream from the

riffle zones. Some juveniles selected the first suitable habitat they encountered, whilst others travelled greater distances.

The preference shown for these localities by the juvenile *E. macrurus* may be explained biologically. Turtles are not streamlined animals and have a low tolerance to sustained exercise (Marvin & Lutterschmidt, 1997; Du, Zheng & Shu, 2006; Clark, Gordos & Franklin, 2008; Micheli-Campbell *et al.*, 2011; 2012), and it is therefore, likely that juvenile *E. macrurus* selected slow flowing water to reduce energy expenditure whilst swimming and surfacing, avoiding displacement from the area by the water current. Inhabiting shallow waters will have two advantages for juvenile *E. macrurus* over deep water. Firstly, it would decrease energy expenditure when surfacing to breathe, and secondly, it would reduce the time that the young turtles would be exposed to predators. Shallow water locations have a higher partial pressure of oxygen at the river substratum than in deep water and because *E. macrurus* satisfy part of their metabolic demands through aquatic respiration, dive duration is increased at higher partial pressures of oxygen (Clark, Gordos & Franklin, 2009). These depth and water flow extents characterise the upstream and downstream vicinities of riffle zones and river margins, and is likely to be the reason for the turtles' preference for these areas. We recognise that other factors such as substratum type and food availability may have also biased turtle preference for these areas, and we recommend these variables be assessed in future studies and incorporated within the habitat suitability characterisation.

The habitat suitability model demonstrated that juvenile *E. macrurus* selected a very narrow range of coinciding environmental variables throughout the first two years of life. These restricted niches are undoubtedly important for juvenile *E. macrurus*, but we are uncertain how significant they are for juveniles of the other five species of turtle inhabiting the Mary River (*Chelodina expansa, Chelodina longicollis, Elseya albagula, Wollumbinia latisternum, Emydura macquarii krefftii*). Evolutionary theory (Gause's Law) predicts that no two species have the same ecological niche at the same time and place (Savage, 1958), and therefore, it would be valuable to clarify to what extent the critical habitat of these other turtle species overlap with *E. macrurus*. These other species have not suffered population declines to the same extent as *E. macrurus*, and such information would reveal if inter-species resource competition occurs at the early life-stages.

Introduction of non-native predators or an increase in native predator populations may have also played a role in the decline of *E. macrurus*. We expected a high level of predation during the study, and indeed 42 % of the twelve tagged turtles monitored by passive telemetry disappeared over the nine months. Although transmitter failure cannot be discounted, we argue that predation was a more likely cause. The lack of any acoustic signal after repeated searches suggested that six turtles and their attached transmitters had been removed from the water. We suggest that the water-rat (*Hydromys chrysogaster*) and white-bellied sea-eagle (*Haliaeetus leucogaster*) were likely predators, because freshwater turtles are known to be part of their diet (Woollard, Vestjens & MacLean, 1978; Woodall, 1982; Olsen, Fuentes & Rose, 2006) and these animals were regularly sighted foraging within the study area (Micheli-Campbell, personal observation). In addition, between seven and eight months post-release the movements of two turtles were observed to suddenly increase, shifting from daily movements of less than 50 m to over 8 km. This rate of movement is improbable for a juvenile freshwater turtle and is more typical of a predatory fish. This data was therefore, not used in the AUC analysis. There are a number of native and introduced predatory fish species inhabiting the Mary River that possess the anatomical features to predate juvenile *E. macrurus*, such as the Australian bass (*Maquaria novemaculatea*), freshwater eel (*Anguilla reinhardtii*), Mary River cod (*Maccullochella peelii mariensis*), fork-tailed catfish (*Arius graeffei*), saratoga (*Scleropages leichardti*) and sooty grunter (*Hephaestus fuliginosus*). The current study presents limited data pertaining to the predation of juvenile *E. macrurus*, and further investigation is required to elucidate the significance of predators upon the declining population.

The findings of the present study contribute to turtle conservation by revealing that juvenile turtles may require a narrow range of habitat types. These may comprise only a limited number of discrete areas throughout the geographical range of the adults and vary greatly from the background environment. The study methodology would be applicable to a wide range of riverine animals, and is particularly useful for identifying critical habitat for species that are difficult to find.

CHAPTER 6

General Discussion

Populations of turtles and tortoises have suffered considerable decline in recent decades and this has been repeated on every continent, and Australia is no exception. Two species endemic to Australia are listed in the top 40 most threatened species of tortoises and freshwater turtles in the world (IUCN 2011). The Mary River turtle (*Elusor macrurus*) is one of those. For the last decade, a local community group has undertaken an extensive nest protection program, and although hundreds of hatchlings enter the river each year, juvenile *E. macrurus* are rarely seen in the wild. The aim of the present study was to investigate the nesting biology and ecology of the early life-history stages of *E. macrurus*, in order to understand the connection between habitat requirements and population recruitment. To achieve this aim, it was necessary to combine field investigations and empirical studies of early-life history stages. The results revealed *E. macrurus* to exhibit nest-site fidelity and to be highly selective of their nesting grounds, laying the eggs in non-vegetated northerly facing sandy banks (Chapter 2; Micheli-Campbell *et al. under review a*). This site-selection indicated a preference by the females for warmer localities to nest. Due to the significant role played by temperature upon embryo development (Deeming and Ferguson 1991; Booth 2006), this was an important avenue for investigation. Empirical studies were carried out to investigate the influences of incubation temperature upon the phenotype of the hatchling *E. macrurus*. In contrast to the hypothesis – that warmer incubation temperatures would improve the performance of the hatchlings – a slight rise in the incubation thermal regime above the mean temperature experienced by the eggs in the wild actually had a detrimental effect upon the morphology and performance of the hatchlings (Chapter 3; Micheli-Campbell *et al.* 2011). Similarly, large temperature fluctuations during incubation caused high embryo mortality and physical impairments (Chapter 4; Micheli-Campbell *et al.* 2012). If the turtles successfully hatch, their next challenge is to find a suitable niche within the river to survive, grow, and attain reproductive age. Using miniaturised telemetry devices, juvenile *E. macrurus* were monitored once they entered the river. Through the use of a predictive distribution model, they were shown to inhabit a very narrow range of habitat characteristics in comparison to those available in the river (Chapter 5; Micheli-Campbell *et al. under review b*). Essentially, the findings presented in this thesis demonstrate the vulnerability of *E. macrurus* to environmental conditions and the importance in protecting specific habitats along

the river bank and in the water, to enable females to nest, embryos to successfully hatch, hatchlings to survive, and juveniles to attain reproductive age.

Visual inspection of the nesting sites preferred by female *E. macrurus* suggested that they select a particular combination of nesting characteristics, and these may result in the warmest available conditions for the incubation of the eggs. First, they prefer sandy soils, which have a high thermal conductivity, i.e. an increased capacity to conduct heat through the soil particles (De Vries 1963; Farouki 1981). Second, the females' preference for non-vegetated nesting sites resulted in the nests to be highly exposed to sunlight. Third, they prefer to nest upon northerly facing slopes, which are generally warmer than slopes facing westerly and southerly in the southern hemisphere. This is because the slope aspect designates both the duration and the angle that the sun rays come into contact with the ground. The greater the amount of radiation in a given surface area, the greater will be the temperature (Radcliffe and Lefever 1981; Rorison, Sutton and Hunt 1986). The same principle applies for regions in the northern hemispheres, where slopes with southern aspects are more exposed to solar radiation than those facing any other direction.

Site-selection based upon thermal conditions has been reported for other freshwater turtle species (Ehrenfeld 1979; Janzen 1994; Booth 2010). However, a large number of turtle species exhibit temperature dependent sex determination (TSD) and therefore, the site selection by the females may have a primary correlation to the offspring sex-ratio (Janzen 1994; Booth 2006). The sex of the *E. macrurus* offspring is determined genetically (Georges and McInnes 1998), and therefore, I hypothesised that the females selected warmer incubation conditions as a strategy to improve the phenotype of the hatchlings.

Prior to the empirical studies, it was firstly important to understand the thermal conditions that *E. macrurus* eggs experience in the wild. The laboratory thermal treatments in which the eggs of *E. macrurus* were incubated were based upon the overall mean and daily fluctuations in temperature recorded from the nests constructed by the females. The study showed that a constant thermal regime of only 1ºC greater than the mean incubation temperature recorded in the wild was detrimental for the offspring. This incubation temperature caused high embryo mortality, and smaller and weaker hatchlings (Chapter 3; Micheli-Campbell *et al.* 2011). Furthermore, large fluctuations in daily temperature fluctuation, which were experienced by *E. macrurus* eggs in the wild at ~ 5 % of the time, induced in the laboratory a 95 % mortality of the embryos (Chapter 4;

Micheli-Campbell *et al.* 2012). The results from these experiments were contradictory to the study hypothesis, and demonstrated the complexity between nest-site selection, natural incubation conditions, and hatchling phenotype. The findings suggest however, that *E. macrurus* eggs may be reaching their upper thermal limit in the wild.

