THE DIVING PHYSIOLOGICAL ECOLOGY OF AUSTRALIAN FRESHWATER TURTLE HATCHLINGS

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DECLARATION

Statement of Originality
The work presented in this thesis is, to the best of my knowledge and belief, original and my own work except where duly acknowledged, and has not been submitted, in part or in whole for any other degree at any other university.

Statement of Contribution to Jointly Published Work
Natalie Clark was the primary contributor of ideas, experimental design, experimentation, analysis and writing in all jointly published work, and as such is first author on publications arising from this work.

Statement of Contribution by Others
Others who contributed to the ideas, design, experimentation and/or analysis of all jointly published work, have been recognised with co-authorship or acknowledgement as noted in each chapter.

I hereby certify the above statements are true

Natalie J Clark Professor Craig E Franklin

April 2008

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As an undergraduate I recall sitting around with friends discussing plans for the future and whilst some already had eyes for further study, I was adamant a PhD was definitely not for me. Then sometime between snorkelling for turtles, measuring cloacal volume and analysing data during my honours year, I accidentally caught the bug and found myself loving research. Needless to say I couldn’t pass up the opportunity to undertake the journey that is a PhD degree. As everyone knows a PhD is full of many good times as well as bad, and as I sit here now at the end and contemplate how far I have come the things that I truly remember are all the wonderful people that helped me along the way and to you all I say the biggest thank you.

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ABSTRACT

Freshwater turtles primarily respire aerially via the lungs, however in some species aquatic respiration can also be achieved by diffusion across the skin, or by active ventilation of the bucco-pharynx and/or cloacal bursae. The ability to supplement aerial respiration with aquatic oxygen allows these highly aquatic reptiles to extend their dive duration and reduce surfacing frequency. Whilst changes in environmental conditions are known to influence the diving physiology and behaviour of adult turtles, very little information is known about potential impacts on hatchlings. Therefore, I examined the relationships between diving physiology and behaviour in five species of hatchling Australian turtles: *Emydura signata*, *Elseya latisternum*, *Elseya albagula*, *Elusor macrurus* and *Rheodytes leukops*. The influence of acute and long-term changes in temperature and aquatic oxygen levels on the diving physiology and behaviour of the Mary River turtle, *Elusor macrurus*, was also investigated. To determine how long-term changes in environmental conditions may impact species survival, I examined the use of aquatic respiration as a predator avoidance strategy.

Diving behaviour, aquatic respiration and blood respiratory properties were compared between hatchling and juveniles from five species of Australian freshwater turtle. Both diving behaviour and physiology differed significantly between species as well as age classes. Dive duration in *R. leukops* was 17 times longer than the other species, with two hatchlings remaining submerged for the entire 72 h recording period. The long dive duration recorded for *R. leukops* was supported by a high reliance on aquatic respiration (63-73%), and high blood oxygen affinity (P_{50} = 17.24). A correlation between dive duration, aquatic respiration and blood respiratory properties was not observed in the remaining turtle species where, despite the longer dive durations of *E. albagula*

and *E. macrurus* compared to *E. signata* and *E. latisternum*, there were no differences observed in percent aquatic respiration or blood oxygen affinity between these species.

Temperature influences the rates of biochemical reactions that underlie animal function and as such directly impacts the physiology and behaviour of ectotherms. Organisms can compensate for these fluctuations through the process of acclimation. The influence of thermal acclimation on diving behaviour, aquatic respiration and locomotor performance was examined in the endangered Mary River turtle, *Elusor macrurus*. After long-term exposure to 28ºC, *E. macrurus* hatchlings were unable to negate the acute effect of increasing temperature on metabolic rate, aquatic respiration, dive duration or burst swimming speed. Diving behaviour was however found to partially acclimate at 17ºC. Turtles acclimated to the cold temperature (17ºC) had a significantly longer dive duration at 17ºC, than the hatchlings acclimated to the warm temperature (28ºC). This increase in dive duration at 17ºC was not a result of physiological alterations in metabolic rate but was due instead to an increase in aquatic oxygen consumption. Increasing aquatic oxygen consumption at 17ºC permitted one cold acclimated hatchling to remain submerged underwater for over 2.5 days.

The acute and long-term effects of aquatic hypoxia on dive duration, oxygen consumption and blood respiratory properties were examined in the endangered Mary River turtle, *Elusor macrurus*. Aquatic respiration in *E. macrurus* was substantially reduced at 30 mmHg compared to 155 mmHg and this resulted in a 51% decrease in dive duration. Contrary to our predictions, *E. macrurus* hatchlings did not acclimate and long-term exposure to hypoxic conditions caused *E. macrurus* to lose significantly more oxygen to the hypoxic water than the normoxic acclimated

turtles. The exacerbation of long-term hypoxia on the respiratory physiology and diving ecology of *E. macrurus* raises concerns about the impacts of long-term environmental change as a result of habitat alteration on the survival of freshwater turtle populations.

Hatchling turtles have extremely high predation rates and their ability to increase dive duration through the use of bimodal respiration suggests that aquatic respiration may function as a predator avoidance strategy in this age class. The diving behaviour of two turtle species, *Elusor macrurus* and *Emydura signata* was examined under control conditions (no predator) and in the presence of a large piscine predator (barramundi - *Lates calcarifer*).   In the presence of the predator, the mean dive duration of *E. macrurus* increased by 35% while maximum dive duration increased by 193%. The mean and maximum dive durations of *E. signata* were not influenced by the presence of the predator. Diving activity levels of both turtle species decreased in the presence of the predator indicating that the risk of predation was perceived to be greater during a dive than at the waters surface. The behavioural response differences between the turtle species may be a result of differences in reliance on aquatic respiration, supporting our hypothesis that aquatic respiration may function as a predator avoidance strategy.

Results from this thesis indicate that the diving physiology and behaviour of hatchling turtles is influenced by changes in environmental conditions. This raises concern about the potential impacts of long-term environmental change as a result of river damming on species survival.

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GENERAL INTRODUCTION

DIVING VERTEBRATES

Vertebrates are thought to have arisen and evolved in the marine environment with the oxygen needed to support aerobic metabolism extracted from the water. The prevalence of warm, hypoxic waters during the Silurian and Devonian periods saw the development of the first air-breathing organs in Paleozoic fish (Barrell, 1916; Schmalhausen, 1968; Graham et al., 1978; Graham, 1994). Air-breathing organs not only allowed fish species to exploit additional aquatic habitat but they also played a key role in the evolution of terrestriality (Westoll, 1938; Westoll, 1943; Romer, 1956; Bray, 1985; Thomson, 1993). The development of paired lungs in amphibians allowed vertebrates to move out of the water and into the terrestrial environment (Schmalhausen, 1968; Gans, 1971; Bray, 1985). The subsequent development of the amniotic egg in reptiles, birds and mammals further contributed to the radiation of vertebrates on land (Gauthier et al., 1988; Packard and Seymour, 1997). Whilst tetrapods became highly adapted to the terrestrial environment, some species returned to the water prompting the reinvasion of the aquatic environment by air-breathing species (Butler and Jones, 1982; Kooyman, 1989).

Aquatic air-breathing vertebrates include members of amphibians, reptiles (eg, turtles, sea snakes and crocodiles), birds (eg, penguins, petrels, murres, auks, cormorants, loons, grebes and waterfowl), and mammals (eg, sea cows, sea otters, seals, sea lions, walrus, whales, dolphins and porpoises) (Butler and Jones, 1982; Kooyman, 1989). These diving vertebrates display a predominantly aquatic existence, but due to their reliance upon pulmonary respiration, they must periodically return to the waters surface to breathe air. Diving vertebrates bear the energetic and temporal costs of surfacing because they achieve some benefit from being submerged underwater (Kramer, 1988). Foraging is the most common diving activity however many animals also utilise the aquatic environment for reproduction and predator avoidance (Butler and Jones, 1982; Kooyman, 1989; Kramer, 1988; Houston and Carbone, 1992; Boyd, 1997; Heithaus and Frid, 2003; Frid et al., 2007; Sparling et al., 2007).

Aerobic Dive Limit

A theoretical estimate of the maximum time an individual can stay submerged underwater based on aerobic metabolism is known as the aerobic dive limit (ADL) and is determined by dividing an individual's oxygen storage capacity by their rate of oxygen utilisation/metabolic rate (Kooyman, 1989). Diving optimality models suggest that diving vertebrates adjust their diving behaviour in order to maximise resource gain during a dive (Kramer, 1988; Thompson et al., 1991; Houston and Carbone, 1992; Mori, 1998; Mori, 1999; Sparling et al., 2007). One strategy employed by air-breathing vertebrates to extend ADL is the 'dive response'. Described by a reduction in heart rate (bradycardia) and peripheral vasoconstriction, the 'dive response' reduces oxygen utilisation during a dive and therefore extends submergence time (Irving, 1939; Butler and Jones, 1982; Seymour, 1982; Kooyman, 1989; Butler and Jones, 1997). The ADL can also be extended through an increase in oxygen storage capacity (Butler and Jones, 1982; Seymour, 1982; Kooyman, 1989; Butler and Jones, 1997). Myoglobin concentrations are 10-30 times greater in aquatic birds and mammals than in terrestrial species (Kooyman, 1989; Butler and Jones, 1997). The oxygen stores of some diving vertebrates can also be supplemented with aquatic respiration. Aquatic oxygen can diffuse across the semi-permeable skin of amphibians and some reptiles thereby extending dive duration beyond that based on pulmonary oxygen stores (Butler and Jones, 1982). Aquatic respiration in air-breathing tetrapods has reached the pinnacle of development in freshwater turtles.

DIVING PHYSIOLOGY AND BEHAVIOUR OF FRESHWATER TURTLES

Aquatic Respiration

Freshwater turtles primarily respire aerially via the lungs, however in some species aquatic respiration can be achieved by diffusion across the skin or by active ventilation of the bucco-pharynx and/or cloacal bursae (Gage and Gage, 1886; Smith and James, 1958; Girgis, 1961; Belkin, 1968; Stone et al., 1992a; King and Heatwole, 1994a). Aquatic respiration via the skin occurs by diffusion and usually sustains only a small proportion of the turtle's total oxygen requirements (Stone et al., 1992a). The skin of some species has however been modified through increased vascularisation and reduction/loss of epidermal scutes as exemplified in the soft-shelled turtles. Aquatic respiration in certain soft-shelled turtle species can support up to 38% of total oxygen consumption (Stone et al., 1992a). Aquatic respiration is highest in turtle species which possess additional respiratory epithelia (bucco-pharynx and/or cloacal bursae) (Gage and Gage, 1886; Legler, 1993; King and Heatwole, 1994a). The bucco-pharynx is a modified region of the buccal cavity, the epithelium of which is lined with filamentous villiform processes (Root, 1949; Girgis, 1961; King and Heatwole, 1994a). Aquatic oxygen uptake via the bucco-pharynx can be as high as 30% in some turtle species (eg, *Trionyx triunguis* and *Elseya latisternum*) (Girgis, 1961; King and Heatwole, 1994b). The cloacal bursae are paired sac-like structures which branch off from the cloaca and are positioned one either side of the bladder (Legler, 1993; King and Heatwole, 1994a; Limpus et al., 2002). Extending from the wall of the bursae sacs are numerous highly vascularised branching processes which increase the surface area available for gas exchange (Legler, 1993; King and Heatwole, 1994a; Limpus et al., 2002). A high surface area combined with a short diffusion distance, large blood supply and active ventilation, make the cloacal bursae the most specialised aquatic respiratory organ supporting up to 70% of total oxygen requirements under laboratory conditions and potentially higher in the natural environment (Legler, 1993; Priest, 1997; Gordos et al., 2003b).

Reliance on aquatic respiration is also influenced by the respiratory properties of blood (Wood and Johansen, 1972; Lomholt and Johansen, 1979; Soivio et al., 1980; Jensen and Weber, 1982; Wells et al., 1989). Blood oxygen affinity determines the ability of the blood to bind and unload oxygen. A high oxygen affinity, as well as high levels of haematocrit and haemoglobin, facilitate the uptake of oxygen from the environment while a low affinity is beneficial for efficient delivery of oxygen to the body tissues (Kooyman, 1989; Gordos et al., 2004b). Due to the high solubility of CO_2 in water, the metabolic CO_2 produced during apnoea can be removed from the blood via the aquatic respiratory organs (Gage and Gage, 1886; Dejours, 1994; Bagatto and Henry, 1999; Crocker et al., 1999; Prassack et al., 2001; Gordos et al., 2006). Reducing the accumulation of CO_2 in the blood during a dive minimises the acid-base disturbance in the turtle and may therefore increasing diving capacity (Jackson, 1976; Jackson et al., 1976; Prassack et al., 2001).

Aquatic respiration in freshwater turtles has a significant effect on diving behaviour. The acquisition of aquatic oxygen during a dive supplements aerial oxygen stores leading to an increased ADL (Belkin, 1968; Bagatto et al., 1997; Bagatto and Henry, 1999; Stone et al., 1992b;

Prassack et al., 2001; Gordos and Franklin, 2002; Maina, 2002; Gordos et al., 2004a; Mathie and Franklin, 2006). Those species that have a high reliance on aquatic respiration are able to dive for significantly longer periods than those with little or no aquatic respiratory ability. For example, Gordos and Franklin (2002), found that *Rheodytes leukops,* a species with a high capacity for aquatic respiration could dive for 623 min compared to 37.1 min for *Emydura macquarri*, a species with a low capacity for aquatic respiration. The relationship between aquatic respiration and diving behaviour in freshwater turtles is influence by a range of environmental factors.

Environmental Factors

Turtle diving physiology and behaviour is dependent upon the interaction of the animal with its environment (Herbert and Jackson, 1985b; Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003a,b; Mathie and Franklin, 2006). The theory of optimal breathing (Kramer, 1988) suggests that the proportion of oxygen extracted from each respiratory medium (air and water) in bimodally respiring animals is dependent upon the costs associated with each respiratory media under specific conditions. Aquatic respiration is generally more energetically expensive than aerial respiration as water is 800 times denser, 60 times more viscous and has a lower oxygen capacitance than air (Dejours, 1994). Aerial respiration does however bear the energetic cost of travelling to the water surfaces and the reduced time available for other activities such as foraging (Dejours, 1994). Environmental factors such as temperature and aquatic PO_2 can influence the costs associated with both aerial and aquatic respiration (Kramer, 1988).

Temperature influences the rates of biochemical reactions that underlie animal function and as such directly impacts the physiology and behaviour of ectotherms (Huey, 1982; Haynie, 2001; Angilletta et al., 2002). For example, a 15ºC increase in temperature results in a five fold decrease in the dive duration of the short-necked turtle *E. macquarii* (Priest and Franklin, 2002). Temperature dependent changes in diving behaviour can be attributed to the physiological processes underlying the ADL (Kooyman, 1989; Schreer and Kovacs, 1997). As temperature increases, metabolic rate also increases while oxygen storage capacity decreases (Kooyman, 1989; Fuster et al., 1997). Given that water contains 30 times less oxygen than air, sustaining an elevated metabolic rate at high temperatures via aquatic respiration may be energetically taxing (Dejours, 1994). As a result, the increased oxygen demand at high temperatures is generally met by increasing aerial respiration which results in reduced dive durations (Herbert and Jackson, 1985a; Fuster et al., 1997; Prassack et al., 2001).

Aquatic respiration and diving behaviour in bimodally respiring animals is also dependent upon aquatic oxygen levels (Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003a,b; Mathie and Franklin, 2006). Decreasing aquatic PO_2 from normoxic (155 mmHg) to anoxic (0 mmHg) conditions results in a 60% decrease in the dive duration of *R. leukops* at 15ºC (Priest and Franklin, 2002). The relationship between dive duration and aquatic PO_2 is a direct result of respiratory partitioning (Yu and Woo, 1985; Mattias et al., 1998; Geiger et al., 2000; Seymour et al., 2004; Randle and Chapman, 2005; Alton et al., 2007). At high levels of aquatic PO_2, diffusion of oxygen across the aquatic respiratory organs is enhanced and reliance on aquatic respiration increases. As aquatic PO_2 decreases, the ventilation rate of the aquatic respiratory organs must increase in order to meet metabolic demands via aquatic respiration. The energetic costs associated with aquatic respiration therefore increases as aquatic PO_2 decreases and as a result reliance on aerial respiration increases (Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003b; Jackson, 2007). For example, aquatic respiration in the bimodally respiring fish *Channa maculate* decreased from 21% in normoxia (130 mmHg) to 13% in hypoxia (20 mmHg) while aerial respiration increased proportionally (Yu and Woo, 1985). The respiratory responses of bimodally respiring turtles to changes in aquatic PO_2 has received little attention however an initial study on *Chrysemys picta* reported a 50% decrease in aquatic respiration under hypoxic conditions (Prassack et al., 2001).

Some animals can compensate for changes in environmental condition through the process of acclimation (laboratory based) or acclimatisation (field based), which is the reversible change in physiological processes in response to environmental variability (Prosser, 1991; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). The beneficial acclimation hypothesis (BAH) suggests that acclimatory responses benefit the organism by increasing individual fitness. That is, in a particular environment, an individual that has not had the chance to acclimate will have lower performance/fitness than an individual that has undergone an acclimatory response to those conditions (Leroi et al., 1994; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006) . For example, at 25ºC the maximum swimming speed of goldfish acclimated to 25º C was greater than fish acclimated to 35º C and vice versa when tested at 35ºC (Fry and Hart, 1948). Acclimation has been reported in all major reptilian groups and may occur at all levels of the organism from gene expression through to whole animal performance (Seebacher, 2005). No studies to date have however investigated if the diving behaviour and physiology of bimodally respring turtles can acclimate to long-term changes in environmental conditions.

Body Size

Past studies on the diving physiology and behaviour of bimodally respiring turtles have primarily focused on adult turtles with little information known about the capabilities of hatchlings. A study by Mathie and Franklin (2006), discovered that *Elseya albagula* hatchlings were able to extract a greater proportion of their total oxygen requirements from the water than adults due to the high surface-area-to-volume ratio of their cloacal bursae. The increased reliance on aquatic respiration seen in these hatchlings allowed them to dive for periods similar to that recorded in the larger adults (Mathie and Franklin, 2006). This study suggests that the influence of aquatic respiration on diving ecology may be greater in hatchling turtles than in adults and consequently, hatchling turtles may be more susceptible to changes in diving physiology and behaviour as a result of variations in environmental conditions.

Hatchling ecology may act as an evolutionary driving force for the observed increased reliance on aquatic respiration and extended dive duration. Hatchling turtles are subject to predation from animals such as water rats, eels, large fish and birds and are most susceptible when at the water surface due to reduced escape ability and decreased visibility (Kramer et al., 1983; Cann, 1998; Heithaus et al., 2002; Heithaus and Frid, 2003). Optimal diving models predict that under the risk of predation an air-breathing diver should either reduced surfacing duration or surfacing frequency depending upon the level of risk over time (Heithaus and Frid, 2003). The ability of hatchling turtles to reduce surfacing frequency through the use of bimodal respiration suggests that aquatic respiration may be used as a predator avoidance strategy in this age class. Predator exposure is known to influence reliance on aquatic respiration in many bimodally respiring fish species as well as the African clawed frog (*Xenopus laevis*) (Baird, 1983; Kramer, 1983; Kramer et al., 1983; Smith and Kramer, 1986; Wolf and Kramer, 1987; Herbet and Wells, 2001; Domenici et al., 2007). The use of aquatic respiration as a predator avoidance strategy has yet to be investigated in hatchling turtles.