Global climatic conditions are changing more rapidly than ever before (Buentgen *et al.* 2011). Australia as a continent is getting dryer and warmer (CSIRO 2007). The mean annual temperature for the Mary River catchment area has risen by 0.4ºC over the past ten years (Queensland Government 2009). This rise in ambient temperature may be the reason why *E. macrurus* nests are approaching the upper thermal limits for the eggs. It seems paradoxical that females would actively select warmer nest-sites under these conditions. A possible explanation may be that the nesting preferences exhibited by *E. macrurus* (Chapter 2) were an evolutionary strategy to improve offspring phenotype (Janzen and Morjan 2002; Booth *et al.* 2004; Hughes and Brooks 2006) and the female *E. macrurus* exhibit natal homing fidelity. Thus, returning to the same location to lay their offspring where they hatched themselves. This phenomenon has been previously reported for both marine and freshwater turtles (Freedberg *et al.* 2005; Lohmann *et al.* 2006; Bowen and Karl 2007; Sheridan *et al.* 2010), and further investigations may explain if the particular nest-site preferences shown by female *E. macrurus* are driven by natal-site fidelity. The preference for warm nesting areas may also be an innate behaviour and *E. macrurus* has a low capacity for behavioural plasticity. Climate models for the Mary River catchment area predict a substantial rise in mean ambient temperature over the next 60 years (CSIRO 2007; Queensland Government 2009). Under such scenarios, the thermal conditions to which the eggs of *E. macrurus* are incubated in their natural environment may also significantly increase. *Elusor macrurus*, as most freshwater turtles, lay shallow nests, which are largely exposed to changes in ambient conditions. Therefore, warmer ambient temperatures due to climate change may seriously compromise the survival of the hatchling and juvenile *E. macrurus* in the wild, and is likely to impact upon the population recruitment.

Investigating the habitat requirements of aquatic animals is often challenging. When the study animal is small, cryptic and shy this can be even more difficult. Juvenile freshwater turtles show all these characteristics, plus inhabit a low-visibility and refuge rich-environment, which are likely to be the reason behind the lack of scientific information regarding their habitat requirements. Such information is however, undoubtedly important for protecting the habitat and ensuring juvenile survival. Identification of critical habitat for hatchling and juvenile *E. macrurus*

was achieved in this study, by populating a presence-only predictive model with location data collected by miniaturised telemetry technology (Chapter 5). The models showed the turtles to inhabit a very narrow range of physical characteristics compared to those available in the river. They preferred areas either upstream or downstream from riffle zones and at the vicinities of the river margins where the water was slow flowing and not very deep. The niche selected by juveniles is often very different from the adults, and it must provide protection from the predators and changes in the physical environment, as well as provide food.

Juvenile freshwater turtles are small and have no parental care, and therefore spend most of their time seeking the protection of the bottom of the river, often under logs and rocks. They do however; need to leave their shelters and travel to the water surface to breathe. Each surfacing event increases their exposure to a host of water and avian predators (Kramer 1988; Heithaus and Frid 2003). The selection of juvenile *E. macrurus* for shallow areas reduces the distance, and therefore the time they would be exposed to predators whilst surfacing to breath. The preference of juvenile *E. macrurus* for areas with slow flowing water would make surfacing easier and less energetically costly, and may therefore also reduce the risk of predation. The proximity to riffle zones and river margins provided a combination of shelter and food source, because these areas have a high concentration of large rocks and submerged logs, and therefore good habitat for aquatic invertebrates, a prey type juvenile *E. macrurus* are known to favour (Flakus 2002). The very narrow range of habitat characteristics, compared to those available in the environment, selected by juvenile *E. macrurus* is likely to be a survival strategy.

Alteration of stream velocity, water depth, temperature, and dissolved oxygen often occur in riverine environments and may result in a loss of the restricted preferred habitats for the juveniles (Allan and Flecker 1993; March *et al.* 2003; Stickler *et al.* 2008; Welch *et al.* 2008; Clark, Gordos and Franklin 2009). In order to aid the maintenance of *E. macrurus* population, the protection of these distinct areas must be incorporated into future conservation acts and management plans.

Implications for turtle management and conservation

The present study demonstrated certain aspects of *Elusor macrurus* ecology that has implications for management and conservation. First, because they exhibit nest-site fidelity to a

few choice river banks (Chapter 2), the protection of these localities is important. Impacts upon these river banks are: sand mining, trampling by livestock, invasive weeds, and because these banks are created by sand deposition during flood events, alteration in the river flood cycle due to damming is likely to be detrimental to these areas. Second, increasing ambient temperatures due to climate change is likely to be detrimental to hatchling phenotype (Chapters 3 and 4), but the management actions for a changing climate are complex with no effective internationally coordinated solution yet in place. Third, juveniles were extremely specific in their habitat selection (Chapter 5). These habitats were formed by a combination of physical characteristics and their occurrence would be severely impacted by river impoundments. Thus, the presence of non-vegetated sandy banks and riffle zones is vital in supporting *E. macrurus* population recruitment. The preservation of these areas needs to be considered in any future management plans for the Mary River.

During this three-year Ph.D. study, a number of methodologies were developed that could be utilised for the study of other freshwater turtle species. The combination of infra-red surveillance cameras deployed on the nesting banks and digitalised image-analysis of the photographs proved a non-invasive and successful technique to identify individuals and monitor their behaviour. I did not capture and mark any of the turtles in this study, but suggest that estimates for nesting females could be determined through mark and recapture statistics, such as the Jolly-Seber method (Jolly 1965). Acoustic telemetry also proved a valuable technique for monitoring the behaviour and habitat selection of hatchling *E. macrurus*. Acoustic telemetry has previously been utilised to investigate the movement patterns of marine turtles (Taquet *et al.* 2006; Scales *et al.* 2011), but for the first time this technology was successfully used to study the habitat requirements of a species of freshwater turtle. The attachment of miniaturised transmitters did not disrupt the normal activities of the young turtles. The data was used to populate a presence-only predictive model, in order to compensate for the false-absences, which are inherent of telemetry studies. This model compared the characteristics of the locality selected by the juveniles against those available in the background environment. This model was efficient for identifying habitat selection at the fine-scale, so was particularly relevant to freshwater turtles that have limited ability for locomotion and inhabit very dynamic environments over temporal and spatial scales.

The population of *E. macrurus* is depleted. In the past, captive breeding programs were considered a viable strategy to increase the recruitment of the populations of threatened species

(Williams and Osentoski 2007; Robert 2009). In few cases, this approach has been successful, but it should be considered only as a last resort. This is because common problems are caused by deleterious genetic changes originated from the captive environment, such as heritable epigenetic effects, inbreeding among relatives and introduction of diseases (see Frankham, Ballou and Briscoe 2002). In the case of *E. macrurus*, the release of captive-bred hatchings into the river should be avoided. There are still a consistent number of nesting females in the wild; hundreds, if not thousands, of eggs are laid, and hundreds of hatchling *E. macrurus* have been recorded entering the river every year. Resources available for conservation would be better invested upon the protection of the clutches and of the nesting areas to ensure that hatchlings from the wild population enter the river each year. The protection of the river itself and, more specifically, the critical habitat for the juveniles is equally important and must be protected to ensure that the juveniles achieve reproductive age.

Directions for future research

There are a number of specific areas within this thesis where further investigation would lead to improved study conclusions. Specific criteria have been discussed in the appropriate chapters, and here I wish to highlight broad-scale approaches for future research. These are: 1/ collaboration and the sharing of data between researchers specialising in different disciplines; 2/ greater integration between controlled empirical studies and field observations; and 3/ monitoring of turtle ecology and associated environmental variables over multiple years.

1. Cross-discipline collaboration

In modern day science, it is becoming impossible for a research group to be able to accurately measure and synthesise data from all aspects of an ecosystem. A productive alternative that is gaining momentum is to combine the knowledge-base and skills from experts in their relative disciplines, to create a more complete picture of the relationship between an animal and its environment. In Chapter 2, some of the research was undertaken in collaboration with geologists, whom directed me in the characterisation of the soil collected from river banks. In Chapter 5, four physical river parameters were measured to identify the critical habitat of juvenile *E. macrurus*. Although I have measured environmental characteristics of the river to the best of my abilities, improved spatial and temporal resolution of the data could have been achieved

relatively easily with the correct, yet expensive, equipment. These were not available to me during my Ph.D. and therefore, I recommend that investigators, whom wish to model the critical habitat of riverine animals, to collaborate with hydrologists or limnologists in order to provide better environmental data to populate the relative models. Furthermore, genetic studies could also provide a more complete picture of population processes when combined with animal movement information.

2. Integration between laboratory and field studies

In order to predict how changes in environmental conditions could influence freshwater turtle populations it is crucial to understand what happens in the field. However, observed correlations in field data do not necessarily mean a direct cause and effect relationship, and empirical studies can reveal the mechanisms involved. For example, there are numerous studies that have investigated the influences of incubation temperature upon turtle embryo development and offspring phenotype. The findings from many of these studies however, have little conservation significance as they have not been compared to the conditions that the animals would be subjected to in the wild (as discussed in Chapter 4). In the present study, *E. macrurus* embryos were exposed to thermal conditions that were based upon actual nest temperatures recorded in the field. I was therefore able to conclude that if predicted rises in ambient temperature do occur in the Mary River catchment area, as a result of global warming, the phenotype of the hatchling *E. macrurus* will be compromised in way that may impact upon the population recruitment. I therefore recommend the ambient conditions to which the turtle embryos are likely to be exposed to in the wild to be monitored before manipulative laboratory studies are conducted. Using this methodology, relevant and realistic laboratory treatments can be trialled and the data generated have significance for the management and conservation.

3. Multiple years studies

Riverine environments are highly dynamic and complex ecosystems. Temporal changes in ambient conditions occur daily, seasonally and extra-annually in most riverine systems, but can be particularly extreme in Australia. The continental weather is influenced by the El Niño and La Niña quasiperiodic climate patterns, resulting in years of drought followed by years of seasonal heavy rain and flooding. Monitoring river characteristics and the ecology of freshwater turtles over a single year may reveal an incomplete description and lead to inaccurate conclusions.

Monitoring of the environment and turtle ecology over multiple years will compensate for inter-year variability and result in a more complete picture of how the turtle population is responding to changes in environmental conditions.

In Chapters 3 and 4, *E. macrurus* eggs were subjected to experimental manipulation of temperature during the incubation period. The experimental thermal regimes were based upon nest data collected from only a single nesting season (2009). Ambient air temperature data collected by the Australian Bureau of Meteorology (2012) showed that the mean maximum temperature in 2009 was 1.5ºC warmer than for the same period in 2010 and 2011. If nest temperatures had been measured in these years, then the daily variation in temperature may have been slightly different, and resulted in the selection of different thermal regimes for the experimental studies. The limited time of a Ph.D. study restricted however, the collection of nesting temperature over multiple years, but I certainly recommend that this be undertaken in future investigations into the effects of nesting temperature upon freshwater turtles.

Conclusions

This thesis combined laboratory and field-based studies to investigate the nesting biology and habitat requirements of an endangered species of freshwater turtle. This integration of physiological and ecological approaches was undertaken with the primary goal of generating scientifically sound information that would aid in on-going management and conservation of the dwindling *Elusor macrurus* population. I believe that this thesis makes a significant contribution to the biology of *E. macrurus*, and all study findings have been submitted for publication in peer-reviewed scientific journals. Whilst undertaking this work, I developed a number of novel techniques and methodologies, and I anticipate that these will be valuable in the investigation of turtle habitat requirements, particularly for hatchlings and juveniles. Such information is urgently required for the conservation and recovery of declining turtle populations worldwide.

References

Ackerman, R.A. (1991). Physical factors affecting the water exchange of buried reptile eggs. In: *Egg incubation : its effects on embryonic development in birds and reptiles*: 193-211. D. C. Deeming and M. W. J. Ferguson (Eds.). Cambridge University Press, Cambridge, Melboune.

Ackerman, R.A., Seagrave, R.C., Dmi'el, R., and Ar, A. (1985). Water and heat exchange between parchment shelled reptile eggs and their surroundings. *Copeia,* **1985:** 703-711.

Afonso, P., Fontes, J., Guedes, R., Tempera, F., Holland, K.N., and Santos, R.S. (2009). A multi-scale study of Red Porgy movements and habitat use, and its application to the design of marine reserve networks. *Tagging and Tracking of Marine Animals with Electronic Devices*, **9**: 423-443.

Allan, J.D., and Flecker, A.S. (1993). Biodiversity conservation in running waters. *Bioscience,* **43:** 32-43.

Armstrong, J.D., and Nislow, K.H. (2006). Critical habitat during the transition from maternal provisioning in freshwater fish, with emphasis on Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*). *Journal of Zoology,* **269:** 403-413.

Ashmore, G.M., and Janzen, F.J. (2003). Phenotypic variation in smooth softshell turtles (*Apalone mutica*) from eggs incubated in constant versus fluctuating temperatures. *Oecologia,* **134,** 182-188.

Australian Greenhouse Office (2003). *Climate change: An Australian guide to the science and potential impacts*. Commonwealth of Australia, Camberra, ACT.

Baillie, J.E.M., Hilton-Taylor, C., and Stuart, S. N. (2004). *2004 IUCN Red List of threatened species. A global species assessment*. Gland, Switzerland and Cambridge: IUCN.

Balmford, A., Green, R.E., and Jenkins, M. (2003). Measuring the changing state of nature. *Trends in Ecology & Evolution,* **18:** 326-330.

Barry, P.M., Carline, R.F., Argent, D.G., and Kimmel, W.G. (2007). Movement and habitat use of stocked juvenile paddlefish in the Ohio River system, Pennsylvania. *North American Journal of Fisheries Management*, **27:** 1316-1325.

Basille, M., Calenge, C., Marboutin, E., Andersen, R., and Gaillard, J.M. (2008). Assessing habitat selection using multivariate statistics: Some refinements of the Ecological-Niche Factor Analysis. *Ecological Modelling,* **211**: 233-240.

Birchard, G.F., and Reiber, C.L. (1996). Heart rate during development in the turtle embryo: Effect of temperature. *Journal of Comparative Physiology B – Biochemical, Systemic and Environmental Physiology,* **166:** 461-466.

Bobyn, M.L., and Brooks, R.J. (1994a). Incubation conditions as potential factors limiting the northern distribution of snapping turtles, *Chelydra serpentina. Canadian Journal of Zoology – Revue Canadienne de Zoologie* **72:** 28-37.

Bobyn, M.L., and Brooks, R.J. (1994b). Inter-clutch and inter-population variation in the effects of incubation conditions on sex, survival and growth of hatchling turtles (*Chelydra serpentina*). *Journal of Zoology* **233:** 233-257.

Bodie, J.R. (2001) Stream and riparian management for freshwater turtles. *Journal of Environmental Management,* **62:** 443-455.

Bonach, K., Lewinger, J.F., Silva, A.P., and Verdade, L.M. (2007). Physical characteristics of Giant Amazon Turtle (*Podocnemis expansa*) nests. *Chelonian Conservation and Biology*, **6**: 252-255.

Booth, D.T. (2000). Incubation of eggs of the Australian broad-shelled turtle, *Chelodina expansa* (Testudinata: Chelidae), at different temperatures: Effects on pattern of oxygen consumption and hatchling morphology. *Australian Journal of Zoology,* **48:** 369-378.

Booth, D.T. (2002). Incubation of rigid-shelled turtle eggs: do hydric conditions matter? *Journal of Comparative Physiology B – Biochemical, Systemic and Environmental Physiology,* **172,** 627-633.

Booth, D.T. (2006). Influence of incubation temperature on hatchling phenotype in Reptiles *Physiological and Biochemical Zoology,* **79:** 274-281.

Booth, D.T. (2010) The natural history of nesting in two Australian freshwater turtles. *Australian Zoologist,* **35:** 198-203.

Booth, D.T., and Astill, K. (2001). Temperature variation within and between nests of the green sea turtle, *Chelonia mydas* (Chelonia: Cheloniidae) on Heron Island, Great Barrier Reef. *Australian Journal of Zoology,* **49:** 71-84.

Booth, D.T. and Yu, C.Y. (2009). Influence of the hydric environment on water exchange and hatchlings of rigid-shelled turtle eggs. *Physiological and Biochemical Zoology,* **82,** 382-387.

Booth, D.T., Burgess, E.A., McCosker, J., and Lanyon, J.M. (2004). The influence of incubation temperature on post-hatching fitness characteristics of turtles. *International Congress Series,* **1275:** 226-233.

Bowen, B.W., and Karl, S.A. (2007). Population genetics and phylogeography of sea turtles. *Molecular Ecology*, **16**: 4886-4907.

Brooks, R.J., Bobyn, M.L., Galbraith, D.A., Layfield J.A., and Nancekivell, E.G. (1991). Maternal and environmental influences on growth and survival of embryonic and hatchling snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology – Revue Canadienne De Zoologie,* **69:** 2667-2676.