FRESHWATER TURTLES OF AUSTRALIA

From the Cainozoic era (66 million years ago) up until the Pleistocene (500 000 years ago) the population of freshwater turtles in Australia was dominated by three main families: the Trionychidea (soft-shelled turtles), the Meiolanidae (horned turtles) and the Chelidae (side-necked turtles) (Cann, 1998). Today the population is comprised only of side-necked turtles in which there are six genera: *Chelodina*, *Emydura*, *Elseya*, *Pseudemydura*, *Rheodytes* and *Elusor*, and one species of two-clawed turtle (family Carettochelyidae) (Cann, 1998). Australian turtle species are highly aquatic and can be found in most river systems, creeks, lakes, lagoons and swamps throughout Australia (except Tasmania) (Cann, 1998). Specialised aquatic respiratory organs (cloacal bursae) appear however to have evolved only once in the Australian Chelidae after the divergence of *Pseudemydura* from the short-necked taxa *Rheodytes, Elseya*, *Emydura*, and *Elusor* (Fig. 1.1) (Seddon et al., 1997; Georges et al., 1998; Georges and Thomson, 2006). Whilst most bimodally respiring turtles obtain less than 20% of their O_2 supply via extra-pulmonary gas exchange (Root, 1949; Gatten, 1980; Stone et al., 1992a; Bagatto et al., 1997; Crocker et al., 1999), aquatic respiratory ability in Australian species range from a low reliance of 10% in *Emydura signata* (Priest, 1997; Priest and Franklin, 2002), through to medium capacities in *Elseya latisternum* (redescribed as *Elseya georgesi*) (27%) (King and Heatwole, 1994b) and *Elseya albagula* (40%) (Mathie and Franklin, 2006) and greater than 70% in *Rheodytes leukops* (Priest, 1997; Gordos et al., 2003b). Consequently, Australian freshwater turtles are an ideal model to further investigate the interactions between diving physiological ecology and the environment.

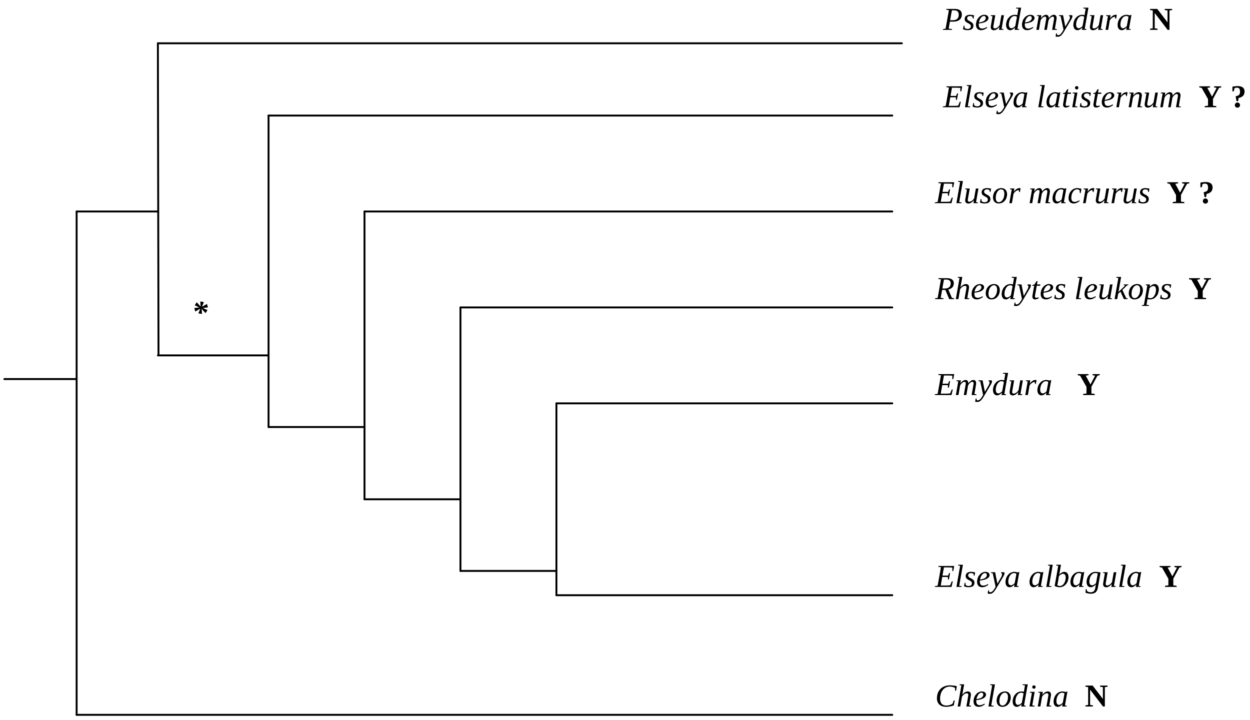

Figure 1.1 - Phylogenetic tree of the relationship of Australian Chelid turtles combining molecular (Georges and Adams, 1992; Georges et al., 1998) and morphological results (Megirian and Murray, 1999). Phylogenetic tree modified from Georges and Thomson, 2006). Y = has cloacal bursae, Y? = thought to have cloacal bursae, N = no cloacal bursae, * = evolution of cloacal bursae.

Conservation Considerations

The river habitats of Australian bimodally respiring turtles have been artificially altered over the years as a result of agriculture, mining and water development (Cogger et al., 1993; Department of Natural Resources, 1993). Changes in environmental conditions such as loss of pool-riffle sequences, reduced water flow, decreased temperature, increased water depth and decreased aquatic oxygen levels, have occurred over a large scale (Ligon et al., 1995; Tucker, 1999; Bodie, 2001; Tucker et al., 2001; Arthington, 2003). Initial reports suggest that generalist turtle species such as *E. signata*, are thriving in these new environments while specialist species like *R. leukops* are declining (Tucker, 1999; Limpus et al., 2002; Arthington, 2003). The differences observed in the sensitivity of these species to environmental change may be attributed to variations in diving physiologies. The study of animal physiology is becoming increasing popular in the field of conservation science due to its ability to provide a detailed mechanistic understanding of the cause of conservation issues as opposite to simple descriptions of the problems (Wikelski and Cooke, 2006). By obtaining knowledge of the relationships between turtle physiological ecology and the environment, we hope to further understand the impacts of environmental change and the consequences of habitat alteration on species survival.

Study Species

Emydura signata

The Brisbane River turtle (*Emydura signata*) is medium size turtle (~25 cm in length) that inhabits the Brisbane River and the lakes, swamps and lagoons in the Brisbane area (Cann, 1998). The cloacal bursae of *E. signata* are relatively undeveloped and as a result adults of this species have a low reliance on aquatic respiration (11%). The maximum dive duration recorded for this species is ~67 min (Priest, 1997; Gordos and Franklin, 2002).

Figure 1.2 - Hatchling Brisbane River turtle, *Emydura signata*.

Elseya latisternum

The saw shelled turtle (*Elseya latisternum*) has a wide distribution down the east coast of Australia from the Cape York Peninsula to the Richmond drainage in New South Wales. A population also occurs in the Northern Territory. This turtle species (20 – 30 cm in length) prefers lagoons, billabongs and creeks with numbers often reduced in large rivers (Cann, 1998). Mean dive duration of this species is ~ 33 min while reliance on aquatic respiration is unknown (Kayes et al., 2005).

Figure 1.3 - Hatchling saw shelled turtle, *Elseya latisternum*.

Elseya albagula

The southern snapping turtle (*Elseya albagula*) is one of Australia's largest turtle species with adult females weighing up to 9 kg. A sexual dimorphism occurs in this species with females being much larger than males (Thomson et al., 2006). *Elseya albagula* has a limited distribution restricted to the Mary, Burnett and Fitzroy Rivers (Thomson et al., 2006). This species is generally found in deep pools (>6 m) either up or down stream from a riffle zone (Hamann et al., 2007). The cloacal bursae of adult *E. albagula* is completely covered in branched but flattened papillae (Legler, 1993) and aquatic respiration in hatchlings supports up to 60% of total oxygen requirements (Mathie and Franklin, 2006). The maximum dive duration of hatchling *E. albagula* is ~110 min (Mathie and Franklin, 2006). This species is listed as a conservation concern by the Queensland Environmental Protection Agency.

Figure 1.4 – Hatchling southern snapping turtle, *Elseya albagula*.

Elusor macrurus

The Mary River turtle (*Elusor macrurus*) is an endangered species that is endemic to the Mary River in Queensland (Cann and Legler, 1994). The Mary River turtle can grow up to 40 cm in length and is generally found in well-oxygenated pools associated with riffle zones (Flakus, 2002). *Elusor macrurus* is known to respire aquatically via cloacal bursae (Legler, 1993) however their reliance on aquatic respiration and diving behaviour has never been investigated.

Figure 1.5 – Hatchling Mary River turtle, *Elusor macrurus*.

Rheodytes leukops

The Fitzroy River turtle (*Rheodytes leukops*) is endemic to the Fitzroy River catchment where it inhabits fast-flowing riffle zones (Legler and Cann, 1980; Cann, 1998). The cloacal bursae of this vulnerable species are extremely specialised with the papillae being highly vascularised and multi-branching (Legler & Cann, 1980; Legler, 1993; Priest, 1997). Aquatic respiration in *R. leukops* can support up to 70% of total oxygen requirements under laboratory conditions and potentially higher in the natural environment (Legler, 1993; Priest, 1997; Gordos et al., 2003b). This species is thought to remain submerged underwater for weeks at time during the winter period (16.5 ± 2ºC) (Gordos et al., 2003b).

Figure 1.6 – Hatchling Fitzroy River turtle, *Rheodytes leukops*.

AIMS OF THESIS

The aim of this thesis was to examine the diving physiological ecology of bimodally respiring freshwater turtle hatchlings. Specifically, the first aim was to determine how diving physiology and behaviour differed between five species of turtle – *Emydura signata*, *Elseya latisternum*, *Elseya albagula*, *Elusor macrurus* and *Rheodytes leukops* (Chapter Two). Aquatic respiration, diving behaviour and blood respiratory properties were measured for both hatchling (1 month old) and yearling (12 months old) turtles. This research provided an insight into the relationships between diving physiology and behaviour and the variation that occurs across species and age classes.

The second aim of this thesis was to examine the influence of changes in environmental conditions on turtle diving physiology and behaviour. Acute and long-term effects of water temperature (Chapter Three) and aquatic oxygen levels (Chapter Four) were investigated in Mary River turtle hatchlings, *E. macrurus.* To determine the ability of the hatchlings to adapt to long-term changes in temperature, hatchling turtles were acclimated to 17ºC and 28ºC for 8 weeks after which aquatic respiration, diving behaviour and locomotor performance were measured and compared to the results from the acute study. Additionally, the effects of aquatic hypoxic on aquatic respiration, diving behaviour and blood respiratory properties were determined and the results used to identify the potential impacts of long-term exposure on species survival.

The third aim was to investigate the consequence of changes in turtle diving behaviour and physiology. Specifically, the use of aquatic respiration as a predator avoidance strategy was examined in *E. macrurus* and *E. signata* hatchlings (Chapter Five). The diving behaviour of the two turtle species was examined in the presence of a predator and without. As *E. macrurus* and *E. signata* hatchlings differ in their abilities to respire aquatically, their behaviour provided an indirect measure of the use of aquatic respiration as a predator avoidance strategy.

The final aim of this thesis was to combine the results of the four experimental chapters and using this enhanced understanding of hatchling diving physiological ecology, discuss the potential impacts of river damming on Australian freshwater turtles (Chapter Six).

STRUCTURE OF THESIS

This thesis is composed of four experimental chapters that investigate the diving physiological ecology of Australian freshwater turtles. The first experimental chapter (Chapter Two: Journal of Zoology 275: 399-406) is a five species comparison of aquatic respiration, diving behaviour and blood respiratory properties. The second experimental chapter (Chapter Three: Physiological and Biochemical Zoology 81: 301-309) examines the thermal plasticity of aquatic respiration, diving behaviour and locomotor performance in *E. macrurus*. Chapter Four (Animal Conservation, accepted) investigates the effects of acute and long-term exposure to aquatic hypoxia on the diving physiology and behaviour of *E. macrurus* hatchlings. The use of aquatic respiration as a predator avoidance strategy is described in the final research chapter (Chapter Five, Behavioral Ecology, in review). This thesis concludes by discussing the findings of the research chapters in relation to the impacts of river damming on Australian freshwater turtle hatchlings (Chapter Six).

CHAPTER TWO

DIVING BEHAVIOUR, AQUATIC RESPIRATION AND BLOOD RESPIRATORY PROPERTIES: A COMPARISON OF HATCHLING AND JUVENILE AUSTRALIAN TURTLES

ABSTRACT

Australia has a number of bimodally respiring freshwater turtle species that use aquatic respiration to extend their aerobic dive limit. Whilst species variations in reliance on aquatic respiration are reflected in the diving behaviour and ecology of adults, it is unknown if these relationships also occur in hatchling and juvenile turtles. This study compared the diving behaviour, aquatic respiration and blood respiratory properties of hatchling and juveniles from five species of Australian freshwater turtles: *Rheodytes leukops*, *Elusor macrurus*, *Elseya albagula*, *Elseya latisternum and Emydura signata.* Both diving behaviour and physiology differed significantly between species as well as age classes. Dive duration in *R. leukops* was 17 times longer than the other species, with two hatchlings remaining submerged for the entire 72 h recording period. The long dive duration recorded for *R. leukops* was supported by a high reliance on aquatic respiration (63-73%), and high blood oxygen affinity (P_{50} = 17.24 mmHg). A correlation between dive duration, aquatic respiration and blood respiratory properties was not observed in the remaining turtle species where, despite the longer dive duration of *E. albagula* and *E. macrurus* compared to *E. signata* and *E. latisternum*, there was no difference observed in percent aquatic respiration or blood oxygen affinity between these species. When compared to adult individuals (data from previous studies), dive duration was positively correlated with body

size in *E. signata*, *E. albagula,* and *R. leukops,* but a negative relationship occurred in *E. latisternum* and *E. macrurus.*

INTRODUCTION

While most animals are limited to one mode of respiration either from air (aerial respiration) or water (aquatic respiration), some species have evolved the ability to exchange respiratory gases in both media (Maina, 2002). Bimodal breathing in vertebrates first evolved in fish during the early Paleozoic and today can also be found in species of amphibians and reptiles including several freshwater turtle species (Boutilier, 1990; Graham, 1994). Aquatic respiration in freshwater turtles occurs by diffusion across the skin, or by active ventilation of the bucco-pharynx and/or cloacal bursae (Gage and Gage, 1886; Smith and James, 1958; Girgis, 1961; Belkin, 1968; Stone et al., 1992a; King and Heatwole, 1994a). In Australia, bimodally respiration occurs within several genera of freshwater turtles, with adult capabilities at temperatures above 20ºC ranging from a low reliance of 10% in *Emydura signata* (Priest, 1997; Priest and Franklin, 2002), through to medium capacities in *Elseya albagula* (40%) (Mathie and Franklin, 2006) and greater than 70% in the Fitzroy River turtle, *Rheodytes leukops* (Priest, 1997; Gordos et al., 2003b).

The ability to supplement aerial respiration with aquatic oxygen allows these highly aquatic reptiles to extend their dive duration and reduce surfacing frequency (Bagatto and Henry, 1999; Gordos and Franklin, 2002). For example, aquatic respiration supports approximately 10% of the total oxygen requirements in *E. signata,* with a maximum dive duration of 166 min being recorded. In contrast, the high reliance of *R. leukops* on aquatic respiration allows this species to remain submerged for days or even weeks at a time (Priest, 1997; Gordos and Franklin, 2002; Priest and Franklin, 2002; Gordos et al., 2003b). Whilst species variations in reliance on aquatic respiration are reflected in the diving behaviour and ecology of adults, it is unknown if these relationships also occur in hatchling and juvenile turtles.

Aquatic respiration and diving behaviour in hatchling and juvenile turtles may be influenced by species morphology, physiology and behaviour. Due to their small size, the mass-specific surface area of hatchling and juvenile turtles is high allowing them to extract a relatively larger amount of oxygen from the water compared to adult turtles (Mathie and Franklin, 2006). Reliance on aquatic respiration is therefore expected to be high in hatchling and juvenile turtles and this is likely to affect dive duration. Species variation in blood respiratory properties may also influence aquatic respiration and dive duration in hatchling and juvenile turtles. A high blood oxygen affinity (low P_{50}) along with high haematocrit and haemoglobin levels would facilitate the uptake of oxygen from the aquatic environment and hence increase dive duration (Gordos et al., 2004b). P_{50} values range from 20.2 to 34.5 mmHg in adult bimodally respiring turtles (*Chrysemys picta, Trachemys scripta, R. leukops* and *E. latisternum*) (Burggren et al., 1977; Maginniss et al., 1983; Gordos et al., 2004b) however, blood respiratory properties have not been investigated in hatchling or juvenile turtles.

The aim of this study was to compare aquatic respiration and diving behaviour of hatchling and juveniles from five species of Australian freshwater turtles: *Rheodytes leukops, Elusor macrurus, Elseya albagula, Elseya latisternum and Emydura signata*. Additionally, blood respiratory properties were analysed for juveniles of each species to determine the relationships among blood properties, aquatic respiration and diving behaviour. We hypothesised that the diving behaviour of the hatchling and juvenile turtles would vary between species and this would be reflected in their reliance on aquatic respiration and blood respiratory properties.

MATERIALS AND METHODS

Turtle Collection and Husbandry

Diving behaviour and physiology were investigated in five Australian turtle species *Rheodytes leukops*, *Elusor macrurus, Elseya albagula*, *Elseya latisternum and Emydura signata*. Eggs of the five species were collected from nests on the banks of the Mary (*E. macrurus*) and Fitzroy (*R. leukops*) Rivers, or from gravid females caught from the Brisbane (*E. signata*), Burnett (*E. latisternum*) and Mary (*E. albagula*) Rivers. A minimum of four clutches were collected for each species to ensure genetic variation. The eggs were transported to The University of Queensland where they were incubated. Upon hatchling the turtles were housed in 1000 L tanks which contained basking platforms and shelters. Tanks that had limited exposure to natural light were provided with Reptiglow UV lights set on a 12:12 light:dark photoperiod. Experiments began at 4 ± 2 weeks of age for the hatchlings and 12 ± 1 month of age for the juveniles. These age classes were chosen to ascertain whether diving behaviour and physiology varied over a small body size scale. The hatchling and juvenile turtles were composed of different egg clutches so that no individual turtle appeared in both age classes. The number of individual turtles within each age class and species group varied according to success of egg collection. This research was approved by Queensland Parks and Wildlife Service (SPP –WISP01477903) and supported by The University of Queensland Animal Ethics Committee (AEC –ZOO/ENT/595/04/URG and ZOO/ENT/731/05/URG).

Diving Behaviour

The diving behaviour of the five turtle species (refer to Table 2.1 for sample sizes and body masses) was examined in a large glass aquarium 150 x 60 x 65 cm (*l* x *w* x *d*). The aquarium contained a pebble substrate and benthic shelters with the water being constantly filtered and maintained at 23ºC (a representative temperature from the Brisbane, Mary, Burnett and Fitzroy Rivers). The photoperiod was set at 12L:12D, with red lights used during the dark period to allow recording. Four individual turtles of a single species were placed into the aquarium and given 24 h to become accustomed to the new environment. Diving behaviour was then recorded for 24 or 72 h (*R. leukops* required a longer recording period due to their long dive durations) using a closed circuit video camera and time lapse VCR. Videotapes were analysed for resting dive durations which were defined as a dive where the turtle sat still on the bottom of the tank for a period of greater than one minute. Mean and maximum resting dive durations were calculated for each turtle along with the frequency of such dives using a custom-written program (M. Gordos). Dive durations were analysed using a generalised linear model with a gamma distribution and inverse link function. After fitting the model, a Tukeys post hoc test was used to determine between and within species comparisons ($P < 0.05$). The dive duration frequency data was analysed using a two-way ANOVA with a Tukeys post hoc test ($P < 0.05$).

Table 2.1 - Body masses (mean ± SEM, grams) and samples sizes (N) of hatchling and juvenile turtles for studies on their diving behaviour, oxygen consumption and blood respiratory properties.

Species	**Diving Behaviour**		**Oxygen Consumption**		**Blood Properties**
	Hatchlings	Juveniles	Hatchlings	Juveniles	Juveniles
Rheodytes leukops	6.28 ± 0.14 (10)	18.41 ± 2.12 (6)	7.61 ± 0.13 (11)	12.83 ± 0.61 (7)	40.19 ± 2.10 (5)
Elusor macrurus	7.99 ± 0.42 (15)	24.88 ± 1.24 (13)	11.08 ± 0.36 (17)	29.41 ± 0.68 (13)	52.38 ± 2.96 (8)
Elseya albagula	23.89 ± 0.85 (11)	106.19 ± 12.79 (4)	25.60 ± 0.72 (15)	125.67 ± 6.42 (4)	38.03 ± 1.07 (8)
Elseya latisternum	6.78 ± 0.07 (12)	29.73 ± 2.70 (14)	6.92 ± 0.09 (14)	41.40 ± 1.46 (14)	52.40 ± 4.25 (8)
Emydura signata	5.89 ± 0.17 (16)	27.29 ± 3.97 (9)	8.03 ± 0.30 (16)	35.01 ± 5.23 (7)	53.44 ± 6.48 (8)

Oxygen Consumption

The aerial and aquatic oxygen consumption rates (VO_2) of the five turtle species were measured using closed-box respirometry (refer to Table 2.1 for sample sizes and body masses). Experiments were conducted at 23°C and red lights used to simulate darkness which aids in reducing turtle activity within the chamber. The respirometers consisted of a 900 ml circular container with an air-tight lid that was filled with 500 ml of water to create an aquatic base chamber. The remaining 400 ml of the respirometer functioned as an aerial chamber into which the turtles could surface to breathe. Two-way taps fitted to the aquatic and aerial chambers permitted water and air sampling. Prior to the beginning of experiments the turtles were weighed and wiped down with a 70% ethanol solution to remove oxygen-consuming bacteria. Turtles were then placed into the respirometers and given 18 h to adjust to the chamber and recover from handling stress before measurements began. During this period, the water in the respirometer was aerated continuously to maintain normoxia. At the beginning of the experimental trial, the aerators were switched off and mineral oil was added to the surface of the water to slow diffusion of gas across phases. The respirometers were then sealed and initial samples of water (5 ml) and air (20 ml) were taken from the sampling ports via a syringe to establish baseline levels of O_2. After an experimental period of either 3 h (hatchlings) or 2 h (juveniles), the final aquatic and aerial gas samples were taken and analysed for oxygen content. Aquatic samples were injected into a water jacket that housed a Cameron oxygen electrode. The oxygen electrode was connected to a Cameron oxygen meter (Cameron Instrument Company model OM200) that measured the aquatic PO_2. The aerial sample passed though H_2O-absorbing Drierite before entering a gas analyser (ADInstruments model ML205) which measured percent O_2 and percent CO_2. The Cameron oxygen meter and gas analyser were connected to a PowerLab/4s, with data recorded on Chart software (v 4.2). Controls were conducted without a turtle in the chamber to account for consumption of oxygen by the electrode and to confirm the effectiveness of the mineral oil as a barrier to gas diffusion. The rate of aerial oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the standard equations of Vleck (1987):

$$VO_2 = \frac{[((V - ViH_2O)\,(Fi - Fe) - Fe\,(VCO_2))/(1-Fe)]}{t}$$

where,

V = volume of air in chamber (ml)

ViH_2O = initial volume of water vapor in chamber (ml)

VCO_2 = volume of CO_2 produced during experiment (ml)

Fi = fractional concentration of O_2 at the start of the experiment (%)

Fe = fractional concentration of O_2 at the end of the experiment (%)

t = length of trial (h)

The rate of aquatic oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the following equation modified from King and Heatwole (1994b):

$$VO_2 = \frac{\Delta DO_2 \times \beta \times VH_2O}{t}$$

where,

t = length of trial (h)

ΔDO_2 = change in dissolved O_2 over trial (mmHg)

VH_2O = volume of water in respirometer (L)

β = oxygen co-efficient of capacitance (ml O_2 L^{-1}mmHg)

Each turtle underwent a total of 4 trials (replicates) with a minimum 2 h period allowed between each trial. The experimental trial that produced the minimum metabolic rate was then used in analysis in order to reduce the variations in oxygen consumption as a result of turtle movement. To account for the allometric scaling of metabolic rate, both aerial VO_2 and aquatic VO_2 were scaled to 0.75 and standardised to an average size turtle (12 g). Percent respiration was estimated by expressing aquatic VO_2 as a proportion of total VO_2. The influence of turtle species and age class on aquatic VO_2 and percent aquatic respiration was determined using a two-way ANOVA (species and age as factors) with a Tukeys post hoc comparison ($P < 0.05$). Percentage data were transformed before analysis.

Blood Respiratory Properties

Blood respiratory properties of the five turtle species was determined using the juvenile turtles only as the small size of the hatchlings prevented blood sampling (refer to Table 2.1 for sample sizes and body masses). A 70-90 µl blood sample was collected from the cervical sinus of the turtles using a 25 gauge needle and 1 ml syringe (Rogers and Booth, 2004). The needle tip and plunger of the syringe were dusted with sodium heparin to prevent coagulation. The blood sample was then transferred into a 0.5 ml Eppendorf tube where sub-samples were collected for analysis of haemoglobin concentration (Hb), haematocrit (Hct) and P_{50}, which was defined as the PO_2 at which 50% of the haemoglobin was saturated.

Concentration of Hb was determining using a spectrophotometer (Pharmacia LKB ultrospec III). Five micro-litres of blood was mixed with 1 ml of Drabkins solution and the absorbance at 540 nm recorded. Hb concentration was then determined from an average absorbance reading (Lewis et al., 2001). A sub-sample of blood collected in a capillary tube was centrifuged at 1000 rpm for 3 min, with Hct determined as a the percent of red blood cells per sample volume. A Hemox analyser Model B (TCS Scientific corp. New Hope, PA) was used to determine the P_{50} values. A 50 µl sample of blood was added to 5 ml of buffered saline (Hemox^{TM} Solution), 20 µl of bovine serum albumin (Additive-A) and 10 µl of antifoaming agent. The blood sample was then added to the Hemox machine where the deoxygenation and oxygenation curves were run at 23ºC, and the P_{50} values recorded at 5% CO_2. Significant differences in Hb, Hct and P_{50} were determined using a one-way ANOVA with a Tukeys post hoc comparison ($P < 0.05$).

RESULTS

Diving Behaviour

There were significant differences in the diving behaviour of the five turtle species and between the hatchling and juvenile turtles (Figs. 2.1 & 2.2).

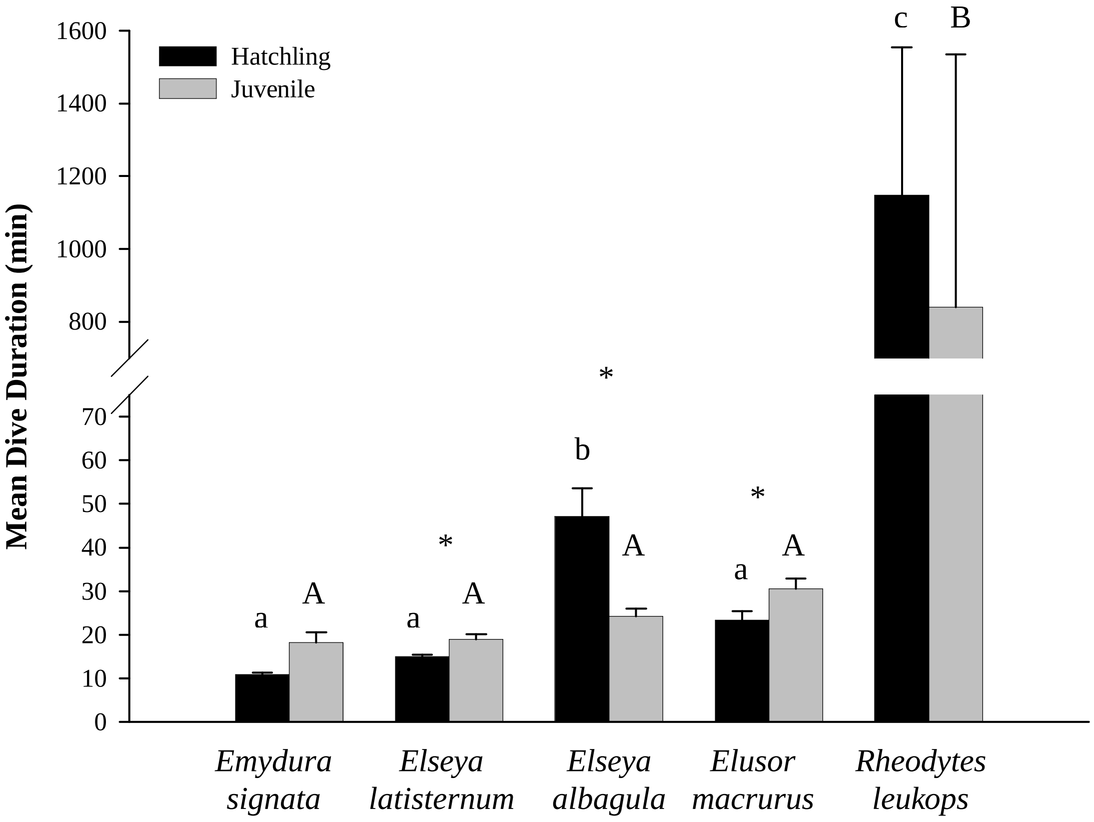

Figure 2.1 - Mean dive duration (min) for five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant difference between juvenile species. Asterisks indicate significant differences between hatchling and juvenile turtles within a species.

Mean and maximum dive durations of *R. leukops* were significantly greater ($P < 0.001$) than the other four turtle species in both the hatchlings (1147 ± 407 min and 2288 ± 341 min, respectively) and juveniles (839 ± 697 min and 1565 ± 554 min, respectively) (Figs. 2.1 & 2.2).

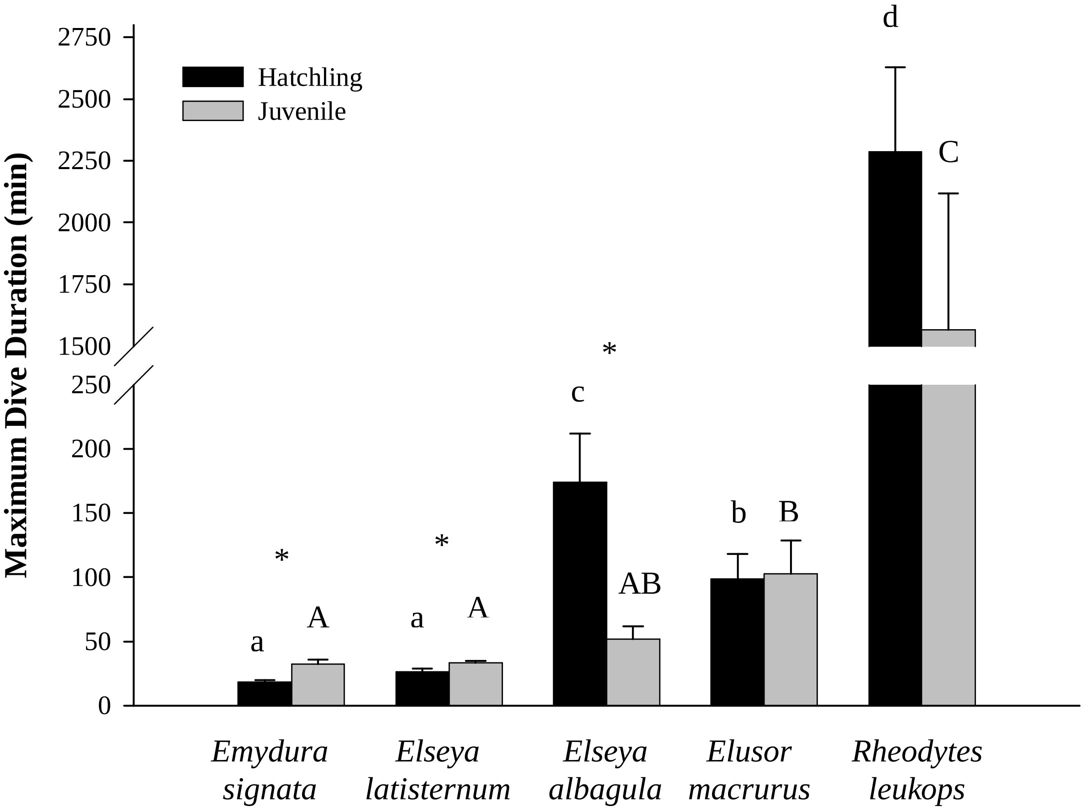

Figure 2.2. - Maximum dive duration (min) for five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant difference between juvenile species. Asterisks indicate significant differences between hatchling and juvenile turtles within a species.

Two hatchling *R. leukops* remained submerged throughout the entire experimental period (72 h), resulting in a maximum dive duration that was seventeen fold longer than values recorded for the other turtle species. Within the hatchlings, the mean and maximum dive durations of *E. albagula* were 47 ± 6 min and 173 ± 38 min, respectively which were significantly greater than that of *E. macrurus* (23 ± 2 min and 98 ± 20 min, $P < 0.05$), *E. latisternum* (15 ± 0.5 min and 26 ± 2 min, $P < 0.001$) and *E. signata* (11 ± 0.5 min and 19 ± 1 min, $P < 0.001$) (Figs. 2.1 & 2.2). However, when comparing the mean and maximum dive durations of juvenile turtles (excluding *R. leukops*) there were no significant differences between species except for the maximum dive duration of *E. macrurus* (103 ± 26 min) which was significantly longer than *E. latisternum* (33 ± 2 min, $P < 0.001$) and *E. signata* (32 ± 4 min, $P < 0.001$) (Figs. 2.1 & 2.2). No statistically significant differences were observed when the dive duration frequency data was compared across the five species for both hatchlings and juveniles, however dives >150 min made up 59% and 37% of the total dive time for hatchling and juvenile *R. leukops* respectively (Figs. 2.3 & 2.4).

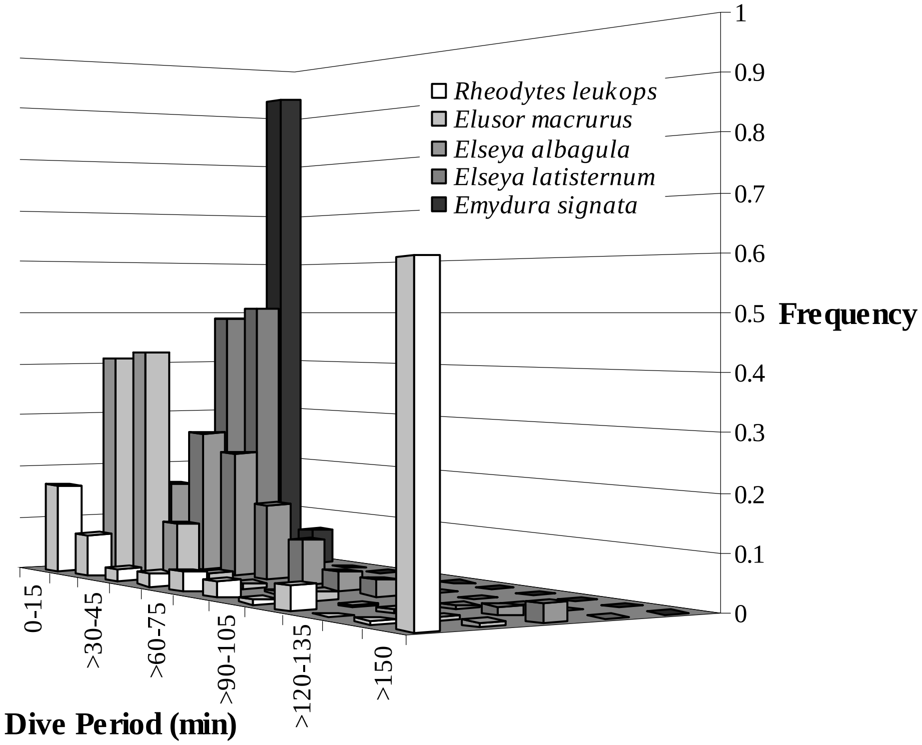

Figure 2.3 - The frequency of dives that occurred within each 15 min dive period from 0 to >150 min for hatchlings of five species of freshwater turtle.

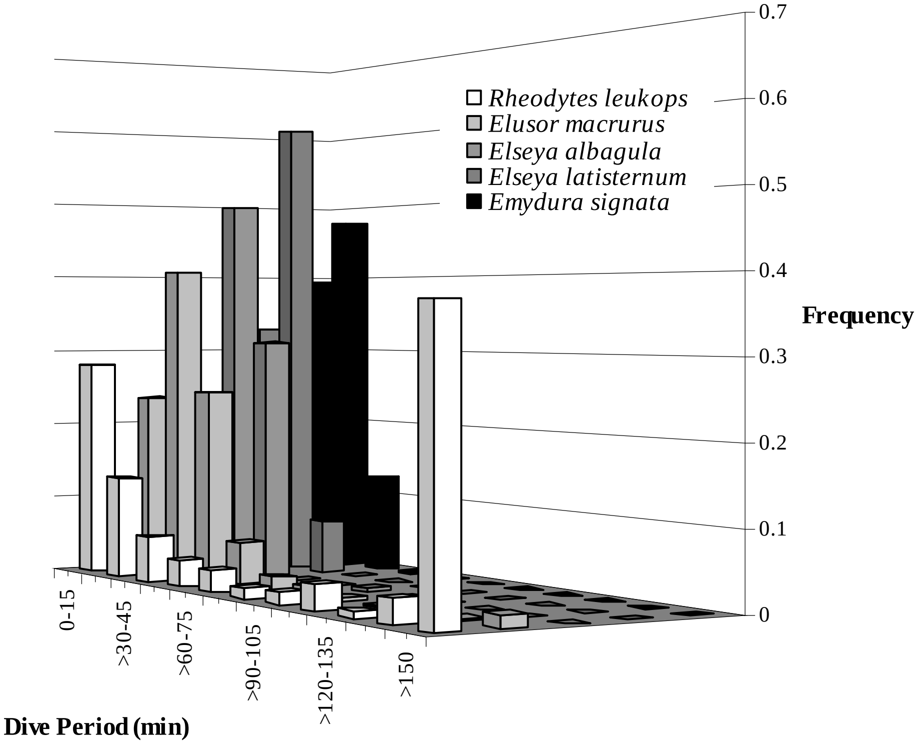

Figure 2.4 - The frequency of dives that occurred within each 15 min dive period from 0 to >150 min for juveniles of five species of freshwater turtle.