Buentgen, U., Tegel, W., Nicolussi, K., McCormick, M., Frank, D., Trouet, V., Kaplan, J.O., Herzig, F., Heussner, K.U., Wanner, H., Luterbacher, J., and Esper, J. (2011). 2500 years of European climate variability and human susceptibility. *Science*, **331**: 578-582.

Burger, J. (1976). Behavior of hatchling diamondback terrapins (*Malaclemys terrapin*) in the field. *Copeia*, **1976:** 742-748.

Burger, J., Zappalorti, R.T., and Gochfeld, M. (1987). Developmental effects of incubation temperature on hatchling pine snakes *Pituophis melanoleucus*. *Comparative Biochemistry and Physiology A – Physiology,* **87:** 727-732.

Burgess, E.A., Booth, D.T., and Lanyon, J.M. (2006). Swimming performance of hatchling green turtles is affected by incubation temperature. *Coral Reefs,* **25:** 341-349.

Burggren, W.W., and Shelton, G. (1979). Gas exchange and transport during intermittent breathing in Chelonian Reptiles. *Journal of Experimental Biology,* **82:** 75-92.

Calenge, C. (2006). The package "adehabitat" for the R software: A tool for the analysis of space and habitat use by animals. *Ecological Modelling,* **197:** 516-519.

Calenge, C., Darmon, G., Basille, M., Loison, A., and Jullien, J.M. (2008). The factorial decomposition of the Mahalanobis distances in habitat selection studies. *Ecology,* **89**: 555-566.

Campbell, H.A., Watts, M.E., Sullivan, S., Read, M.A., Choukroun, S., Irwin, S.R., and Franklin, C.E. (2010). Estuarine crocodiles ride surface currents to facilitate long-distance travel. *Journal of Animal Ecology,* **79:** 955-964.

Campbell, H.A., Hewitt, M., Watts, M.E., Peverell, S., and Franklin, C.E. (2012). Short- and long-term movement patterns in the freshwater whipray (*Himantura dalyensis*) determined by the signal processing of passive acoustic telemetry data. *Marine and Freshwater Research,* **63**: 341-350.

Cann, J. (1998). *Australian freshwater turtles*. John Cann & Beaumont Publishing Pte Ltd, Singapore.

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**: 81-96.

Chazdon, R.L. (2008). Beyond deforestation: Restoring forests and ecosystem services on degraded lands. *Science,* **320:** 1458-1460.

Choo, B.L., and Chou, L. M. (1987). Effect of temperature on the incubation period and hatchling ability of *Trionyx sinensis* Weigmann eggs. *Journal of Herpetology,* **21:** 230-232.

Chown, S.L. (2012) Trait-based approaches to conservation physiology: forecasting environmental change risks from the bottom up. *Philosophical Transactions of the Royal Society: Biological Sciences,* **367:** 1615-1627.

Clark, D.B., and Gibbons, J. W. (1969). Dietary shift in turtle *Pseudemys scripta* (Schoepff) from youth to maturity. *Copeia,* **1969:** 704-706.

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:** 399-406.

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:** 301-309.

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:** 147-154.

Commonwealth Scientific and Industrial Research Organisation – CSIRO & Bureau of Meteorology – BoM (2007). *Climate Change in Australia: Technical Report 2007*, CSIRO, Melbourne, VIC, Australia.

Commonwealth Scientific and Industrial Research Organisation – CSIRO (2007). Regional climate change projections (2007). CSIRO, Melbourne, VIC, Australia.

Congdon, J.D., and Gibbons, J.W. (1985). Egg components and reproductive characteristics of turtles – Relationships to body size. *Herpetologica,* **41,** 194-205.

Congdon, J.D., Tinkle, D.W., Breitenbach, G.L., and Sels, R.C.V. (1983). Nesting ecology and hatchingsuccess in the turtle *Emydoidea blandingi*. *Herpetologica*, **39**: 417-429.

Congdon, J.D., Nagle, R.D., Dunham, A.E., Beck, C.W., Kinney, O.M., and Yeomans, S.R. (1999). The relationship of body size to survivorship of hatchling snapping turtles (*Chelydra serpentina*): An evaluation of the "bigger is better"' hypothesis. *Oecologia*, **121:** 224-235.

Cooke, S.J., Hinch, S.G., Farrell, A.P., Patterson, D.A., Miller-Saunders, K., Welch, D.W., Donaldson, M.R., Hanson, K.C., Crossin, G.T., Mathes, M.T., Lotto, A.G., Hruska, K.A., Olsson, I.C., Wagner, G.N., Thomson, R., Hourston, R., English, K.K., Larsson, S., Shrimpton, J.M., and Van der Kraak, G. (2008). Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult fraser river sockeys salmon. *Fisheries*, **33:** 321-338.

Cooke, S.J., Hinch, S.G., Donaldson, M.R., Clark, T.D., Eliason, E.J., Crossin, G.T., Raby, G.D., Jeffries, K.M., Lapointe, M., Miller, K., Patterson, D.A., and Farrell, A.P. (2012). Conservation physiology in practice: how physiological knowledge has improved our ability to sustainably manage Pacific salmon during up-river migration. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **367:** 1757-1769.

De Vries, D.A. (1963). Thermal properties of soils. In: *Physics of Plant Environment*: 210-235. W.R.V. Wijk (Ed.). North-Holland Publishing Co., Amsterdam.

Deeming, D.C. (2004). Post-hatching phenotypic effects of incubation in reptiles. In: *Reptilian Incubation Environment, Evolution and Behaviour*: 211-228. D.C. Deeming (Ed.). Nottingham University Press, Nottingham.

Deeming, D.C., and Ferguson, M.W.J. (1988). Environmental-regulation of sex determination in Reptiles. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, **322:** 19-39.

Deeming, D.C., and Ferguson, M.W.J. (1989). The mechanism of temperature-dependent sex determination in crocodilians – A hypothesis. *American Zoologist* **29:** 973-985.

Deeming, D.C., and Ferguson, M.W.J. (1991). Physiological effects of incubation temperature on embryonic development in reptiles and birds. In: *Egg Incubation: Its Effects on Embryonic development in birds and reptiles*: 147-171. D.C. Deeming & M.W.J. Ferguson (Eds.). Cambridge University Press, Cambridge.

Delmas, V., Baudry, E., Girondot M., and Prevot-Julliard, A.C. (2007). The righting response as a fitness index in freshwater turtles. *Biological Journal of the Linnean Society*, **91:** 99-109.

Demuth, J.P. (2001). The effects of constant and fluctuating incubation temperatures on sex determination, growth, and performance in the tortoise *Gopherus polyphemus. Canadian Journal of Zoology – Revue Canadienne de Zoologie* **79:** 1609-1620.

Dettki, H., Lofstrand, R., and Edenius, L. (2003). Modelling habitat suitability for moose in coastal northern Sweden: empirical vs. process-oriented approaches. *Ambio*, **32:** 549-556.

Donovan, T.M., Freeman, M., Abouelezz, H., Royar, K., Howard, A., and Mickey, R. (2011). Quantifying home range habitat requirements for bobcats (*Lynx rufus*) in Vermont, USA. *Biological Conservation*, **144**: 2799-2809.

Du, W., and Ji, X. (2003). The effects of incubation thermal environments on size, locomotor performance and early growth of hatchling soft-shelled turtles, *Pelodiscus sinensis. Journal of Thermal Biology*, **28:** 279-286.

Du, W.G., Zheng, R.Q., and Shu, L. (2006). The influence of incubation temperature on morphology, locomotor performance, and cold tolerance of hatchling chinese three-keeled pond turtles, *Chinemys reevesii*. *Chelonian Conservation and Biology*, **5**: 294-299.

Du, W., Hu, L., Lu J., and Zhu, L. (2007). Effects of incubation temperature on embryonic development rate, sex ratio and post-hatching growth in the Chinese three-keeled pond turtle, *Chinemys reevesii*. *Aquaculture*, **272:** 747-753.

Du, W.G., Shen, J.W., and Wang, L. (2009). Embryonic development rate and hatchling phenotypes in the Chinese three-keeled pond turtle (*Chinemys reevesii*): The influence of fluctuating temperature versus constant temperature. *Journal of Thermal Biology*, **34:** 250-255.

Dwyer, Ross.G., Watts, M., Campbell, H.A. & Franklin, C.E. (2012). V-Track: An R package for analysing and visualising data generated from the VEMCO suite of passive and active acoustic receivers. In: *R package version 0.94*. http://www.uq.edu.au/eco-lab/v-track.

Ehrenfeld, D.W. (1979). Behavior associated with nesting. In: *Turtles. Perspectives & Research:* 417-434. M. Harless and H. Morlock (Eds.). John Wiley & Sons, New York.