The relationships between hatchling and juvenile dive durations varied considerable between species. Juvenile mean and maximum dive durations were significantly greater than that of the hatchlings in *E. macrurus* (P < 0.01, mean dive duration only), *E. latisternum* (P < 0.01) and *E. signata* (P < 0.001). Hatchling dive durations were however greater than for juveniles in *E. albagula* (P < 0.001) while no differences in age class was recorded for *R. leukops* (Figs. 2.1 & 2.2). Seventy percent of dives undertaken by both hatchling and juvenile turtles were short in duration (< 30 min) for all species (Figs. 2.3 & 2.4). A significantly higher number of dives occurred within the 0-15 min dive period than in the longer dive periods for both the hatchlings and juveniles (P < 0.01, except when compared to the 15-30 min dive period) (Fig. 2.3). Juvenile turtles also had significantly more dives occurring within 15-30 min dive period than for the longer dive periods ($P < 0.01$) (Fig. 2.4).

Oxygen Consumption

Percent aquatic respiration and aquatic oxygen consumption were significantly greater in *R. leukops* than in all other species ($P < 0.001$) (Table 2.2 & Fig. 2.5). Aquatic respiration in *R. leukops* supported 63 ± 3% and 73 ± 10% of the total oxygen requirement in hatchling and juveniles respectively. Percent aquatic respiration did not differ between the remaining species in either the hatchling or juvenile age class (Fig. 2.5).

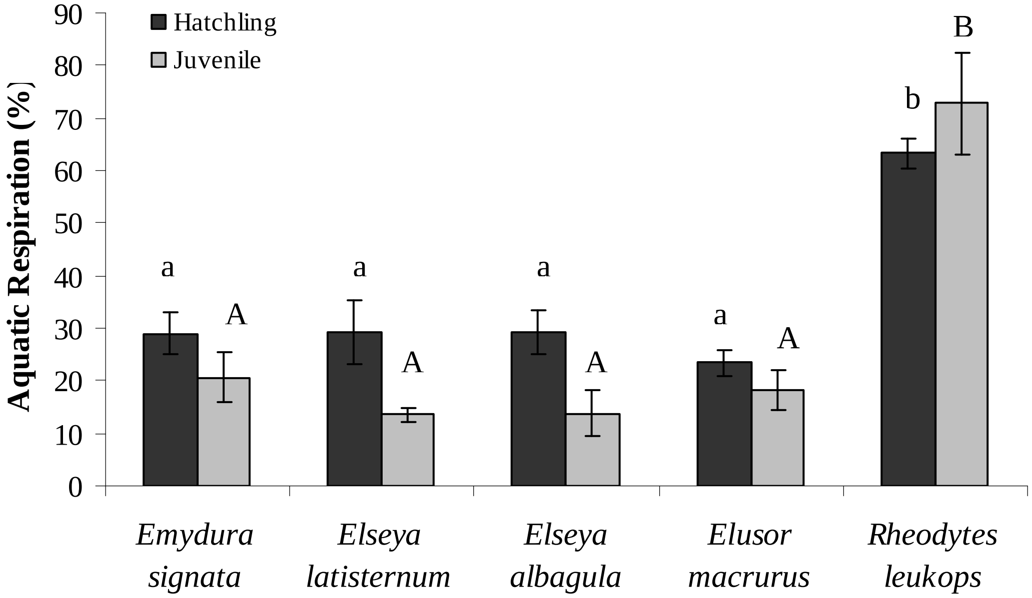

Figure 2.5 - Aquatic respiration in five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant difference between juvenile species.

The aquatic oxygen consumption of *E. macrurus* (0.109 ± 0.008 mlO_2h^{-1}) was greater than *E. latisternum* (0.088 ± 0.011 mlO_2h^{-1}, $P < 0.05$) and *E. albagula* (0.068 ± 0.007 mlO_2h^{-1}, $P < 0.05$) while that of *E. signata* (0.122 ± 0.017 mlO_2h^{-1}) was also higher than *E. latisternum* ($P < 0.05$) (Table 2.2). Overall, aquatic oxygen consumption and percent aquatic respiration did not significantly differ between age classes; however the aquatic oxygen consumption of hatchling *R. leukops* (0.447 ± 0.014 mlO_2h^{-1}) was greater than that of the juveniles (0.259 ± 0.037 mlO_2h^{-1}) ($P < 0.001$) (Table 2.2 & Fig. 2.5).

Aerial oxygen consumption differed between species within the juvenile age class only where *R. leukops* (0.110 ± 0.039 $ml\ O_2\ h^{-1}$) had a significantly lower oxygen consumption than *E. latisternum* (0.512 ± 0.033 $ml\ O_2\ h^{-1}$, $P < 0.01$), *E. albagula* (0.789 ± 0.054 $ml\ O_2\ h^{-1}$, $P < 0.001$) and *E. macrurus* (0.926 ± 0.188 $ml\ O_2\ h^{-1}$, $P < 0.001$) (Table 2.2). Aerial oxygen consumption in hatchling turtles was generally less than that of the juveniles however this was only significant in *E. albagula* ($P < 0.01$) and *E. macrurus* ($P < 0.05$) (Table 2.2).

Table 2.2 - Aerial and aquatic oxygen consumption (mlO_2h^{-1}) in hatchlings and juveniles of five species of freshwater turtle. VO_2 aerial and VO_2 aquatic were scaled to 0.75 then standardised to a 12 g turtle. Values represent means ± SEM.

Species	**Aquatic Oxygen Consumption (ml O_2h^{-1})**		**Aerial Oxygen Consumption (ml O_2h^{-1})**	
	Hatchlings	Juveniles	Hatchlings	Juveniles
Rheodytes leukops	0.45 ± 0.01	0.26 ± 0.04	0.27 ± 0.03	0.11 ± 0.04
Elusor macrurus	0.11 ± 0.01	0.14 ± 0.02	0.47 ± 0.08	0.93 ± 0.19
Elseya albagula	0.07 ± 0.01	0.13 ± 0.041	0.24 ± 0.04	0.79 ± 0.05
Elseya latisternum	0.09 ± 0.01	0.08 ± 0.01	0.31 ± 0.05	0.51 ± 0.03
Emydura signata	0.12 ± 0.02	0.10 ± 0.01	0.35 ± 0.06	0.49 ± 0.12

Blood Respiratory Properties

Blood respiratory properties differed significantly among the five turtle species (Hct, $P < 0.001$; Hb, $P < 0.001$; P_{50}, $P < 0.001$). Hct and Hb levels were significantly greater in *R. leukops* than in the other turtle species ($P < 0.001$) (Figs. 2.6a & b). The Hb levels of *E. macrurus* (1.6 ± 0.1 mmolL^{-1}) were also significantly higher than *E. signata* (1.0 ± 0.1 mmolL^{-1}, $P < 0.05$) and *E. albagula* (1.0 ± 0.1 mmolL^{-1}, $P < 0.05$) (Fig. 2.6b). The P_{50} values of the turtles varied significantly among species ($P < 0.001$) (Fig. 2.6c). *Rheodytes leukops* recorded the lowest P_{50} of 17.24 ± 0.45 mmHg which was significantly different to all of the other species ($P < 0.05$) except *E. albagula* which recorded a value of 21.81 ± 1.56 mmHg. The P_{50} of *E. albagula* was lower than that of *E. signata* (29.03 ± 2.44 mmHg, $P < 0.01$) and *E. macrurus* (29.71 ± 1.30 mmHg, $P < 0.01$) in which P_{50} values did not differ ($P > 0.05$), and *E. latisternum* which had the highest P_{50} value of 33.79 ± 1.42 mmHg ($P < 0.001$).

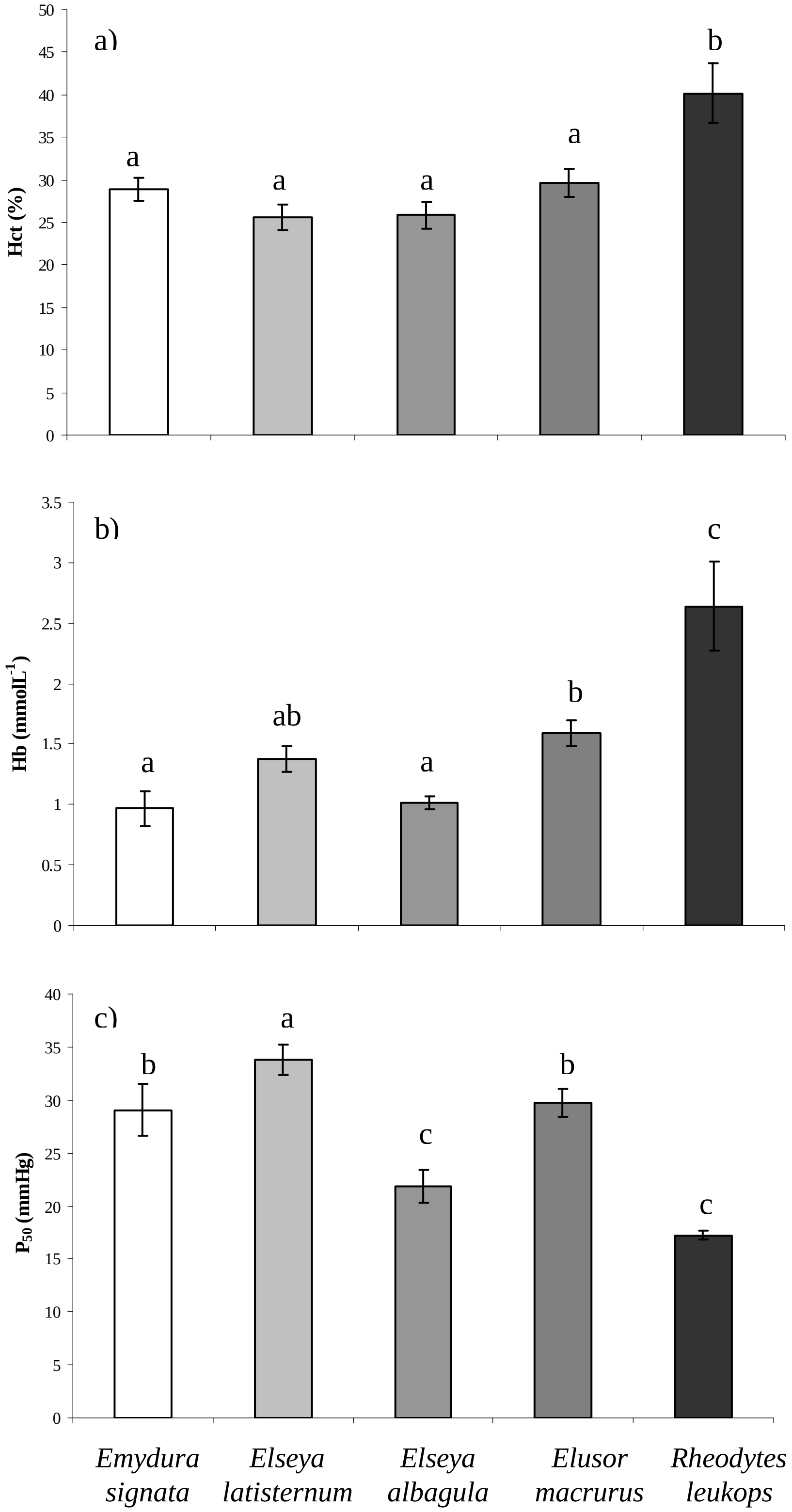

Figure 2.6 - Blood respiratory properties of juvenile freshwater turtles; (a) percent haematocrit, (b) haemoglobin, and (c) P_{50}. Values represent means ± SEM. Letter differences indicate significant differences

DISCUSSION

The diving behaviour of the hatchling and juvenile turtles differed markedly among the five turtle species however the relationships between diving behaviour and physiology were not as apparent as predicted. The long dive duration recorded for *R. leukops* was supported by a high reliance on aquatic respiration (63-73%) and high blood oxygen affinity (P_{50} = 17.24 mmHg). A correlation between dive duration, aquatic respiration and blood respiratory properties was not however observed in the remaining turtle species where, despite the longer dive duration of *E. albagula* and *E. macrurus* compared to *E. signata* and *E. latisternum*, there was no difference observed in percent aquatic respiration or blood oxygen affinity between these species.

Dive duration in air-breathing vertebrates is influenced by the magnitude of the species aerobic dive limit (ADL). The ADL provides a theoretical estimate of the maximum aerobic dive possible and is calculated by divided an individual's oxygen storage capacity by their rate of oxygen utilization/metabolic rate (Kooyman, 1989). However, the ability to acquire oxygen from the aquatic environment during a dive allows bimodally respiring turtles to extend their ADL thereby influencing a species' diving behaviour (Belkin, 1968; Stone et al., 1992a; King and Heatwole, 1994a,b; Bagatto et al., 1997; Bagatto and Henry, 1999; Prassack et al., 2001; Maina, 2002; Gordos et al., 2004b). Percent aquatic respiration in *R. leukops* reached a mean value of 67% which was approximately three times greater than that of the other turtle species. The high reliance of *R. leukops* on aquatic respiration supports the long dive durations observed in this species. A correlation between dive duration and reliance upon aquatic respiration was not however observed in the remaining turtle species.

Obtaining accurate measures of a species maximum ability to respire aquatically is generally difficult due to the turtle's ability to voluntarily change their reliance on aerial and aquatic oxygen consumption (Mathie and Franklin, 2006). Due to the difficulty in measuring aquatic respiration, the morphology, perfusion and ventilation of the cloacal bursae may provide a better indication of a species capacity to respire aquatically. The turtle species used in this study all possess cloacal bursae which are dorso-lateral diverticula of the cloaca. The degree of morphological development in this respiratory organ differs dramatically between species (Legler, 1993; Legler and Georges, 1993). The cloacal bursae of adult *E. signata, E. latisternum* and *E. macrurus* are the least developed with the bursal lining only partly covered by papillae (Legler and Georges, 1993; Cann and Legler, 1994; King and Heatwole, 1994a). The cloacal bursae of adult *E. albagula* is completely covered in branched but flattened papillae, while that of adult *R. leukops* is the most specialised with the papillae being highly vascularised and multi-branching (Legler and Cann, 1980; Legler, 1993; Priest, 1997). However, further investigations are required into the morphology of cloacal bursae and how bursae structure varies with development and across size classes.

Dive duration can be influenced by blood respiratory properties (Kooyman, 1989). Blood oxygen affinity determines the ability of the blood to bind and unload oxygen. A high oxygen affinity facilitates oxygen loading at the respiratory organs while a low affinity is beneficial for efficient delivery of oxygen to the body tissues (Kooyman, 1989). Blood oxygen affinity was predicted to be high in bimodally respiring turtles as this would facilitate the uptake of oxygen from the aquatic environment (Gordos et al., 2004b). A study by Gordos et al. (2004b) however found that the P_{50}, Hb and Hct levels of adult *R. leukops* were no different to values reported for other freshwater turtles that display a low reliance on aquatic respiration. The results of this study however showed that blood oxygen affinity, Hb and Hct levels of juvenile *R. leukops* were all significantly higher than that of the other turtle species and this supports the high levels of aquatic respiration and long dive durations recorded for this species. These results suggest that an increase in blood oxygen affinity and oxygen carrying capacity may confer an adaptive advantage regarding the uptake of oxygen from the aquatic environment. The differences in blood oxygen affinity seen between this study and that of Gordos et al. (2004b) may be due to differences in turtle body size and species developmental rates. Haemoglobin composition in sea turtles is known to differ between hatchlings and adults with the development of the adult component occurring between 14 and 90 days old in green sea turtle (*Chelonia mydas mydas*) hatchlings (Isaacks et al., 1978) but between 4 and 7 months old in the Kemp's Ridley turtle (*Lepidochelys kempi*) (Davis, 1991).

Species ecology may also act as an evolutionary driving force for reliance on aquatic respiration and diving behaviour. The remarkable ability of *R. leukops* to extend dive duration through the use of aquatic respiration is thought be a key factor in the ability of this species to inhabit fast-flowing riffle zones (Gordos, 2004). A high reliance on aquatic respiration decreases the frequency and therefore the costs associated with surfacing in a high velocity environment. Within riffle zones, *R. leukops* has reduced competition from other turtle species for food resources as well as reduced predator exposure (Gordos, 2004). The maximum dive durations of *E. macrurus* and *E. albagula* (4 h) are eight times longer than that of *E. signata* and *E. latisternum* (30 min) suggesting they too may use an increased reliance on aquatic respiration to further exploit the aquatic environment.

Table 2. 3 - Mean aquatic respiration and mean dive duration for hatchlings, juveniles and adults in five species of freshwater turtle.

Species	**Mean Aquatic Respiration (%)**			**Mean Dive Duration (min)**			**Citation**
	Hatchlings	**Juveniles**	**Adults**	**Hatchlings**	**Juveniles**	**Adults**	
Emydura signata	29.0 ± 4.0	20.7 ± 4.8	13.0 ± 3.0	10.9 ± 0.4	18.2 ± 2.4	6.7 ± 1.2	This study; Priest, 1997; Priest & Franklin, 2002
Elseya latisternum	29.2 ± 6.1	13.6 ± 1.4		15.0 ± 0.5	19.0 ± 1.1	32.9 ± 11.5	This study; Kayes, 2005
Elseya albagula	29.2 ± 4.2	13.8 ± 4.5	17.0 ± 3.0	47.1 ± 6.5	24.3 ± 1.8	35.0 ± 3.0	This study; Mathie & Franklin, 2006
Elusor macrurus	23.4 ± 2.4	18.2 ± 3.7		23.3 ± 2.0	30.5 ± 2.5	70.6 ± 8.8	This study; Sandjian, 2007
Rheodytes leukops	63.4 ± 2.9	72.8 ± 9.7	38.0 ± 3.5	1147 ± 407	839 ± 697	38.0 ± 5.0	This study; Priest, 1997; Priest & Franklin, 2002

The relationships between diving behaviour and physiology have primarily been studied in adult turtles with very little information known about the capabilities of hatchlings and juveniles. Dive duration in air-breathing vertebrates generally increases with body mass as larger animals have a higher oxygen storage capacity and a lower mass-specific metabolic rate (Kleiber, 1961; Butler and Jones, 1982; Kooyman, 1989; Schmidt-Nielsen, 1984; Schreer and Kovacs, 1997; Kooyman and Ponganis, 1998). Dive duration is however predicted to correlate negatively with body mass in bimodally respiring turtles as smaller turtles have a relatively higher reliance on aquatic respiration and higher predation pressures (Stone et al., 1992a; Bagatto et al., 1997; Mathie and Franklin, 2006). The completion of this study on hatchling and juvenile turtles allows for the relationship between body size and diving behaviour in bimodally respiring turtles to be investigated further. Table 2.3 reports mean aquatic respiration and mean dive duration for hatchlings, juveniles and adults recorded in captivity between 23ºC and 25ºC. Aquatic respiration was higher in hatchlings than adults for *E. signata*, *E albagula*, and *R. leukops*. Consequently, the dive duration of these three species were longer in the hatchlings. This trend was not supported for all species however with the dive durations of hatchlings *E. latisternum* and *E. macrurus* being shorter than the adults.