Elnitsky, M.A., and Claussen, D.L. (2006). The effects of temperature and inter-individual variation on the locomotor performance of juvenile turtles. *Journal of Comparative Physiology B – Biochemical, Systemic and Environmental Physiology*, **176:** 497-504.

Ewert, M.A. (1979). The embryo and its egg: development and natural history. In: *Turtles: perspectives and research*: 333-413. M. Harless and M. Morlock (Eds.). John Wiley & Sons, New York.

Ewert, M.A., and Nelson, C.E. (1991). Sex determination in turtles: diverse patterns and some possible adaptive values. *Copeia*, **1991:** 50-69.

Farouki, O.T. (1981). The thermal properties of soils in cold regions. *Cold Regions Science and Technology*, **5**: 67-75.

Ferguson, M.W.J. (1985). Reproductive biology and embryology of the crocodilians. In: *Biology of the reptilia*: 329-491. C. Gans, F. Billett and P.F.A. Maderson (Eds.). John Wiley and Sons, New York.

Ficetola, G.F. (2007). The influence of beach features on nesting of the hawksbill turtle *Eretmochelys imbricata* in the Arabian Gulf. *Oryx*, **41**: 402-405.

Finkler, M.S. (1999). Influence of water availability during incubation on hatchling size, body composition, desiccation tolerance, and terrestrial locomotor performance in the snapping turtle *Chelydra serpentina*. *Physiological and Biochemical Zoology*, **72**: 714-722.

Flakus, S.P. (2002). Ecology of the Mary River Turtle, *Elusor macrurus*. Master of Sciences Thesis (Zoology), Department of Zoology and Entomology, The University of Queensland, Brisbane, QLD, Australia.

Flakus, S.P., and Connell, M. (2008). The Mary River turtle: Yesterday, today, tomorrow... Tiaro & District Landcare Group, Toowoomba, QLD, Australia.

Frankham, R., Ballou, J.D., and Briscoe, D.A. (2002). *Introduction to Conservation Genetics*. Cambridge University Press, Cambridge.

Freedberg, S., Ewert, M.A., Ridenhour, B.J., Neiman, M., and Nelson, C.E. (2005). Nesting fidelity and molecular evidence for natal homing in the freshwater turtle, *Graptemys kohnii*. *Proceedings of the Royal Society B: Biological Sciences*, **272**: 1345-1350.

Freedberg, S., Stumpf, A.L., Ewert, M.A., and Nelson, C.E. (2004). Developmental environment has long-lasting effects on behavioural performance in two turtles with environmental sex determination. *Evolutionary Ecology Research*, **6:** 739-747.

Gallego, D., Canovas, F., Esteve, M.A., Galian, J., and Schwerdtfeger, F. (2004). Descriptive biogeography of Tomicus (Coleoptera: Scolytidae) species in Spain. *Journal of Biogeography*, **31**: 2011-2024.

Galparsoro, I., Borja, A., Bald, J., Liria, P., and Chust, G. (2009) Predicting suitable habitat for the European lobster (*Homarus gammarus*), on the Basque continental shelf (Bay of Biscay), using Ecological-Niche Factor Analysis. *Ecological Modelling*, **220**: 556-567.

Geiger, R. (1965). *The climate near the ground*, rev. ed. (Translated by Scripta Technica, Inc.). Harvard University Press, Cambridge.

Georges, A. (1983). Reproduction of the Australian freshwater turtle *Emydura krefftii* (Chelonia, Chelidae). *Journal of Zoology*, **201:** 331-350.

Georges, A., and McInnes, S. (1998). Temperature fails to influence hatchling sex in another genus and species of Chelid turtle, *Elusor macrurus*. *Journal of Herpetology*, **32:** 596-598.

Goode, J., and Russell, J. (1968). Iucubation of eggs of three species of Chelid tortoises and notes on their embryological development. *Australian Journal of Zoology*, **16**: 749-761.

Greene, C.H., Block, B.A., Welch, D., Jackson, G., Lawson, G.H., and Rechisky, E.L. (2009). Advances in conservation oeanography new tagging and tracking technologies and their potential for transforming the science underlying fisheries management. *Oceanography*, **22**: 210-223.

Guderley, H., and Seebacher, F. (2011). Thermal acclimation, mitochondrial capacities and organ metabolic profiles in a reptile (*Alligator mississippiensis*). *Journal of Comparative Physiology B – Biochemical, Systemic and Environmental Physiology*, **181:** 53-64.

Guisan, A., and Zimmermann, N.E. (2000). Predictive habitat distribution models in ecology. *Ecological Modelling*, **135**: 147-186.

Gutzke, W.H.N., Packard, G.C., Packard, M.J., and Boardman, T.J. (1987). Influence of the hydric and thermal environments on eggs and hatchlings of painted turtles (*Chrysemys picta*). *Herpetologica*, **43:** 393-404.

Hart, D.R. (1983). Dietary and habitat shift with size of red-eared turtles (*Pseudemys scripta*) in southern Louisiana population. *Herpetologica*, **39:** 285-290.

Haskell, A., Graham, T.E., Griffin, C.R., and Hestbeck, J.B. (1996). Size related survival of headstarted redbelly turtles (*Pseudemys rubriventris*) in Massachusetts. *Journal of Herpetology*, **30:** 524-527.

Heithaus, M.R., and Frid, A. (2003). Optimal diving under the risk of predation. *Journal of Theoretical Biology*, **223:** 79-92.

Heupel, M.R., Reiss, K.L., Yeiser, B.G., and Simpfendorfer, C.A. (2008). Effects of biofouling on performance of moored data logging acoustic receivers. *Limnology and Oceanography-Methods*, **6**: 327-335.

Hicks, J.W., and Wang, T. (2004). Hypometabolism in reptiles: behavioural and physiological mechanisms that reduce aerobic demands. *Respiratory Physiology & Neurobiology*, **141:** 261-271.

Hirzel, A.H., Hausser, J., Chessel, D., and Perrin, N. (2002). Ecological-Niche Factor Analysis: How to compute habitat-suitability maps without absence data? *Ecology*, **83:** 2027-2036.

Hirzel, A.H., Posse, B., Oggier, P.A., Crettenand, Y., Glenz, C., and Arlettaz, R. (2004). Ecological requirements of reintroduced species and the implications for release policy: the case of the bearded vulture. *Journal of Applied Ecology*, **41**: 1103-1116.

Hoffmann, M., Hilton-Taylor, C., Angulo, A., Bohm, M., Brooks, T.M., Butchart, S.H.M., Carpenter, K.E., Chanson, J., Collen, B., Cox, N.A., Darwall, W.R.T., Dulvy, N.K., Harrison, L.R., Katariya, V., Pollock, C.M., Quader, S., Richman, N.I., Rodrigues, A.S.L., Tognelli, M.F., Vie, J.C., Aguiar, J.M., Allen, D.J., Allen, G.R., Amori, G.,

Ananjeva, N.B., Andreone, F., Andrew, P., Ortiz, A.L.A., Baillie, J.E.M., Baldi, R., Bell, B.D., Biju, S.D., Bird, J.P., Black-Decima, P., Blanc, J.J., Bolanos, F., Bolivar, W., Burfield, I.J., Burton, J.A. Capper, D.R., Castro, F., Catullo, G., Cavanagh, R.D., Channing, A., Chao, N.L., Chenery, A.M., Chiozza, F., Clausnitzer, V., Collar, N.J., Collett , L.C., Collett e, B.B., Cortez Fernandez, C.F., Craig, M.T., Crosby, M.J., Cumberlidge, N., Cutt elod, A., Derocher, A.E., Diesmos, A.C., Donaldson, J.S., Duckworth, J.W., Dutson, G., Dutt a, S.K., Emslie, R.H., Farjon, A., Fowler, S., Freyhof, J., Garshelis, D.L., Gerlach, J., Gower, D.J., Grant, T.D., Hammerson, G.A., Harris, R.B., Heaney, L.R., Hedges, S.B., Hero, J.-M., Hughes, B., Hussain, S.A., Icochea M., J., Inger, R.F., Ishii, N., Iskandar, D.T., Jenkins, R.K.B., Kaneko, Y., Kott elat, M., Kovacs, K.M., Kuzmin, S.L., Marca, E.L., Lamoreux, J.F., Lau, M.W.N., Lavilla, E.O., Leus, K., Lewison, R.L., Lichtenstein, G., Livingstone, S.R., Lukoschek, V., Mallon, D.P., McGowan, P.J.K., McIvor, A., Moehlman, P.D., Molur, S., Alonso, A.M., Musick, J.A., Nowell, K., Nussbaum, R.A., Olech, W., Orlov, N.L., Papenfuss, T.J., Parra-Olea, G., Perrin, W.F., Polidoro, B.A., Pourkazemi, M., Racey, P.A., Ragle, J.S., Ram, M., Rathbun, G., Reynolds, R.P., Rhodin, A.G.J., Richards, S.J., Rodríguez, L.O., Ron, S.R., Rondinini, C., Rylands, A.B., Sadovy de Mitc heson, Y., Sanciangco, J.C., Sanders, K.L., Santos-Barrera, G., Schipp er, J., Self-Sullivan, C., Shi, Y., Shoemaker, A., Short, F.T., Sillero-Zubiri, C., Silvano, D.L., Smith, K.G., Smith, A.T., Snoeks, J., Stattersfield, A.J., Symes, A.J., Taber, A.B., Talukdar, B.K., Temple, H.J., Timmins, R., Tobias, J.A., Tsytsulina, K., Tweddle, D., Ubeda, C., Valenti, S.V., van Dijk, P.P., Veiga, L.M., Veloso, A., Wege, D.C., Wilkinson, M., Williamson, E.A., Xie, F., Young, B.E., Akçakaya, H.R., Bennun, L., Blackburn, T.M., Boitani, L., Dublin, H.T., Fonseca, G.A.B. da, Gascon, C., Lacher, T.E., Jr., Mace, G.M., Mainka, S.A., McNeely, J.A., Mitt ermeier, R.A., Reid, G.M., Rodriguez, J.P., Rosenberg, A.A., Samways, M.J., Smart, J., Stein, B.A., and Stuart, S.N. (2010). The impact of conservation on the status of the world's vertebrates. *Science*, **330**: 1503-1509.