This study demonstrates that the diving behaviour and physiology of Australian freshwater turtles does differ between species. *Rheodytes leukops* is the obvious standout species recording the maximum reliance of aquatic respiration, highest blood oxygen affinity and longest dive duration. The relationships among the other four species however remain unclear. The current phylogenetics of Australian freshwater turtles does suggest that developed aquatic respiratory organs (eg, cloacal bursae with papillae) have evolved only once in the short-necked taxa, which indicates that a common factor has contributed to the evolution of aquatic respiration and extended dive duration. To date *Emydura*, *Elseya*, *Elusor* and *Rheodytes* remain as an unresolved polytomy and further phylogenetic, morphological and ecological data is required to understand the among species differences that occur (Georges et al., 1998).

CHAPTER THREE

THERMAL PLASTICITY OF DIVING BEHAVIOUR, AQUATIC RESPIRATION AND LOCOMOTOR PERFORMANCE IN THE MARY RIVER TURTLE, *ELUSOR MACRURUS*

ABSTRACT

Locomotion is a common measure of performance used in studies of thermal acclimation due to its correlation with predator escape and prey capture. However, for sedentary animals like freshwater turtles, we propose that diving behaviour may be a more ecologically relevant measure of performance. Increasing dive duration in hatchling turtles reduces predator exposure and therefore functions as an ecological benefit. Diving behaviour is thermally dependent and in some species of freshwater turtles, is also reliant upon aquatic respiration. This study examined the influence of thermal acclimation on diving behaviour, aquatic respiration, and locomotor performance in the endangered, bimodally respiring Mary River turtle, *Elusor macrurus*. Diving behaviour was found to partially acclimate at 17ºC, with turtles acclimated to a cold temperature (17ºC) having a significantly longer dive duration than hatchlings acclimated to a warm temperature (28ºC). This increase in dive duration at 17ºC was not a result of physiological alterations in metabolic rate but was due instead to an increase in aquatic oxygen consumption. Increasing aquatic oxygen consumption permitted cold acclimated hatchlings to remain submerged for significantly longer periods, with one turtle undertaking a dive of over 2.5 days. When burst swimming speed was used as the measure of performance, thermal acclimation was not detected. Overall, *E. macrurus* demonstrated a partial ability to acclimate to changes in environmental temperature.

INTRODUCTION

Temperature influences the rates of biochemical reactions that underlie animal function and as such, directly impacts the physiology and behaviour of ectotherms (Huey, 1982; Haynie, 2001; Angilletta et al., 2002). Organisms can compensate for these fluctuations through the process of acclimation (laboratory based) or acclimatisation (field based), which is the reversible change in physiological processes in response to environmental variability (Prosser, 1991; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). The beneficial acclimation hypothesis (BAH) suggests that these acclimatory responses benefit the organism by increasing individual fitness (Leroi et al., 1994; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006).

Locomotion is one of the primary performance measures used in studies of thermal acclimation due to its correlation with predator escape and prey capture (Bennett, 1990; Bennett and Huey, 1990; Johnson and Bennett, 1995; Domenici and Blake, 1997; Johnston and Temple, 2002; O'Steen and Bennett, 2003; Wilson, 2005). Whilst locomotion is an ideal performance measure for active species like fish, an alternative measure may be required for sedentary animals like freshwater turtles. Hatchling turtles spend the majority of their time sheltering on the river bottom, with surfacing events for aerial respiration increasing the hatchlings’ exposure to predation in both the water column and at the waters surface (Kramer, 1988; Kramer et al., 1983; Heithaus and Frid, 2003). Rather than maximising burst swimming speed, hatchling turtles are thought to reduce predator exposure by maximising their dive duration and hence reducing surfacing frequency. Therefore, we propose that diving behaviour is a more ecologically relevant measure of performance for hatchling chelonians compared to locomotion.

Diving behaviour in freshwater turtles is known to be thermally dependent (Herbert and Jackson, 1985b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003a,b). For example, a 15ºC increase in temperature resulted in a five fold decrease in the dive duration of the short-necked turtle (*Emydura macquarii*) (Priest and Franklin, 2002). Temperature dependent changes in diving behaviour can be attributed to the physiological processes underlying the aerobic dive limit (ADL) (Kooyman, 1989; Schreer and Kovacs, 1997). As temperature increases, metabolic rate also increases while oxygen storage capacity decreases, thereby resulting in shorter dive durations for chelonians (Kooyman, 1989; Fuster et al., 1997). Diving behaviour in freshwater turtles may also be dependent upon aquatic respiration (Gordos and Franklin, 2002; Mathie and Franklin, 2006). Certain freshwater turtle species display the ability to respire aquatically, which allows them to undertake extended dives compared to primarily air-breathing species (Belkin, 1968; Stone et al., 1992a; King and Heatwole 1994a,b; Bagatto et al., 1997; Bagatto and Henry, 1999; Maina, 2002; Prassack et al., 2001). For the Fitzroy River turtle, *Rheodytes leukops*, aquatic respiration supports up to 70% of total VO_2, which translates into extended dives of several days (Gordos and Franklin, 2002). However, aquatic respiration in freshwater turtles is also thermally dependent. As temperature increases, aquatic respiration cannot fulfil the increased metabolic demands of the turtle, thus resulting in an elevated reliance upon aerial respiration (Herbert and Jackson, 1985b; Yu and Woo, 1985; Prassack et al., 2001).

Despite the high dependence of dive duration on temperature, there have been no studies to date that have investigated if the diving behaviour of turtles can thermally acclimate. We hypothesise that it is beneficial for hatchling turtles to maximise dive duration in order to reduce predator exposure. Acclimation to a high temperature will therefore result in the turtles increasing their dive duration at that temperature through a reduction in metabolic rate and increase in aquatic respiration. Acclimation to a cold temperature however may actually result in a decrease in dive duration as a trade off occurs between performance and metabolic rate. Acute exposure to cold temperatures has a depressive effect on metabolism and as a result the animals may increase their energy expenditure in order to maintain metabolic capacity (Wood et al., 1978; Evans, 1990; Seebacher et al., 2003; Seebacher et al., 2004; Glanville and Seebacher, 2006). For example in winter, the metabolic enzymes of the Australian freshwater turtle (*Chelodina longicollis*) acclimatised so that activity levels could be maintained despite the drop in body temperature (Seebacher et al., 2004). We hypothesised that the metabolic rate of cold acclimated *Elusor macrurus* turtles may also increase at cold temperatures and as a result dive duration will decrease. The aim of this study was to examine the influence of thermal acclimation on diving behaviour, aquatic respiration and swimming performance in the Mary River turtle, *Elusor macrurus*.

MATERIAL AND METHODS

Turtle Collection and Husbandry

Elusor macrurus eggs were collected from nesting sites along the Mary River near the town of Tiaro (25.72606ºS, 152.58265ºE) and transported to The University of Queensland where they were incubated. Once hatched, the turtles were housed in a large holding tank (1000 L) which contained basking platforms and shelters. The holding tanks were maintained at a constant temperature of 23ºC with a photoperiod of 12L:12D. At 10 months of age, the hatchling turtles were randomly divided into two groups and place into either a cold (17ºC, N = 6, 29.88 ± 1.70 g) or warm (28ºC, N = 7, 30.04 ± 2.32 g) acclimation treatment. These temperatures were selected as representatives of winter and summer water temperatures in the Mary River (Pusey et al., 1993). The acclimation tank setup (140 L) was identical between treatments, consisting of basking platforms, shelters, aerators and a 12L:12D photoperiod. The hatchlings were acclimated for a minimum of 8 weeks at which time diving behaviour, aquatic respiration and swimming performance of both acclimation groups were examined at the two test temperatures, 17ºC and 28ºC. This research was approved by Queensland Parks and Wildlife Service (SPP-WISP-01477903) and supported by the University of Queensland Ethics Committee (AEC – ZOO/ENT/595/04/URG and ZOO/ENT/731/05/URG.

Diving Behaviour

The diving behaviour of the cold and warm acclimated turtles was examined at both 17ºC and 28ºC test temperatures. The behavioural experiments were conducted in a large glass aquarium 150 x 60 x 65 cm (*l* x *w* x *d*). A Perspex sheet was placed down the middle of the aquarium to divide the tank into two halves, allowing for two groups of turtles to be recorded simultaneously. The aquarium contained a pebble substrate and benthic shelters, with the water being constantly filtered (Aqua One hang-on filter). The photoperiod was set at 12L:12D, with red lights used during the dark period to facilitate recording. Turtles were placed into each side of the tank in groups of three and given 24 h to become accustomed to the new environment. Diving behaviour was then recorded using a closed circuit video camera and time lapse VCR. Recording of diving behaviour ranged between 12 h and 86 h depending on the activity levels of turtles within a group. At 17°C, a longer recording period was required so that an adequate number of dives were recorded, while less time was required at the 28°C due to the short dive lengths. Turtles were given a minimum of one week to rest before being tested at the remaining experimental temperature. Video tapes were analysed for resting dive durations. A resting dive was defined as a dive in which the turtle sat relatively still on the bottom of the tank for a period of greater than one minute.

Respiration

Resting metabolic rate, mean aerial and aquatic respiration and percent aquatic respiration of acclimated animals were measured using closed-box respirometry. Respiration experiments were conducted in controlled temperature rooms set at 17ºC or 28ºC, with red lights used to simulate darkness to ensure that measures were of resting metabolic rate. The respirometers consisted of a 900 ml circular container with an air-tight lid that was filled with 500 ml of water to create an aquatic base chamber. The remaining 400 ml of the respirometer functioned as an aerial chamber into which the turtles could surface to breathe. Two-way taps fitted to the aquatic and aerial chambers permitted water and air sampling, respectively. Prior to the beginning of experiments the turtles were weighed and wiped down with a 70% ethanol solution to remove oxygen-consuming bacteria. Turtles were given 18 h to adjust to the respirometers and recover from handling stress before measurements began. The water in the respirometer was aerated continuously to maintain normoxia prior to respiratory measurements. At the beginning of the experimental trial, the aerators were switched off and mineral oil was added to the surface of the water to prevent diffusion of gas across phases. The respirometers were then sealed and initial samples of water (5 ml) and air (20 ml) were taken from the sampling ports via a syringe to establish baseline levels of O_2. Turtles were then left for either 1 h (28ºC) or 2 h (17ºC) before the final aquatic and aerial gas samples were taken. Each turtle underwent a total of 4 trials with a minimum 1 h period allowed between each trial. The hatchlings were given a minimum of three days rest before being tested at the other experimental temperature.

For gas analysis, water samples were injected into a water jacket that housed a Cameron oxygen electrode. The oxygen electrode was connected to a Cameron oxygen meter (Cameron Instrument Company model OM200) that measured the aquatic PO_2. The aerial sample was passed through H_2O-absorbing Drierite before entering a gas analyser (ADInstruments model ML205), which measured percent oxygen. The Cameron oxygen meter and gas analyser were connected to a PowerLab/4s and data recorded on Chart software (v 4.2). Controls were conducted to account for consumption of oxygen by the electrode and to confirm the effectiveness of the mineral oil as a barrier to gas diffusion.

The rate of aerial oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the standard equations of Vleck (1987):

$$VO_2 = \frac{[((V - ViH_2O)(Fi - Fe) - Fe(VCO_2))/(1-Fe)]}{t}$$

where,

V = volume of air in chamber (ml)

ViH_2O = initial volume of water vapor in chamber (ml)

VCO_2 = volume of CO_2 produced during experiment (ml)

Fi = fractional concentration of O_2 at the start of the experiment

Fe = fractional concentration of O_2 at the end of the experiment

t = length of trial (h)

The rate of aquatic oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the following equation modified from King and Heatwole (1994b):

$$VO_2 = \frac{\Delta DO_2 \times \beta \times VH_2O}{t}$$

where,

t = length of trial (h)

ΔDO_2 = change in dissolved O_2 over trial (mmHg)

VH_2O = volume of water in respirometer (L)

β = oxygen co-efficient of capacitance (ml O_2 L^{-1}mmHg)

For analysis, mean VO_2 aerial and VO_2 aquatic were standardised to a 35g turtle then presented on a per gram basis (ie VO_2 in ml O_2 $h^{-1}g^{-1}$). Total VO_2 (metabolic rate) (ml O_2 h^{-1} g^{-1}) equaled the sum of minimum aerial and minimum aquatic VO_2, while percent aquatic respiration was estimated by expressing maximum aquatic VO_2 as a proportion of total $VO_{2.}$

Swimming Performance

Swimming performance was examined in a 30 cm x 31cm x 14.5 cm (*l* x *w* x *h*) temperature-controlled tank. Pilot studies revealed that the burst swimming response of the turtles was to push off with their hind legs and swim along the bottom of the tank rather than to swim within the water column. To provide the gripping surface that is needed for this type of locomotion, a layer of plastic mesh was glued to the bottom of the tank.

The burst swimming performance of the cold and warm acclimated turtles was examined at 17ºC and 28ºC. Turtles were allowed to adjust to the test temperature overnight and were then placed individually into the experimental tank and allowed to settle on the bottom. A burst swimming response was generated by tapping the bottom of the tank behind the turtle. This action startled the turtle, resulting in 2-3 burst swimming strokes. A mirror positioned above the tank at a 90º angle reflected the movement of the turtle which was captured by a high speed camera at 100 frames s^{-1} (Red Lake Imaging Corporation USA). The camera was connected to a computer which recorded the video on Motion Scope software. Maximum speed was calculated by documenting the (x,y) position of the turtle for each frame of movement beginning from the first sign of locomotion. The distance between the frames was divided by time to produce speed (cm s^{-1}). Four burst swimming sequences were analysed for each individual and the maximum speed recorded. To account for body size difference between acclimation groups the maximum speed for each individual was divided by carapace length and then multiplied by 7 to standardise velocity to a 7 cm turtle.

Statistical Analysis

The effects of test temperature and acclimation temperature on diving behaviour, respiration and swimming performance were analysed using two-way repeated measures analysis of variance (ANOVA). Where normality failed, data was log-transformed before analysis. Significant differences were further analysed using the Student-Newman-Keuls post hoc test. All results are presented as mean ± standard error unless otherwise stated, with statistical significant differences considered at the level $P < 0.05$.

RESULTS

Diving Behaviour

There was a significant effect of thermal acclimation on mean and maximum dive durations of *E. macrurus* (mean dice duration - $F_{1,25}$ = 14.30, P = 0.003; maximum dive duration - $F_{1,25}$ = 41.26, $P < 0.001$) (Fig. 3.1). However, the effect of thermal acclimation on dive durations was highly dependent upon test temperature (mean dive duration - $F_{1,25}$ = 21.19, $P < 0.001$; maximum dive duration - $F_{1,25}$ = 19.34, $P < 0.001$). Mean dive duration at 17ºC equaled 765 ± 213 min in the cold acclimated turtles compared to 125 ± 21 min in the warm acclimated group ($P < 0.001$) (Fig. 3.1). At 17 ºC, maximum dive duration of the cold acclimated turtles was 2667 ± 420 min which was significantly greater than the maximum value of 366 ± 71 min recorded for the warm acclimated turtles (Fig. 3.1).

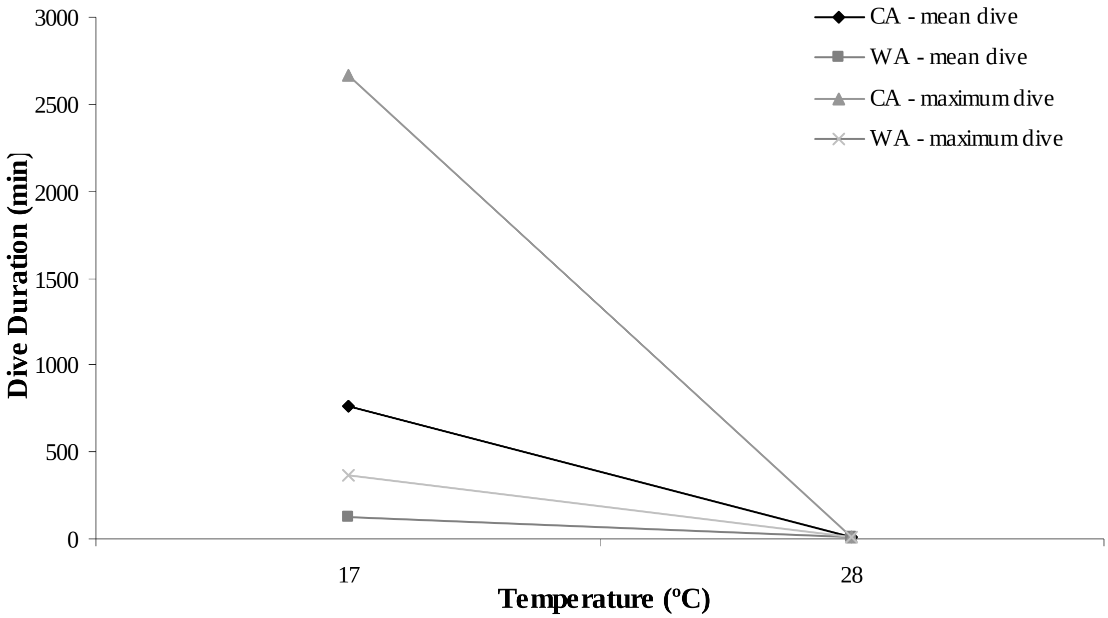

Figure 3.1 - Effect of temperature on mean and maximum dive duration in cold (CA) and warm (WA) acclimated turtles.

There was no effect of thermal acclimation on mean or maximum dive duration at the 28ºC test temperature where mean dive durations were 8.36 ± 0.58 min and 6.78 ± 0.96 min for the cold and warm acclimated groups respectively ($P > 0.05$). Mean and maximum dive durations were greater at 17ºC than at 28ºC for both the cold and warm acclimated turtles (mean dive duration - $F_{1,25} = 655.45$, $P < 0.001$, maximum dive duration - $F_{1,25} = 486.51$, $P < 0.001$) (Fig. 3.1).

Respiration

The resting metabolic rate of *Elusor macrurus* hatchlings did not thermally acclimate ($F_{1,25} = 0.24$, $P > 0.05$) (Fig. 3.2). The metabolic rate of both acclimation groups was however influenced by test temperature ($F_{1,25} = 50.84$, $P < 0.001$) (Fig. 3.2). Metabolic rate increased from 0.08 ± 0.008 ml O_2 h^{-1} g^{-1} to 0.16 ± 0.012 ml O_2 h^{-1} g^{-1} in the cold acclimated turtles and 0.06 ± 0.009 ml O_2 h^{-1} g^{-1} to 0.17 ± 0.017 ml O_2 h^{-1} g^{-1} in the warm acclimated turtles at 17ºC and 28ºC respectively. The Q_{10} value for the cold and warm acclimated animals was 1.80 and 2.46, respectively.