Huck, M., Jedrzejewski, W., Borowik, T., Milosz-Cielma, M., Schmidt, K., Jedrzejewska, B., Nowak, S., and Myslajek, R.W. (2010). Habitat suitability, corridors and dispersal barriers for large carnivores in poland. *Acta Theriologica,* **55:** 177-192.

Hughes, E.J., and Brooks, R.J. (2006). The good mother: Does nest-site selection constitute parental investment in turtles? *Canadian Journal of Zoology – Revue Canadienne de Zoologie*, **84**: 1545-1554.

Intergovernmental Panel on Climate Change, IPCC. (2007). *Climate change 2007: Synthesis report. Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change*. C.W. Team, R.K. Pachauri and A. Reisinger (Eds.). IPCC, Geneva, Switzerland.

International Union for Conservation of Nature, IUCN (2011). Tortoise & Freshwater Turtle Specialist Group 1996. In: *IUCN Red List of Threatened Species*.

Iverson, J.B., Brown, R.M., Akre, T.S., Near, T.J., Le, M., Thomson, R.C., and Starkey, D.E. (2007). In search of the tree of life for turtles. *Chelonian Research Monographs,* **4:** 85-106.

Jackson, D.R., and Walker, R.N. (1997). Reproduction in the Suwannee cooter, *Pseudemys concinna suwanniensis*. *Bulletin of the Florida Museum of Natural History,* **41**: 69-167.

Jammalamadaka, S.R., and SenGupta, A. (2001). *Topics in circular statistics*. World Scientific Press, Singapore.

Janzen, F.J. (1993). The influence of incubation temperature and family on eggs, embryos, and hatchlings of the Smooth Softshell turtle (*Apalone mutica*). *Physiological Zoology,* **66:** 349-373.

Janzen, F.J. (1994). Vegetational cover predicts the sex-ratio of hatchling turtles in natural nests. *Ecology,* **75:** 1593-1599.

Janzen, F.J., and Morjan, C.L. (2002). Egg size, incubation temperature, and posthatching growth in painted turtles (*Chrysemys picta*). *Journal of Herpetology*, **36**: 308-311.

Janzen, F.J., Packard, G.C., Packard, M.J., Boardman, T.J., and Zumbrunnen, J.R. (1990) Mobilization of lipid and protein by embryonic snapping turtles in wet and dry environments. *Journal of Experimental Zoology*, **255:** 155-162.

Janzen, F.J., Tucker, J.K., and Paukstis, G.L. (2000). Experimental analysis of an early life-history stage: Selection on size of hatchling turtes. *Ecology*, **81:** 2290-2304.

Ji, X., Chen, F., Du, W.G., and Chen, H.L. (2003). Incubation temperature affects hatchling growth but not sexual phenotype in the Chinese soft-shelled turtle, *Pelodiscus sinensis* (Trionychidae). *Journal of Zoology*, **261**: 409-416.

Johnson, A.L. (1996). The avian ovarian hierarchy: A balance between follicle differentiation and atresia. *Poultry and Avian Biology Reviews,* **7:** 99-110.

Jolly, G.M. (1965). Explicit estimates from capture-recapture data with both death and immigration-stochastic model. *Biometrika,* **52**: 225-&.

Kingsford, R.T. (2000). Ecological impacts of dams, water diversions and river management on floodplain wetlands in Australia. *Austral Ecology,* **25:** 109-127.

Kinney, J.L., Matsuura, D.T., and White, F.N. (1977). Cardiorespiratory effects of temperature in the turtle, *Pseudemys florida na*. *Respiration Physiology,* **31,** 309-325.

Kinney, M.J., and Simpfendorfer, C.A. (2009). Reassessing the value of nursery areas to shark conservation and management. *Conservation Letters*, **2**: 53-60.

Kolbe, J.J., and Janzen, F.J. (2001). The influence of propagule size and maternal nest-site selection on survival and behaviour of neonate turtles. *Functional Ecology*, **15:** 772-781.

Kolbe, J.J., and Janzen, F.J. (2002). Impact of nest-site selection on nest success and nest temperature in natural and disturbed habitats. *Ecology*, **83**: 269-281.

Kolbe, J.J., Janzen, F.J., and Gatten Jr, R.E. (2002). Experimental analysis of an early life-history stage: Water loss and migrating hatchling turtles. *Copeia*, **2002:** 220-226.

Koorevaar, P., Menelik, G., and Dirksen, C. (1983). *Elements of soil physics*. Elsevier, New York.

Kramer, D.L. (1988). The behavioral ecology of air breathing by aquatic animals. *Canadian Journal of Zoology – Revue Canadienne de Zoologie*, **66**: 89-94.

Legler, J.M. (1985) Australian chelid turtles: reproductive patterns in wide-ranging taxa. In: *Biology of Australian frogs and reptiles*: 117-123. G. Grigg, R. Shine and H. Ehmann (Eds.). Surrey Beatty & Sons, in association with The Royal Zoological Society of New South Wales, Sydney.

Legler, J.M., and Cann, J. (1980). *Rheodytes leukops* – New genus, new species of chelid turtle from Queensland, Australia. *Contributions in Science (Los Angeles)***,** 1-18.

Les, H.L., Paitz, R.T., and Bowden, R.M. (2007). Experimental test of the effects of fluctuating incubation temperatures on hatchling phenotype. *Journal of Experimental Zoology A – Ecological Genetics and Physiology*, **307A:** 274-280.

Les, H.L., Paitz, R.T., and Bowden, R.M. (2009). Living at extremes: development at the edges of viable temperature under constant and fluctuating conditions. *Physiological and. Biochemical Zoology*, **82:** 105-112.

Ligon, F.K., Dietrich, W.E., and Trush, W.J. (1995). Downstream ecological effects of dams. *Bioscience*, **45:** 183-192.

Lohmann, K.J., Horner, A.J., Akins, L., and Lohmann, C.M.F. (2006). Detection of coastal magnetic fields by sea turtles: a possible mechanism underlying natal homing. *Integrative and Comparative Biology*, **46**: E86-E86.

Loncke, D.J., and Obbarde, M.E. (1977). Tag success, dimensions, clutch size and nesting-site fidelity for snapping turtle, *Chelydra serpentina* (Reptilia, Testudines, Chelydridae) in Algonquin-Park, Ontario, Canada. *Journal of Herpetology*, **11**: 243-244.

Lucas, J.F.R., Tavares, M.H.F., Cardoso, D.L., and Cassaro, F.A.M. (2011). Soil-water retention curve by the filter paper method. *Revista Brasileira de Ciencia do Solo*, **35**: 1967-1973.

Magnusson, W.E. (1980). Habitat required for nesting by *Crocodylus porosus* (Reptilia, Crocodilidae) in Northern Australia. *Australian Wildlife Research*, **7**: 149-156.

March, J.G., Benstead, J.P., Pringle, C.M., and Scatena, F.N. (2003). Damming tropical island streams: Problems, solutions, and alternatives. *Bioscience,* **53:** 1069-1078.

Marchand, M.N., and Litvaitis, J.A. (2004). Effects of landscape composition, habitat features, and nest distribution on predation rates of simulated turtle nests. *Biological Conservation,* **117:** 243-251.

Marvin, G.A., and Lutterschmidt, W.I. (1997). Locomotor performance in juvenile and adult box turtles (*Terrapene carolina*): A reanalysis for effects of body size and extrinsic load using a terrestrial species. *Journal of Herpetology*, **31:** 582-586.

McArdle, B.H. (1990). When are rare species not there? *Oikos,* **57**: 276-277.

Metcalfe, J.D., Le Quesne, W.J.F., Cheung, W.W.L., and Righton, D.A. (2012). Conservation physiology for applied management of marine fish: an overview with perspectives on the role and value of telemetry. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **367:** 1746-1756.

Micheli-Campbell, M.A., Campbell, H.A., Cramp, R.L., Booth, D.T., and Franklin, C.E. (2011). Staying cool, keeping strong: incubation temperature affects performance in a freshwater turtle. *Journal of Zoology*, **285**: 266-273.

Micheli-Campbell, M.A., Gordos, M.A., Campbell, H.A., Booth, D.T., and Franklin, C.E. (2012) The influence of daily temperature fluctuations during incubation upon the phenotype of a freshwater turtle. *Journal of Zoology.* doi/10.1111/j.1469-7998.2012.00934.

Mickelson, L.E., and Downie, J.R. (2010). Influence of incubation temperature on morphology and locomotion performance of Leatherback (*Dermochelys coriacea*) hatchlings. *Canadian Journal of Zoology – Revue Canadienne de Zoologie,* **88:** 359-368.

Miller, J.D., and Dinkelacker, S.A. (2008). Reproductive structures and strategies of turtles. In: *Biology of turtles*: 225-278. J. Wyneken, M.H. Godfrey and V. Bels (Eds.). CRC Press, London.

Miller, K., Packard, G.C., and Packard, M.J. (1987) Hydric conditions during incubation influence locomotor performance of hatchling snapping turtles. *Journal of Experimental Biology*, **127:** 401-412.

Mogensen, S., and Post, J.R. (2012). Energy allocation strategy modifies growth-survival trade-offs in juvenile fish across ecological and environmental gradients. *Oecologia,* **168:** 923-933.

Moll, D. (1976). Food and feeding strategies of Ouachita map turtle (*Graptemys pseudogeographica ouachitensis*). *American Midland Naturalist*, **96:** 478-482.

Monk, J., Ierodiaconou, D., Bellgrove, A., Harvey, E., and Laurenson, L. (2011) Remotely sensed hydroacoustics and observation data for predicting fish habitat suitability. *Continental Shelf Research*, **31**: S17-S27.

Moret-Fernandez, D., Vicente, J., Latorre, B., Lera, F., Castaneda, C., Lopez, M.V., and Herrero, J. (2012). TDR pressure cell for monitoring water content retention and bulk electrical conductivity curves in undisturbed soil samples. *Hydrological Processes*, **26**: 246-254.

Mortimer, J.A. (1990). The influence of beach sand characteristics on the nesting-behavior and clutch survival of green turtles (*Chelonia mydas*). *Copeia,* **1990**: 802-817.

Myers, N., and Knoll, A.H. (2001). The biotic crisis and the future of evolution. *Proceedings of the National Academy of Sciences of the United States of America,* **98:** 5389-5392.

Olsen, J., Fuentes, E., and Rose, A.B. (2006). Trophic relationships between neighbouring white-bellied sea-eagles (*Haliaeetus leucogaster*) and wedge-tailed eagles (*Aquila audax*) breeding on rivers and dams near Canberra. *Emu,* **106:** 193-201.

Otto, S.R.L., and Alcaide, M. (2001). Utilization of the TDR-tensiometer method to obtain the soil water retention curve. *Revista Brasileira de Engenharia Agricola e Ambiental*, **5**: 265-269.

Packard, G.C. (1991). Physiological and ecological importance of water to embryos of oviparous reptiles. In: *Egg incubation: its effects on embryonic development in birds and reptiles*: 213-228. D.C. Deeming and M.W.J. Ferguson (Eds.). Cambridge University Press, Cambridge.

Packard, G.C. (1999). Water relations of Chelonian eggs and embryos: Is wetter better? *American Zoologist,* **39:** 289-303.

Packard, M.J., Packard, G.C., and Boardman, T.J. (1982). Structure of eggshells and water relations of reptilian eggs. *Herpetologica,* **38:** 136-155.

Packard, G.C., Packard, M.J., Miller, K., and Boardman, T.J. (1987). Influence of moisture, temperature and substrate on snaping turtle eggs and embryos. *Ecology*, **68:** 983-993.

Packard, G.C., Miller, K., Packard, M.J., and Birchard, G.F. (1999). Environmentally induced variation in body size and condition in hatchling snapping turtles (*Chelydra serpentina*). *Canadian Journal of Zoology – Revue Canadienne de Zoologie*, **77**: 278-289.

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

Paterson, J.E., Steinberg, B.D., and Litzgus, J.D. (2012). Revealing a cryptic life-history stage: differences in habitat selection and survivorship between hatchlings of two turtle species at risk (*Glyptemys insculpta* and *Emydoidea blandingii*). *Wildlife Research,* **39:** 408-418.

Perez, M., Collado, E., and Ramo, C. (1979). Crecimiento de *Mauremys caspica leprosa* (Schweigger, 1812) (Reptilia, Testudines) en la Reserva Biologica de Donana. *Donana Acta Vertebrata,* **6:** 161-178.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar D., and the R Development Core Team (2010). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3. 1-97.

Plummer, M.V., and Farrar, D.B. (1981). Sexual dietary differeces in a population of *Trionyx muticus*. *Journal of Herpetology,* **15:** 175-179.

Queensland Government (2009). *Climate change in the Wide Bay Burnett Region: Summary Report*, Brisbane, QLD, Australia.

R Development Core Team (2011). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Radcliffe, J.E., and Lefever, K.R. (1981). Aspect influences on pasture microclimate at Coopers Creek, North Canterbury. *New Zealand Journal of Agricultural Research*, **24**: 55-66.

Reagan, D.P. (1974). Habitat selection in the three-toed box turtle, *Terrapene carolina triunguis*. *Copeia,* **1974:** 512-527.

Reed, D.A., Porro, L.B., Iriarte-Diaz, J., Lemberg, J.B., Holliday, C.M., Anapol, F., and Ross, C.F. (2011). The impact of bone and suture material properties on mandibular function in *Alligator mississippiensis:* testing theoretical phenotypes with finite element analysis. *Journal of Anatomy*, **218:** 59-74.

Rhen, T., and Lang, J.W. (1995). Phenotypic plasticity for growth in the common snapping turtle – effects of incubation temperature, clutch, and their interaction. *American Naturalist,* **146,** 726-747.

Rhen, T., and Lang, J.W. (1999). Temperature during embryonic and juvenile development influences growth in hatchling snapping turtles, *Chelydra serpentina*. *Journal of Thermal Biology*, **24:** 33-41.

Rhodin, A.G.J., Van Dijk, P.P., Iverson, J.B. & Shaffer, H.B. (2010). Turtles of the world, 2010 update: Annoted checklist of taxonomy, synonymy, distribution, and conservation status. In: *Conservation biology of freshwater turtles and tortoises: A compilation project of the*

IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. (eds.) A.G.J. Rhodin, P.C.H. Pritchard, P.P. van Dijk, R.A. Saumure, K.A. Buhlmann, J.B. Iverson and R.A. Mittermeier *Chelonian Research Monographs,* **5**: 085-164.

Rhodin, A.G.J., Walde, A.D., Horne, B.D., Van Dijk, P.P., Blanck, T., and Hudson, R. (2011). *Turtles in trouble: The world's 25+ most endangered tortoises and freshwater turtles - 2011.* IUCN/SSC Tortoise and Freshwater Turtle Specialist Group, Turtle Conservation Fund, Turtle Survival Alliance, Turtle Conservancy, Chelonian Research Foundation, Conservation International, Wildlife Conservation Society, and San Diego Zoo Global. Lunenburg, MA.

Robert, A. (2009). Captive breeding genetics and reintroduction success. *Biological Conservation,* **142**: 2915-2922.

Roosenburg, W.M. (1996). Maternal condition and nest site choice: An alternative for the maintenance of environmental sex determination? *American Zoologist,* **36**: 157-168.

Roosenburg, W.M., and Kelley, K.C. (1996). The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin. Journal of Herpetology,* **30:** 198-204.

Rorison, I.H., Sutton, F., and Hunt, R. (1986). Local climate, topography and plant growth in Lathkill Dale NNR. I. A twelve-year summary of solar radiaton and temperature. *Plant Cell and Environment,* **9**: 49-56.

Rowlingson, B.S., and Diggle, P.J. (1993). Splancs - Spatial point pattern-analysis code in S-Plus. *Computers & Geosciences,* **19:** 627-655.