The observed increase in metabolic rate at 28ºC was a direct result of an increase in both aerial and aquatic oxygen consumption (aerial - $F_{1,25} = 54.15$, $P < 0.001$; aquatic - $F_{1,25} = 27.95$, $P < 0.001$) (Fig. 3.3). Aerial oxygen consumption increased from 0.08 ± 0.006 and 0.065 ± 0.008 ml O_2 h^{-1} g^{-1} at 17ºC to 0.158 ± 0.012 and 0.178 ± 0.021 ml O_2 h^{-1} g^{-1} at 28ºC in the cold and warm acclimated turtles respectively. As test temperature increased from 17ºC to 28ºC, aquatic oxygen consumption of the cold acclimated turtles increased from 0.018 ± 0.0008 ml O_2 h^{-1} g^{-1} to 0.023 ± 0.002 ml O_2 h^{-1} g^{-1}, while that of the warm acclimated turtles increased from 0.014 ± 0.0008 ml O_2 h^{-1} g^{-1} to 0.018 ± 0.001 ml O_2 h^{-1} g^{-1}. There was no effect of acclimation temperature on aerial oxygen consumption at either 17ºC or 28ºC ($F_{1,25} = 0.013$, $P > 0.05$). Aquatic oxygen consumption was however significantly influenced by acclimation temperature with consumption higher in the cold acclimated turtles than in the warm acclimated turtles at both test temperatures (17ºC - $P = 0.036$, 28ºC – $P < 0.05$) (Fig. 3.3).

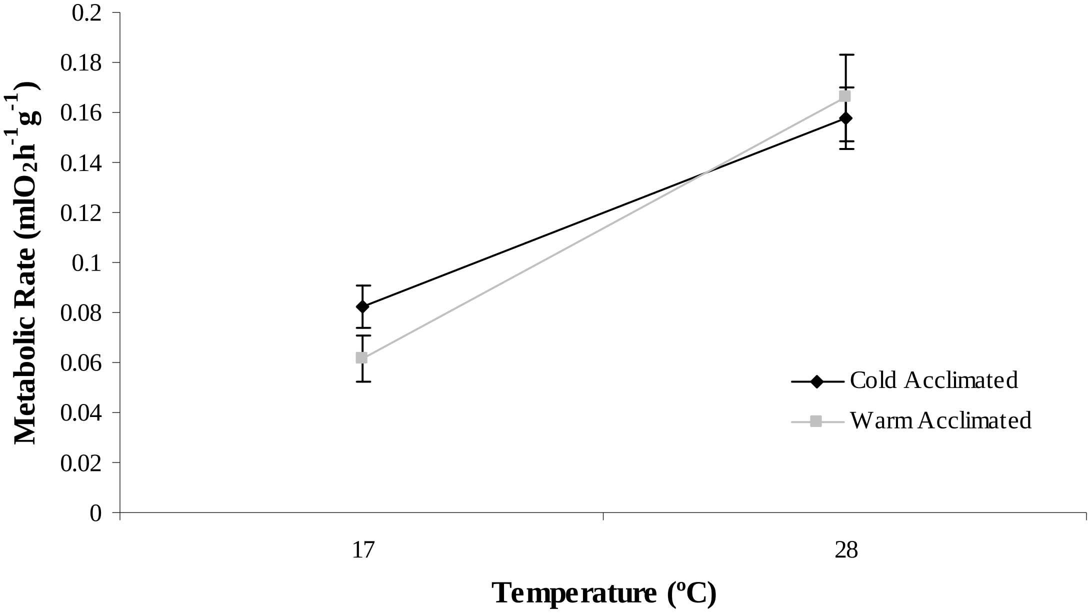

Figure 3.2 - The effect of temperature on metabolic rate in cold and warm acclimated turtles. Values represent mean ± SEM.

Unlike aquatic oxygen consumption, percent aquatic respiration ((maximum aquatic oxygen consumption / total MR) x 100) did not thermally acclimate at either test temperatures ($F_{1,25} = 0.007$, $P > 0.05$). There was however a significant effect of temperature on percent aquatic respiration in the warm acclimated *E. macrurus* but no effect in the cold acclimated turtles ($P > 0.05$). At 17ºC aquatic respiration was 21.54% in cold acclimated turtles and 24.20% in warm acclimated turtles. When tested at 28ºC, aquatic respiration in the cold and warm acclimated turtles was 16.07% and 13.73% respectively.

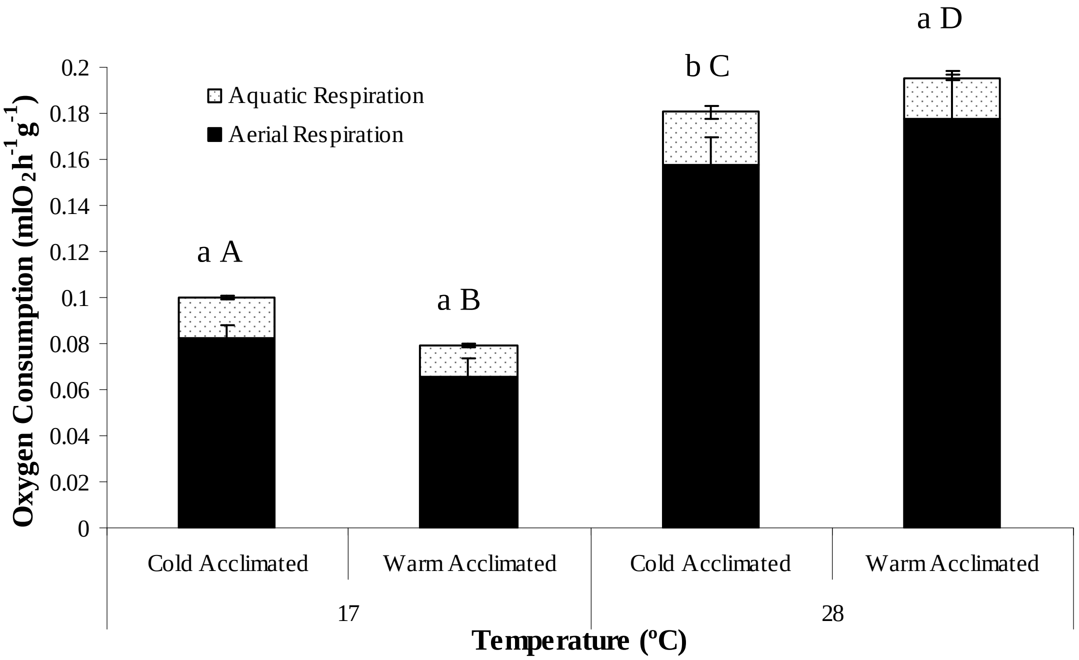

Figure 3.3 - The influence of temperature on mean aerial and aquatic oxygen consumption in cold and warm acclimated Mary River turtles. Values represent mean ± SEM.

Swimming Performance

Burst swimming speed of *E. macrurus* did not thermally acclimate ($F_{1,25} = 0.03$, $P > 0.05$) (Fig. 3.4). Burst swimming speed was however significantly faster at 28°C than 17°C in both cold and warm acclimated turtles ($F_{1,25} = 33.52$, $P < 0.001$) (Fig. 3.4). Maximum velocity of the cold acclimated turtles increased from 68.0 ± 2.5 cm s^{-1} at 17°C to 79.1 ± 2.9 cm s^{-1} at 28°C. Maximum velocity of the warm acclimated turtles increased from 67.3 ± 1.5 cm s^{-1} to 78.8 ± 1.6 cm s^{-1} as temperature increased. The Q_{10} for this relationship was 1.15 for both acclimation groups.

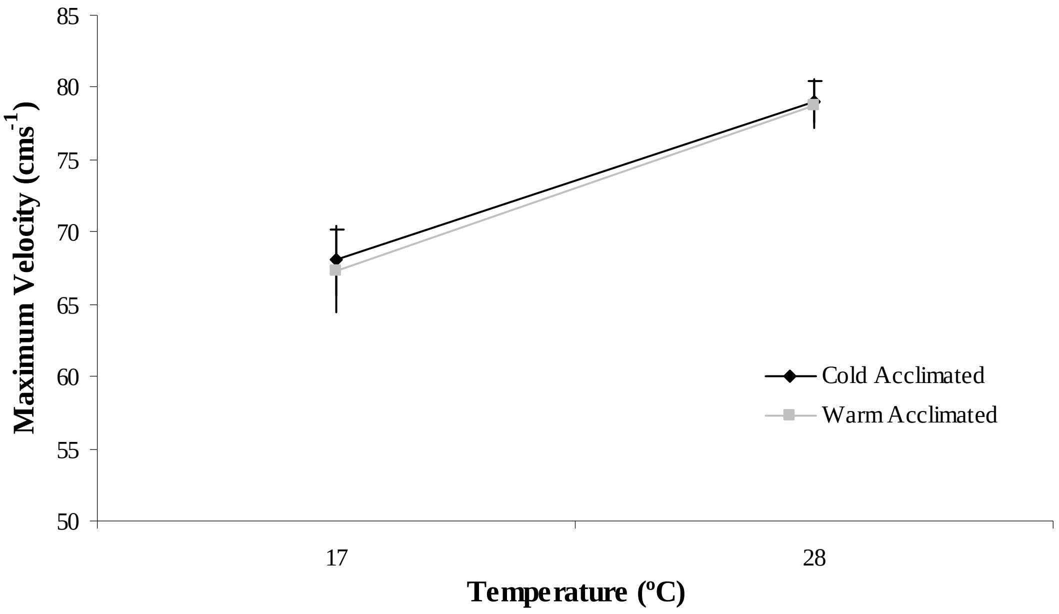

Figure 3.4 - The effect of temperature on the swimming performance of cold and warm acclimated turtles. Values represent mean ± SEM.

DISCUSSION

Elusor macrurus hatchlings displayed partial thermal acclimation with respect to their diving behaviour however, the turtles did not acclimate according to our hypothesis. At 28ºC, dive duration was expected to increase after warm acclimation due to a decrease in metabolic rate and increase in aquatic respiration. However, thermal acclimation was not observed for *E. macrurus* hatchlings maintained at a warm temperature with regards to either of these two parameters. As a result, no difference in dive duration was observed between cold and warm acclimated turtles at 28ºC. Alternatively, hatchlings acclimated to a cold temperature were expected to display a decreased dive duration at 17ºC as a result of an increased metabolic rate. Yet acclimation to a cold temperature resulted in a six fold increase in dive duration.

High costs associated with aquatic respiration at 28ºC may explain why warm acclimated turtles did not thermally acclimate dive duration. Aquatic respiration in *Elusor macrurus* can occur via diffusion across the skin or by active ventilation of the cloacal bursae. Tidal ventilation associated with the cloacal bursae requires a continual change in the kinetic energy of water, which compared to air is 60 times as viscous and contains 30 times less oxygen per volume (Dejours, 1994). In accordance with the theory of optimal breathing, as metabolic rate increases with increasing temperature, turtles are likely to become more reliant upon aerial respiration equating to shorter dive durations (Kramer, 1988). For the softshell turtle, *Apalone ferox*, reliance upon aquatic respiration decreased from 44.1% to 17.4% as temperature increased from 15ºC to 25ºC (Prassack et al., 2001). Additionally, surfacing frequency for the softshell turtle increased over three fold with the associated temperature increase (Prassack et al., 2001). Aquatic respiration in the warm acclimated *E. macrurus* hatchlings accounted for only 14-16% of total oxygen consumption suggesting that at 28ºC hatchlings were primarily relying upon pulmonary oxygen supplies.

At 17ºC, the reduction in metabolic rate of both the cold and warm acclimated turtles resulted in an increased reliance upon aquatic respiration. This increase in aquatic respiration permitted an increase in dive duration of both acclimation groups however, the dive duration of the cold acclimated turtles was six times longer than that of the warm acclimated hatchlings. Some species of freshwater turtles are known to show an inverse compensatory response at low temperatures by entering a state of dormancy (Guppy and Withers, 1999). For example, snapping turtles, *Chelydra serpentina*, acclimated at 10ºC showed a 13% reduction in metabolic rate at 25ºC compared to turtles acclimated at 25ºC (Gatten, 1978). However, our results suggest that at

17ºC, *E. macrurus* hatchlings do not enter a state of metabolic depression (ie, metabolic rate did not differ between cold and warm acclimated animals at 17ºC). Instead, the increase in dive duration observed in cold acclimated turtles tested at 17ºC may be due to an increased reliance on aquatic respiration which was opposite to our original hypothesis.

Aquatic oxygen consumption of the cold acclimated turtles was significantly greater than that of the warm acclimated turtles at both test temperatures. This result suggests that the cold acclimated turtles underwent a physiological and or morphological adaptation that increased their efficiency to extract aquatic oxygen. Obtaining accurate measures of percent aquatic respiration is generally difficult due to the turtle's ability to voluntarily change their reliance on both aerial and aquatic oxygen consumption. In a shallow respirometry chamber, hatchlings are likely to increase their reliance on aerial oxygen consumption due to the costs associated with aquatic respiration under these conditions (Mathie and Franklin, 2006). Measures of percent aquatic respiration are therefore likey to be underestimated. Percent aquatic respiration in *E. macrurus* did not differ between acclimation groups, however the diving behaviour data supports the increase in aquatic oxygen consumption seen in the cold acclimated turtles.

The dramatic increase in dives at 17°C indicates a greater reliance on aquatic oxygen consumption, as exemplified by one cold acclimated hatchling that recorded a dive of over 2.5 days. This extended dive duration marks one of the longest submergences recorded for any freshwater turtle in captivity and was five times longer than the maximum dive duration of any warm acclimated *E. macrurus* hatchling. Similarly, in a study on the natural diving behaviour of *Rheodytes leukops*, dive durations of over three weeks where recorded during winter (16.5ºC) compared to a maximum duration of only 2.39 days in summer (27.3ºC) (Gordos et al., 2003b). Based upon these results, Gordos et al. (2003b) suggested that freshwater turtles can use aquatic respiration as an effective overwintering strategy at cooler temperatures.

Traditionally, studies of the beneficial acclimation hypothesis have used locomotion as the performance measure (Bennett, 1990; Johnson and Bennett, 1995; Wilson and Franklin, 1999; O'Steen and Bennett, 2003; Wilson, 2005). In this study, the burst swimming speed of *E. macrurus* hatchlings did not thermally acclimate. This result supports the hypothesis that thermal acclimation of locomotor performance in reptiles is minimal in its extent (Bennett, 1990; Angilletta et al., 2002). However, research into this area is limited and generally restricted to terrestrial species. Instead, thermal acclimation is believed to apply more to aquatic species that are protected from large daily variations in temperature due to the thermal stability of water, but are exposed to marked changes in seasonal temperature (Seebacher, 2005). This hypothesis is supported by a study on amphibians where the highly aquatic larval form of the stripped marsh frog, *Limnodynastes peronii*, was found to thermally acclimate locomotor performance, while no difference was observed in swimming or jumping performance of adults (Wilson and Franklin, 2000). Although *E. macrurus* fits into the category that is most likely to benefit from thermal acclimation (highly aquatic, no thermoregulation), no differences were observed in burst locomotor performance. Burst locomotor performance is however a measure of anaerobic capacity which is known to be less thermally dependent than aerobic metabolism (Bennett, 1990; O'Steen and Bennett, 2003). The effect of thermal acclimation on sustained swimming performance may differ to that of burst swimming performance and therefore further research is required to support the lack of acclimation in *E. macrurus*.

This study has demonstrated that the respiratory physiology of the Mary River turtle is influenced by water temperature and this has a significant effect on hatchling behaviour. This dependence upon aquatic respiration in this species raises concerns about the potential impact of river regulation on the species' survival. Water storage impoundments are known to significantly change the ecological properties of rivers by converting narrow flowing systems into large stagnant reservoirs (Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001; Arthington, 2003). Environmental costs of impoundments include loss of pool-riffle sequences, reduced water velocity, decreased aquatic oxygen levels, increased water depth and increased temperature stratification (Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001; Turner and Erskine, 2005). Temperature stratification in impoundments results in the formation of a warmer than usual surface layer (Turner and Erskine, 2005). Hatchling turtles are likely to occur near the surface margins of reservoirs and hence will more likely inhabit this warmer stagnant water. This study has shown that at higher temperatures the turtle's reliance on aerial respiration will increase leading to a reduction in dive duration and increase in surfacing frequency. Hatchlings that inhabit the warm surface layers of impoundments may therefore potentially increase their exposure to predators due to this change in respiratory physiology and diving behaviour.

CHAPTER FOUR

IMPLICATIONS OF RIVER DAMMING: THE INFLUENCE OF AQUATIC HYPOXIA ON THE DIVING PHYSIOLOGY AND BEHAVIOUR OF THE ENDANGERED MARY RIVER TURTLE, *ELUSOR MACRURUS*

ABSTRACT

River impoundments are characterised by low oxygen levels as a result of reduced water velocity and increased water depth. Bimodally respiring turtle species are likely to be highly sensitive to changes in aquatic PO_2 with decreases in oxygen levels impacting upon their diving ability. The acute and long-term effects of aquatic hypoxia on dive duration, oxygen consumption and blood respiratory properties were examined in hatchlings of the endangered Mary River turtle, *Elusor macrurus*. It was hypothesised that acute exposure to aquatic hypoxia would cause a decrease in dive duration as a consequence of a decrease in reliance on aquatic respiration. With long-term exposure to hypoxia, we predicted that *E. macrurus* would have the capacity to compensate for the acute effect of hypoxia and that dive duration would increase due to an increase in aquatic respiration, haemoglobin concentration and oxygen affinity (P_{50}). When exposed to hypoxic conditions, aquatic respiration in *E. macrurus* was substantially reduced resulting in a 51% decrease in dive duration. Contrary to our predictions, *E. macrurus* hatchlings did not acclimate and long-term exposure to hypoxic conditions caused *E. macrurus* to lose significantly more oxygen to the hypoxic water than the normoxic acclimated turtles. The exacerbated effect of long-term hypoxia on the respiratory physiology and diving ecology of *E. macrurus* raises

concerns about the impacts of long-term environmental change as a result of habitat alteration, on the survival of freshwater turtle populations.

INTRODUCTION

River damming is known to significantly change the ecological properties of rivers by converting narrow flowing systems into large stagnant reservoirs (Ward and Stanford, 1989; Tucker, 1999; Bodie, 2001; Arthington, 2003). Environmental impacts associated with river damming include loss of pool-riffle sequences, reduced water flow, increased water depth and decreased aquatic oxygen levels (Ward and Stanford, 1989; Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001). The impacts of these physio-chemical changes have largely been investigated in fish populations (Park et al., 2003; Antonio et al., 2007; Clarke et al., 2007; Fukushima et al., 2007; Xie et al., 2007) with very few studies considering the effects on freshwater turtles. In Australia, population monitoring results suggest the impacts of river damming on freshwater turtles are variable, with some species declining in numbers and others flourishing (Tucker, 1999; Limpus et al., 2002; Arthington, 2003). Identifying the cause of these declines has been identified as an important aspect of species conservation (Tucker, 1999). Conservation physiology is a relatively new field of scientific research which through the use of physiological tools, aims to provide a detailed mechanistic understanding of the causes of conservation problems (Wikelski and Cooke, 2006). Knowledge of the physiological relationships between an animal and their environment provides an understanding of the consequences of habitat alteration and can be used to predict the effects of future environmental change (Wikelski and Cooke, 2006). With increasingly more water infrastructure proposed for the future, understanding how freshwater turtles interact with a changing environment is essential for species conservation.

Freshwater turtles spend the majority of their time underwater surfacing occasionally to respire aerially via their lungs. Some species of turtle also possess the ability to respire aquatically acquiring oxygen from the water via their skin, buccopharynx and/or cloacal bursae (Gage and Gage, 1886; Smith and James, 1958; Girgis, 1961; Belkin, 1968; Stone et al., 1992a; King and Heatwole, 1994a). By supplementing their punctuated aerial oxygen supply with aquatic respiration, the turtles are able to extend their dive duration and reduce surfacing frequency (Belkin, 1968; Stone et al., 1992b; Bagatto et al., 1997; Bagatto and Henry, 1999; Prassack et al., 2001; Gordos and Franklin, 2002; Gordos et al., 2004a; Mathie and Franklin, 2006). Ecological benefits associated with an extended dive duration include increased time available for foraging and decreased predator exposure (Kramer, 1988).

Aquatic respiration and diving behaviour in bimodally respiring animals is however dependent upon environmental conditions such as aquatic oxygen levels (Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003a,b; Mathie and Franklin, 2006). Decreasing aquatic PO_2 from normoxic (155 mmHg) to anoxic (0 mmHg) conditions results in a 60% decrease in the dive duration of *R. leukops* at 15ºC (Priest and Franklin, 2002). The relationship between dive duration and aquatic PO_2 is a direct result of respiratory partitioning (Yu and Woo, 1985; Mattias et al., 1998; Geiger et al., 2000; Seymour et al., 2004; Randle and Chapman, 2005; Alton et al., 2007; Clark et al., 2007). At high levels of aquatic PO_2, diffusion of oxygen across the aquatic respiratory organs is enhanced and reliance on aquatic respiration increases. As ambient PO_2 decreases, the PO_2 diffusion gradient (water : blood) is reduced and energetic costs associated with aquatic respiration increase, decreasing the effectiveness of aquatic respiration (Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003b;

Jackson, 2007). For example, aquatic respiration in the bimodally respiring fish, *Channa maculate*, decreased from 21% in normoxia (130 mmHg) to 13% in hypoxia (20 mmHg) while aerial respiration increased proportionally (Yu and Woo, 1985). The respiratory responses of bimodally respiring turtles to changes in aquatic PO_2 has received little attention however an initial study on *Chrysemys picta* reported a 50% decrease in aquatic respiration under hypoxic conditions (Prassack et al., 2001).

The relationship between aquatic PO_2 and the diving physiology and behaviour of bimodal breathers suggests that river damming may have a serious impact on turtle physiological ecology as a result of the low oxygen levels in this environment. However, with long-term exposure to hypoxia, a number of animals have been reported to initiate a range of physiological responses that can compensate for the acute effects of aquatic hypoxia (Prosser, 1991; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). For example, after long-term exposure to aquatic hypoxia, some fish species are able to increase their aquatic oxygen uptake efficiency by increasing the haemoglobin concentration and oxygen affinity of their blood (Manwell, 1960; Grigg, 1969; Wood and Johansen, 1972; Lomholt and Johansen, 1979; Soivio et al., 1980; Jensen and Weber, 1982; Wells et al., 1989; Val et al., 1998; Wu, 2002). The beneficial acclimation hypothesis suggests that in a particular environment, an individual that has had the chance to acclimate to those conditions will have a higher performance/fitness than an individual that has not undergone an acclimatory response to those conditions (Prosser, 1991; Leroi et al., 1994; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). The benefits of hypoxic acclimation have been demonstrated in male mosquito fish (*Gambusia holbrooki*). Fish acclimated to hypoxic conditions exhibited an increase in sustained swimming performance and decrease in reliance on aquatic surface respiration in hypoxic conditions which resulted in an increase in reproductive performance compared to non-acclimated males (Carter and Wilson, 2006).

The aim of this study was to examine the influence of acute and long-term exposure to aquatic hypoxia on the diving physiology and behaviour of the bimodally respiring turtle *Elusor macrurus*. *Elusor macrurus* is an endangered turtle species that is endemic to the Mary River, Australia. It was hypothesised that compared to normoxic conditions, acute exposure to aquatic hypoxia would cause a decrease in dive duration as a consequence of a decrease in reliance on aquatic respiration. After long-term exposure to aquatic hypoxia, the dive duration of the turtles was expected to increase under hypoxic conditions due to an increased reliance on aquatic respiration and increase in blood respiratory properties. The effects of aquatic hypoxia on turtle diving physiology and behaviour were examined in hatchling turtles. Hatchling turtles have a higher reliance on aquatic respiration than adults and hence may be more susceptible to changes in environmental oxygen levels.

MATERIALS AND METHODS

Turtle Collection and Husbandry

Elusor macrurus eggs were collected from nesting sites along the Mary River near the town of Tiaro (25.72606ºS, 152.58265ºE) and transported to The University of Queensland. The eggs were incubated in river bank sand in a Styrofoam box and temperature was allowed to fluctuate naturally. One week after hatching the turtles were placed into normoxic (N=10) or hypoxic (N=10) acclimation treatments. Acclimation tanks (140 L) were identical, consisting of basking platforms, shelters and aerators. Turtles were exposed to a photoperiod of 12L:12D and water temperature of 23ºC. The normoxic acclimation treatment was bubbled with air (PO_2 = 150 - 155 mmHg) while the hypoxic acclimation treatment was bubbled with nitrogen to achieve aquatic hypoxia ($PO_2 < 30$ mmHg). Fifty percent of the water surface in the tanks was covered by polystyrene to minimise gas exchange across the air-water interface. The hatchlings were acclimated for a minimum of five months at which time diving behaviour, aquatic respiration and blood oxygen affinity of both acclimation treatment groups were examined at the two aquatic oxygen levels, 30 mmHg and 155 mmHg. This research was approved by Queensland Parks and Wildlife Service (SPP-WISP-01477903) and supported by the University of Queensland Ethics Committee (AEC – ZOO/ENT/731/05/URG and ZOO/ENT/133/07/URG).

Oxygen Consumption

The aerial and aquatic oxygen consumption of the turtles was measured using closed-box respirometry. Experiments were conducted at both 155 mmHg (normoxic) and 30 mmHg (hypoxic) for each of the acclimation groups. The measurements were recorded at 23ºC and a red light was used to simulate darkness for the purpose of reducing turtle activity within the chamber. The respirometers consisted of a 1000 ml circular container with an air-tight lid that was filled with 500 ml of water to create an aquatic base chamber. The remaining 500 ml of the respirometer functioned as an aerial chamber into which the turtles could surface to breathe. Two-way taps fitted to the aquatic and aerial chambers permitted water and air sampling.

Prior to the beginning of experiments the turtles were weighed and wiped down with a 70% ethanol solution to remove oxygen-consuming bacteria. Turtles were then placed into the respirometers and given 18 h to adjust to the chamber and recover from handling stress before measurements began. During this period, the water in the respirometer was bubbled with either air or nitrogen to achieve an aquatic PO_2 of 155 ± 10 mmHg or 30 ± 10 mmHg, respectively. Mineral oil was added to the surface of the water during this period to prevent diffusion of gas across phases. At the beginning of the experimental trial, the gas was switched off and the respirometers were then sealed and initial samples of water (5 ml) and air (20 ml) were taken from the sampling ports via a syringe to establish baseline levels of O_2. After an experimental period of 2 h, the final aquatic and aerial gas samples were taken and analysed for oxygen content. Aquatic samples were injected into a water jacket that housed a Cameron oxygen electrode. The oxygen electrode was connected to a Cameron oxygen meter (Cameron Instrument Company model OM200) that measured aquatic PO_2. The aerial sample passed though H_2O-absorbing Drierite before entering a gas analyser (ADInstruments model ML205) which measured percent oxygen. The Cameron oxygen meter and gas analyser were connected to a PowerLab/4s, with data recorded on Chart software (v 4.2). Controls were conducted to account for consumption of oxygen by the electrode and to adjust for gas diffusion between the aerial and aquatic chambers. Each turtle underwent a total of 4 trials with a minimum 2 h period allowed between each trial.

The rate of aerial oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the standard equation of Vleck (1987):

$$VO_2 = \frac{[((V - ViH_2O)(Fi - Fe) - Fe(VCO_2))/(1-Fe)]}{t}$$

where,

V = volume of air in chamber (ml)

ViH_2O = initial volume of water vapor in chamber (ml)

VCO_2 = volume of CO_2 produced during experiment (ml)

Fi = fractional concentration of O_2 at the start of the experiment (%)

Fe = fractional concentration of O_2 at the end of the experiment (%)

t = length of trial (h)

The rate of aquatic oxygen consumption (VO_2 ml O_2 h^{-1}) was determined using the following equation modified from King and Heatwole (1994b):

$$VO_2 = \frac{\Delta DO_2 \times \beta \times VH_2O}{t}$$

where,

t = length of trial (h)

ΔDO_2 = change in dissolved O_2 over trial (mmHg)

VH_2O = volume of water in respirometer (L)

β = oxygen co-efficient of capacitance (ml O_2 L^{-1}mmHg)

To account for the allometric scaling of metabolic rate both aerial VO_2 and aquaticVO_2 were scaled to 0.75 and standardised to an average sized turtle (12 g). Percent respiration was estimated by expressing aquatic VO_2 as a proportion of total VO_2.

Diving Behaviour

The diving behaviour of the normoxic and hypoxic acclimated turtles was examined at two test PO_2 levels (155 ± 10 mmHg and 30 ± 10 mmHg) in a large glass aquarium 150 x 60 x 65 cm (*l* x *w* x *d*). The aquarium was divided in half by a sheet of clear Perspex. The Perspex physically divided the aquarium however water was able to diffuse across the tank via holes that were drilled into the sheet. The front section of the tank functioned as the turtle observational area, while the back section contained the air stones and was covered by a sheet of polystyrene that limited diffusion of gas across the air-water interface. Air was bubbled into the tank via the air-stones for the 155 mmHg treatment while nitrogen was bubbled into the tanks for the 30 mmHg treatment. The aquarium contained a pebble substrate and benthic shelters with water temperature maintained at 23°C. The photoperiod was set at 12L:12D, with red lights used during the dark period to allow recording. Four individual turtles from one acclimation treatment were placed into the aquarium and given 24 h to become accustomed to the new environment. Diving behaviour was then recorded for 24 h using a networked video camera connected to a remote PC with Milestone Surveillance software. Turtles were allowed a minimum of one weeks rest before being tested at the second PO_2 level. The exposure of both acclimation groups to the test oxygen levels was randomised. Video files were analysed for resting dive durations which were defined as dives where the turtle sat still on the bottom of the tank for a period of greater than one minute. Mean and maximum dive durations were calculated for each turtle using a custom-written program (M. Gordos).

Blood Respiratory Properties

To determine the blood respiratory properties of the normoxic and hypoxic acclimated turtles a 70-90 µl blood sample was collected from the cervical sinus of the turtles using a 25 gauge needle and 1 ml syringe (Rogers and Booth, 2004). The needle tip and plunger of the syringe were dusted with sodium heparin to prevent coagulation. The blood sample was then transferred into a 0.5 ml Eppendorf tube where sub-samples were collected for analysis of haemoglobin concentration (Hb), haematocrit (Hct), and the determination of P_{50}, which was defined as the PO_2 at which 50% of the haemoglobin was saturated.

Hb concentration was determined using a spectrophotometer (Pharmacia LKB ultrospec III). Five micro-litres of blood were mixed with 1 ml of Drabkins solution, with the absorbance being recorded at 540 nm. Hb concentration was then determined from an average absorbance reading (Lewis et al., 2001). A sub-sample of blood collected in a capillary tube was centrifuged at 1000 rpm for 3 min, with Hct determined as the percent of red blood cells per sample volume. A Hemox analyser Model B (TCS Scientific corp. New Hope, PA) was used to determine the P_{50} values. A 50 µl sample of blood was added to 5 ml of buffered saline (Hemox^{TM} Solution), 20 µl of bovine serum albumin (Additive-A) and 10 µl of antifoaming agent. The blood sample was then added to the Hemox machine where the deoxygenation and oxygenation curves were run at 23ºC and the P_{50} values recorded at 5% CO_2.

Statistical Analysis

The effects of acute and long-term exposure to aquatic hypoxia on diving behaviour and oxygen consumption were analysed using a two-way repeated measures analysis of variance (ANOVA).

Where normality or equal variance failed, data was transformed before analysis. Percentage aquatic respiration was transformed using an inverse hyperbolic tangent transformation due to the negative percentages in this variable. Significant differences were further analysed using the Student-Newman-Keuls post hoc test. The influence of oxygen acclimation on Hb, Hct and P_{50} were analysed using a one-way ANOVA. All results are presented as mean ± standard error unless otherwise stated, with statistical significant differences considered at the level $P < 0.05$.

RESULTS

Oxygen Consumption

Acute and long-term exposure to aquatic hypoxia had a significant effect on the respiratory physiology of the *E. macrurus* hatchlings.

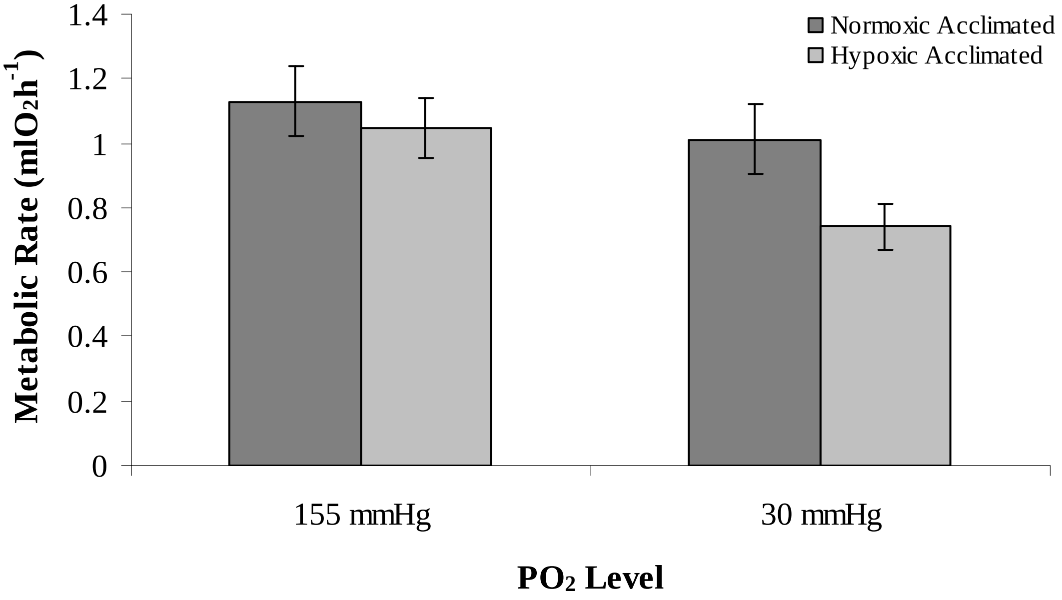

Figure 4.1 - The influence of aquatic PO_2 on metabolic rate (mlO_2h^{-1}) in normoxic and hypoxic acclimated hatchlings. Values represent mean ± SEM.

Overall, the metabolic rate of the hatchlings was not influenced by either aquatic oxygen level ($F_{[1,36]} = 3.1$, $P > 0.05$) or acclimation group ($F_{[1,36]} = 3.5$, $P > 0.05$) (Fig. 4.1), however a difference was recorded when the total oxygen consumption was partitioned according to respiratory medium. When the normoxic acclimated *E. macrurus* hatchlings were exposed to an aquatic PO_2 of 155 mmHg, aquatic respiration supported 9.5 ± 1.81% of the total metabolic rate (Fig. 4.2). Aquatic respiration was significantly reduced when the turtles were exposed to an aquatic PO_2 of 30 mmHg with the normoxic acclimated turtles losing -9.8 ± 4.99% of their total oxygen consumption to the water ($P < 0.001$). Long-term exposure to aquatic hypoxia did not influence aquatic respiration at 155 mmHg (11 ± 1.86%, $P > 0.05$) however there was a significant acclimation effect at 30 mmHg with the hypoxic acclimated turtles losing a higher percentage of aquatic oxygen (-28.4 ± 3.89%) than the normoxic acclimated turtles ($P < 0.001$) (Fig. 4.2).

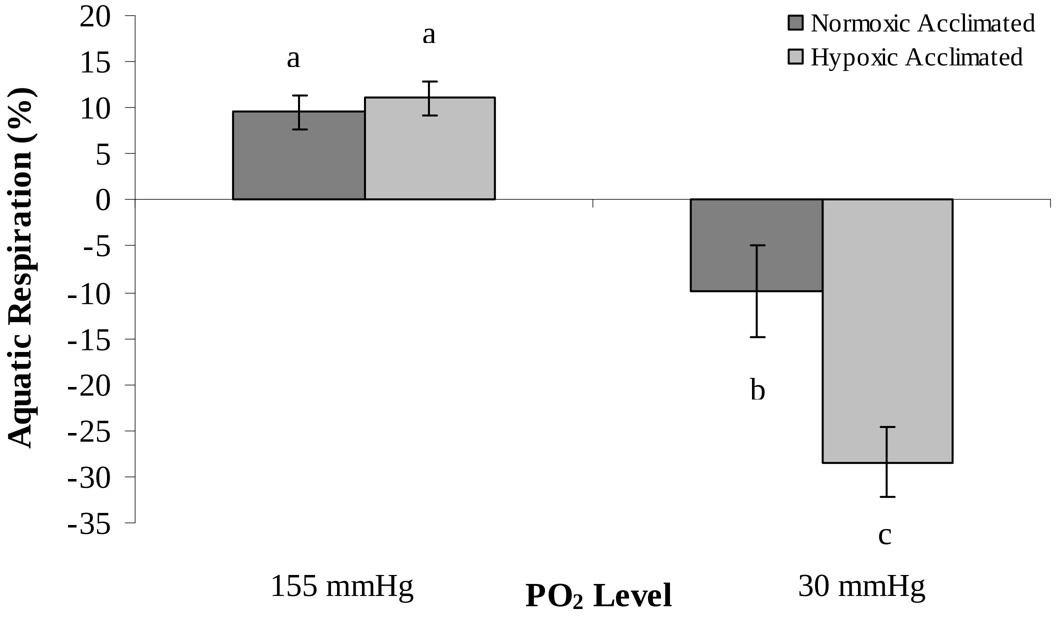

Figure 4.2 - The influence of aquatic PO_2 on aquatic respiration (%) in normoxic and hypoxic acclimated hatchlings. Values represent mean ± SEM. Letter differences indicate significant differences.