Savage, J.M. (1958). The concept of ecological niche: With reference to the theory of natural coexistence. *Evolution,* **12**: 111-112.

Scales, K.L., Lewis, J.A., Lewis, J.P., Castellanos, D., Godley, B.J., and Graham, R.T. (2011). Insights into habitat utilisation of the hawksbill turtle, *Eretmochelys imbricata* (Linnaeus, 1766), using acoustic telemetry. *Journal of Experimental Marine Biology and Ecology,* **407**: 122-129.

Schwarzkopf, L., and Brooks, R.J. (1985). Sex determination in Northern Painted turtle - Effect of incubation at constant and fluctuating temperatures. *Canadian Journal of Zoology – Revue Canadienne de Zoologie,* **63:** 2543-2547.

Seebacher, F., and Franklin, C.E. (2012). Determining environmental causes of biological effects: the need for a mechanistic physiological dimension in conservation biology. *Philosophical Transactions of the Royal Society B: Biological Sciences,* **367:** 1607-1614.

Sheridan, C.M., Spotila, J.R., Bien, W.F., and Avery, H.W. (2010). Sex-biased dispersal and natal philopatry in the diamondback terrapin, *Malaclemys terrapin*. *Molecular Ecology*, **19**: 5497-5510.

Simpfendorfer, C.A., Wiley, T.R., and Yeiser, B.G. (2010). Improving conservation planning for an endangered sawfish using data from acoustic telemetry. *Biological Conservation*, **143:** 1460-1469.

Sing, T., Sander, O., Beerenwinkel, N., and Lengauer, T. (2009). ROCR: Visualizing the performance of scoring classifiers. In: *R package version 1.0-4*.

Sokal, R.R., and Rohlf, F.J. (1981). *Biometry: The principles and practice of statistics in biologcal research.*, second edn. W. H. Freeman and Co., San Francisco, USA.

Soulé, M.E. (1985). What is Conservation Biology? *Bioscience*, **35:** 727-734.

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

Spotila, J. R., Zimmerman, L.C., Binckley, C.A., Grumbles, J.S., Rostal, D.C., List Jr., A., Beyer, E.C., Phillips, K.M., and Kemp, S.J. (1994). Effects of incubation conditions on sex determination, hatching success, and growth of hatchling desert tortoises, *Gopherus agassizii*. *Herpetological Monographs*, **8:** 103-116.

Steyermark, A.C., and Spotila, J.R. (2001). Effects of maternal identity and incubation temperature on Snapping turtle (*Chelydra serpentina*) growth. *Functional Ecology*, **15:** 624-632.

Stickler, M., Enders, E.C., Pennell, C.J., Cote, D., Alfredsen, K.T., and Scruton, D.A. (2008). Habitat use of atlantic salmon *Salmo salar* parr in a dynamic winter environment: The influence of anchor-ice dams. *Journal of Fish Biology*, **73**: 926-944.

Tamplin, J. (2006) Response of hatchling wood turtles (*Glyptemys insculpta*) to an aquatic thermal gradient. *Journal of Thermal Biology*, **31:** 400-405.

Taquet, C., Taquet, M., Dempster, T., Soria, M., Ciccione, S., Roos, D., and Dagorn, L. (2006). Foraging of the green sea turtle *Chelonia mydas* on seagrass beds at Mayotte Island (Indian Ocean), determined by acoustic transmitters. *Marine Ecology-Progress Series*, **306**: 295-302.

Tittensor, D.P., Baco, A.R., Brewin, P.E., Clark, M.R., Consalvey, M., Hall-Spencer, J., Rowden, A.A., Schlacher, T., Stocks, K.I., and Rogers, A.D. (2009) Predicting global habitat suitability for stony corals on seamounts. *Journal of Biogeography*, **36**: 1111-1128.

Valenzuela, N. (2004). Temperature-dependent sex determination. In: *Reptilian Incubation Environment, Evolution and Behaviour*: 229-252. D.C. Deeming (Ed.). Nottingham University Press, Nottingham.

Valenzuela, N., and Janzen, F.J. (2001). Nest-site philopatry and the evolution of temperature-dependent sex determination. *Evolutionary Ecology Research*, **3**: 779-794.

Vestjens, W.J.M. (1969) Nesting, egg-laying and hatching of the snake-necked tortoise at Canberra, A.C.T. Australia. *Australian Zoologist,* **15,** 141-149.

Vinegar, A. (1973). The effects of temperature on growth and development of embryos of Indian python, *Python molorus* (Reptilia: Serpentes: Boidae). *Copeia***, 1973:** 171-173.

Vinegar, A. (1974). Evolutionary implications of temperature induced anomalies of development in snake embryos. *Herpetologica,* **30:** 72-74.

Wang, T., and Hicks, J.W. (1996). Cardiorespiratory synchrony in turtles. *Journal of Experimental Biology*, **199,** 1791-1800.

Ward, J.V., and Stanford, J.A. (1989). Riverine ecossystems: the influence of man on catchment dynamics and fish ecology. *Canadian Special Publication of Fisheries and Aquatic Sciences,* **106:** 56-64.

Ward, D.M., Nislow, K.H., and Folt, C.L. (2011). Seasonal shift in the effects of predators on juvenile atlantic salmon (*Salmo salar*) energetics. *Canadian Journal of Fisheries and Aquatic Sciences,* **68**: 2080-2089.

Webb, G.J.W., Sack, G.C., Buckworth, R., and Manolis, S.C. (1983). An examination of *Crocodylus porosus* nests in two northen Australian freshwater swamps, with an analysis of embryo mortality. *Australian Wildlife Research*, **10:** 571-605.

Welch, D.W., Ward, B.R., and Batten, S.D. (2004). Early ocean survival and marine movements of hatchery and wild steelhead trout (*Oncorhynchus mykiss*) determined by an acoustic array: Queen Charlotte Strait, British Columbia. *Deep-Sea Research Part Ii-Topical Studies in Oceanography*, **51:** 897-909.

Welch, D.W., Rechisky, E.L., Melnychuk, M.C., Porter, A.D., Walters, C.J., Clements, S., Clemens, B.J., Mckinley, R.S., and Schreck, C. (2008). Survival of migrating salmon smolts in large rivers with and without dams. *PLoS Biology*, **6**: 2101-2108.

Welch, D.W., Melnychuk, M.C., Rechisky, E.R., Porter, A.D., Jacobs, M.C., Ladouceur, A., Mckinley, R.S., and Jackson, G.D. (2009). Freshwater and marine migration and survival of endangered Cultus Lake sockeye salmon (*Oncorhynchus nerka*) smolts using POST, a large-scale acoustic telemetry array. *Canadian Journal of Fisheries and Aquatic Sciences,* **66**: 736-750.

White, F.N., Hicks, J.W., and Ishimatsu, A. (1989). Relationship between respiratory state and intracardiac shunts in turtles. *American Journal of Physiology,* **256,** R240-R247.

Wikelski, M., and Cooke, S.J. (2006). Conservation physiology. *Trends in Ecology & Evolution,* **21:** 38-46.

Wilbur, H.M., and Morin, P.J. (1988). Life history evolution in turtles. *Biology of Reptilia,* **16:** 387-439.

Williams, D.A., and Osentoski, M.F. (2007). Genetic considerations for the captive breeding of tortoises and freshwater turtles. *Chelonian Conservation and Biology,* **6**: 302-313.

Wilson, D.S. (1998). Nest-site selection: Microhabitat variation and its effects on the survival of turtle embryos. *Ecology,* **79:** 1884-1892.

Wood, D.W., and Bjomdal, K.A. (2000). Relation of temperature, moisture, salinity, and slope to nest site selection in loggerhead sea turtles. *Copeia,* **2000**: 119-128.

Woodall, P.F. (1982). White-bellied sea-eagle feeding on freshwater turtles. *Sunbird,* **12**: 11-14.

Woollard, P., Vestjens, W.J.M., and MacLean, L. (1978). The ecology of the eastern water rat *Hydromys chrysogaster* at Griffith, N.S.W.: Food and feeding habits. *Australian Wildlife Research,* **5**: 59-73.

Yntema, C.L. (1960). Effects of various temperatures on the embryonic development of *Chelydra serpentina*. *Anatomical Record,* **136:** 305-306.

Yntema, C.L. (1968). A series of stages in embryonic development of *Chelydra serpentina*. *Journal of Morphology,* **125:** 219-251.

Yntema, C.L. (1978). Incubation times for eggs of turtle *Chelydra serpentina* (Testudines: Chelydridae) at various temperatures. *Herpetologica*, **34:** 274-277.

Zuur, A.F., Ieno, E.N., Walker, N., Savelievn, A.A., and Smith, G.M. (2009). *Mixed Effects Models and Extensions in Ecology with R.* 1st edn. Springer, New York.