Diving Behaviour

Acute exposure to aquatic hypoxia had a significant effect on the diving behaviour of *E. macrurus* hatchlings (mean dive duration – $F_{[1,37]} = 10.21$, $P < 0.01$; maximum dive duration - $F_{[1,36]} = 10.65$, $P < 0.01$). At an aquatic PO_2 of 30 mmHg, the mean dive duration of the normoxic acclimated turtles (7.54 ± 1.4 min) was 51% shorter than the dive durations recorded at 155 mmHg (15.43 ± 4.7 min, $P < 0.01$) (Fig. 4.3). Maximum dive duration was also significantly reduced at 30 mmHg with the normoxic ($P < 0.05$) acclimated turtles recording a dive duration of only 12.8 ± 2.7 min compared to 50.7 ± 20.5 min at 155 mmHg (Fig. 4.4). Long-term exposure to aquatic hypoxia did not significantly affect the diving behaviour of the *E. macrurus* hatchlings (mean dive duration - $F_{[1,37]} = 0.681$, $P > 0.05$; maximum dive duration - $F_{[1,36]} = 0.471$, $P > 0.05$).

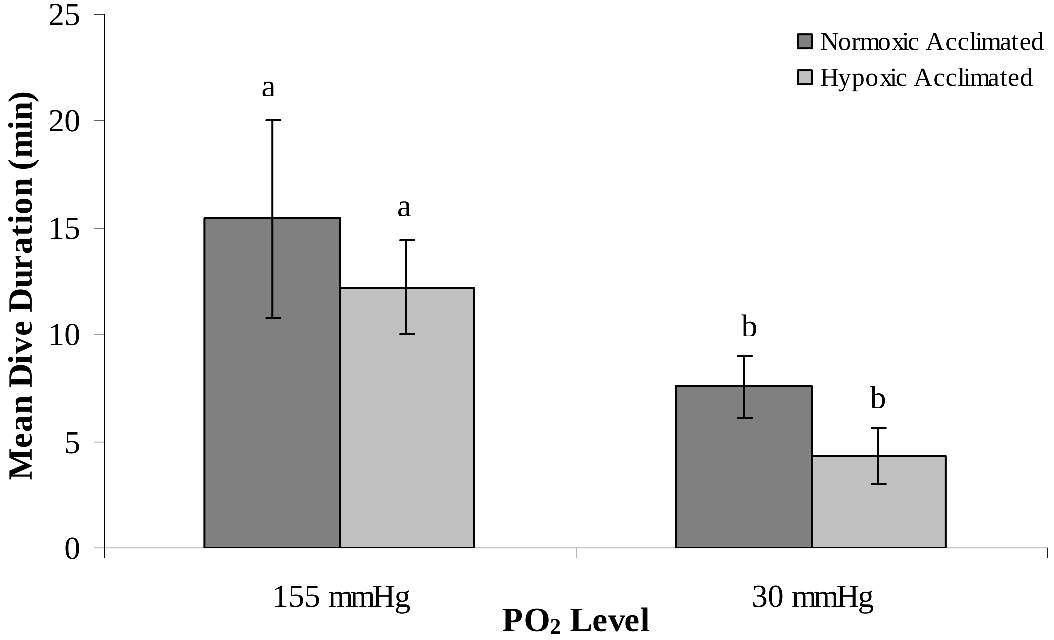

Figure 4.3 - The influence of aquatic PO_2 on mean dive duration in normoxic and hypoxic acclimated hatchlings. Values represent mean ± SEM. Letter differences indicate significant differences.

Mean and maximum dive durations of the hypoxic acclimated turtles did not differ from the normoxic acclimated turtles at either 155 mmHg (mean dive duration - 12.20 ± 2.2 min; maximum dive duration - 36.6 ± 9.5 min) or 30 mmHg (mean dive duration - 4.30 ± 1.3 min; maximum dive duration - 9.0 ± 2.7 min) (Figs. 4.3 & 4.4).

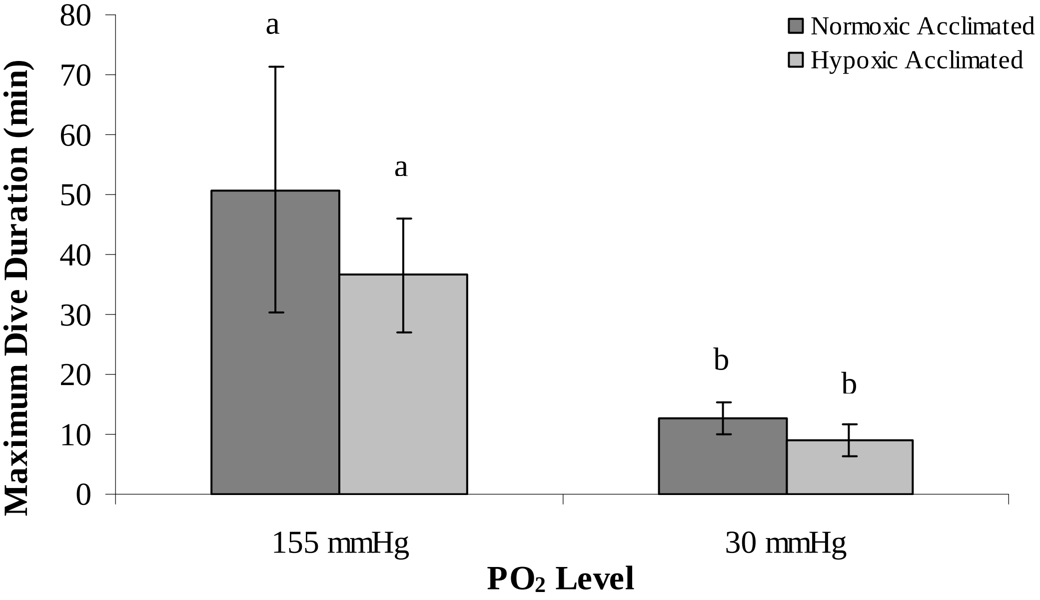

Figure 4.4 - The influence of aquatic PO_2 on maximum dive duration in normoxic and hypoxic acclimated hatchlings. Values represent mean ± SEM. Letter differences indicate significant differences.

Blood Respiratory Properties

Hypoxic acclimated turtles had significantly lower Hb concentrations (1.51 ± 0.04 mmolL^{-1}) than the normoxic acclimated hatchlings (1.65 ± 0.05 mmolL^{-1}) ($F_{[1,19]} = 5.07$, $P < 0.05$) (Fig. 4.5a). Hct levels were not influenced by aquatic hypoxia with no difference between the normoxic (33.4 ± 1.5%) and hypoxic (34.3 ± 0.99%) acclimated turtles ($F_{[1,18]} = 0.281$, $P > 0.05$) (Fig. 4.5b). P_{50} values of the normoxic and hypoxic acclimated turtles were 41.9 ± 1.08 mmHg and 41.0 ± 0.63 mmHg respectively and were not significantly different ($F_{[1,19]} = 0.530$, $P > 0.05$) (Fig. 4.5c).

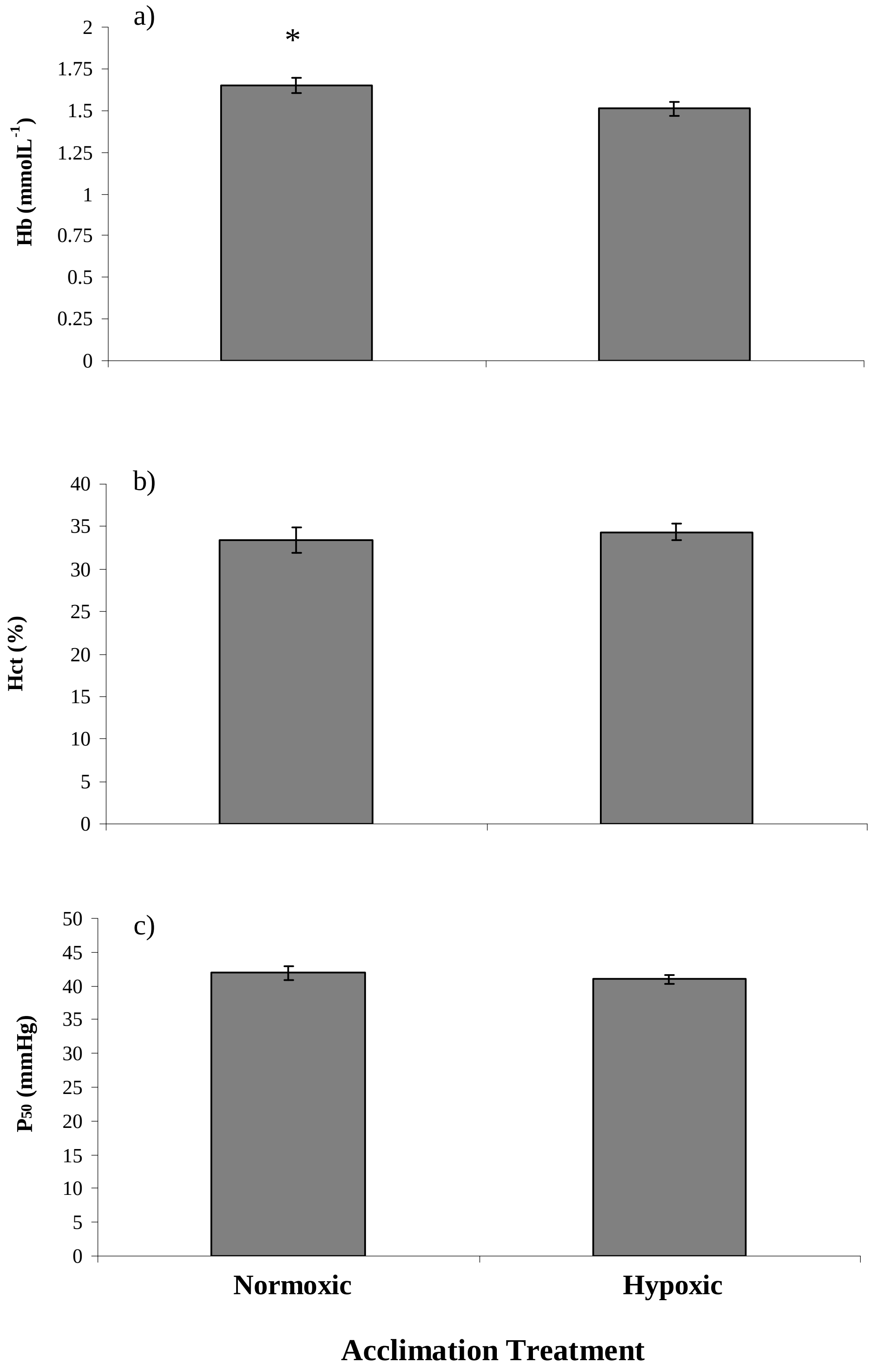

Figure 4.5 - The influence of oxygen acclimation on a) Hb, B) Hct and c) P$_{50}$, in *E. macrurus* hatchlings. Values represent mean ± SEM. Asterisks indicates significant difference.

DISCUSSION

Knowledge of the relationships between animals and their environment is essential for understanding the conservational implications of long-term environmental change (Wikelski and Cooke, 2006). This study has demonstrated that the diving physiology and behaviour of *Elusor macrurus* hatchlings is significantly affected by acute exposure to aquatic hypoxia and that these affects are exacerbated after long-term exposure. Diving physiology and behaviour in bimodally respiring turtles is directly related to ecological activities such as foraging, reproduction and predator exposure (Kramer, 1988). Variations in diving ecology as a result of changes in environmental conditions therefore have the potential to seriously influence species survival. In the natural environment, aquatic oxygen levels can vary acutely on a temporal and spatial scale or long-term as a result of human influence such as river damming (Walker, 1985; Wu, 2002 Gordos et al., 2003b).

Acute Effects of Aquatic Hypoxia

The aquatic habitats of freshwater turtles are highly diverse. Factors such as water depth, water velocity and aquatic vegetation can all influence how aquatic oxygen levels vary on a spatial and temporal scale. For bimodally respiring turtles, variations in aquatic PO_2 can directly influence species ecology as a result of respiratory partitioning. The theory of optimal breathing (Kramer, 1988) states that reliance on aerial and aquatic respiration in bimodally respiring animals is dependent upon the costs associated with each form of respiration under specific conditions. Aquatic respiration is generally more energetically expensive than aerial respiration as water is 800 times denser, 60 times more viscous and has a lower oxygen capacitance than air (Dejours, 1994). Aerial respiration does however bear the energetic cost of travelling to the water surface and the reduced time available for other activities such as foraging (Dejours, 1994). Aquatic oxygen levels can influence the costs associated with both aerial and aquatic respiration and can therefore influence respiratory partitioning (Kramer, 1988). Our results support Kramer's theory of optimal breathing with the respiratory partitioning of the turtles significantly influenced by aquatic PO_2. At 155 mmHg, aquatic respiration supported 9.5% of the turtle's total oxygen consumption. This percentage is much lower than that reported in previous studies of *E. macrurus* where aquatic respiration supported ~25% of the total oxygen consumption (Chapter Two). At 30 mmHg, the respiratory partitioning of the turtles shifted and aquatic respiration decreased significantly to the point where the turtles were actually losing oxygen to the surrounding water. To compensate for this loss of oxygen, percent aerial respiration increased to above 100% so that overall metabolic rate remained constant. A similar result was found for the bimodally respiring fish, *Megalops cyprinoides*, where at 45 mmHg the fish were 100% reliant upon aerial respiration (Seymour et al., 2007).

The change in respiratory partitioning of *E. macrurus* hatchlings in response to acute exposure to aquatic hypoxia had a direct effect on the turtle's dive duration. Dive duration in bimodally respiring turtles is positively correlated with reliance on aquatic respiration (Belkin, 1968; Stone et al., 1992b; Bagatto et al., 1997; Bagatto and Henry, 1999; Prassack et al., 2001; Maina, 2002; Gordos et al., 2004b). For example, hatchling *Rheodytes leukops* have a significantly higher reliance on aquatic respiration (63%) than *Elseya albagula* (29%) and as a result, mean dive duration in this species is over twenty four times longer than that of *E. albagula* (Chapter Two). The change in respiratory partitioning that occurred during acute exposure to aquatic hypoxia meant that the turtle's dives were supported entirely by aerial respiration and this decreased mean dive duration in aquatic hypoxia by 51%. The effect of acute exposure to aquatic hypoxia on dive duration is known to be dependent upon reliance on aquatic respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*) and mud turtles (*Kinosternon subrubrum*) (Stone et al., 1992b). Dive duration is independent of aquatic PO_2 in stinkpot and mud turtles, which have a relative low reliance on aquatic respiration (26% and 14% respectively), but positively correlated with aquatic oxygen level in the soft-shelled turtle, in which reliance on aquatic respiration is higher (38%) (Stone et al., 1992a,b). The large decrease in dive duration that occurred in the *E. macrurus* hatchlings during acute exposure to aquatic hypoxia suggests that despite the low values recorded for aquatic respiration in normoxia, the turtles were actually highly reliant upon this form of respiration during the normoxic diving behaviour trials. The cost of aerial respiration is likely to have been higher in the diving behaviour tank compared to the respirometry chamber due to a nearly seven fold increase in water depth. The increased depth may have resulted in an increased reliance on aquatic respiration for the turtle, as has been demonstrated in the honey gourami (*Colisa chuna*) (Bevan and Kramer, 1986).

Long-Term Effects of Aquatic Hypoxia

Long-term exposure to aquatic hypoxia is known to elicit a range of physiological responses in fish species that allow them to compensate for the acute effect of hypoxia and establish a more efficient uptake of aquatic oxygen under hypoxic conditions (Prosser, 1991; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). This can be demonstrated in the Antarctic fish, *Pagothenia borchgrevinki*, that after long-term exposure to hypoxic conditions, increased oxygen carrying capacity by 40% as a result of a 66% increases in Hb and a 34% decrease in P_{50} (Wells et al., 1989). P_{50} and Hct in the *E. macrurus* hatchlings did not differ between the two acclimation groups indicating that this species does not possess the capacity to acclimate these parameters to aquatic hypoxia. Hb concentrations were slightly lower in the hypoxic acclimated turtles and although this was a statistically significant difference, it is likely that this is not functionally significant.

The high P_{50} values recorded in this study demonstrate that at 30 mmHg the ability of the blood to bind aquatic oxygen was severely reduced and the respirometry results show that the hatchlings were actually losing oxygen to the aquatic environment at this level of PO_2. Long-term exposure to aquatic hypoxia exacerbated this effect with the hypoxic acclimated turtles losing significantly more oxygen to the environment in hypoxic conditions than the normoxic acclimated hatchlings. This result may indicate that a beneficial acclimatory response had been initiated in the hatchlings in response to the low oxygen levels but at the level of hypoxia tested it may have been too severe for the changes to function beneficially (Huey et al., 1999; Loeschcke and Hoffmann, 2002; Wilson and Franklin, 2002a,b; Woods and Harrison, 2002). For example, an increase in reliance on aquatic respiration in the hypoxic acclimated turtles as a result of changes in cloacal bursae morphology or ventilation rate would benefit the hatchlings at mild levels of hypoxia but at severe levels would result in higher losses of oxygen from the turtle to the environment. Increases in gill surface area as a result of increases in gill filament length, the number of gill filaments and the size of secondary lamellae, is a common response of fish species to long-term exposure to aquatic hypoxia (Chapman et al., 2000; Chapman and Hulen, 2001; Schaack and Chapman, 2003; Sollid et al., 2003). It is not known whether the cloacal bursae of bimodally respiring turtles can display this kind of morphological plasticity.

Despite the increased loss of oxygen in the hypoxic acclimated turtles, dive duration did not differ between the two acclimation groups. The higher loss of oxygen in the hypoxic acclimated hatchlings should have resulted in an increase in surfacing for aerial respiration and hence decrease in dive duration in hypoxic conditions. Diving vertebrates do not however always dive to their aerobic limit and it is possible that in the hypoxic conditions the normoxic acclimated turtles did not dive to their full aerobic capacity resulting in a reduced dive duration (Thompson et al., 1991; Chappell et al., 1993; Thompson and Fedak, 2001; Heithaus and Frid, 2003). Alternatively, after long-term exposure to aquatic hypoxia the metabolic rate of the hatchlings may have decreased in order to reduce oxygen demand and hence increase dive duration. Metabolic depression is a known acclimatory response to aquatic hypoxia in fish (Brauner et al., 1995; Wu, 2002) and has also been reported in some species of freshwater turtle (Jackson, 1968; Ultsch, 1985; Storey, 1996; Jackson, 2000; Costanzo et al., 2001; Hicks and Wang, 2004; Bickler and Buck, 2007; Storey, 2007). Whilst most turtle species enter a metabolic depression at low temperatures only (overwintering strategy), *Trachemys* and *Chrysemys* species are able to survive 12-24 h of anoxic forced submergence at high temperatures (20ºC-25ºC) by achieving a 90% reduction in metabolic rate (Jackson, 1968; Herbert and Jackson, 1985a,b; Milton and Prentice, 2007; Stecyk and Farrell, 2007). The results of this study show that *E. macrurus* hatchlings do not undergo a metabolic depression in response to aquatic hypoxia but rather increase their reliance on aerial respiration in order to support and maintain metabolic rate.

Conservation Implications

Installation of large water impoundments are often associated with marked decreases in aquatic oxygen levels within the reservoir and in some cases throughout the immediate downstream reaches (Walker, 1985; Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001; Arthington, 2003; Snoussi et al., 2007). Such physio-chemical changes are likely to impact negatively upon bimodally respiring turtle species due to their respiratory physiology and diving ecology. Past studies on the impacts of river damming on freshwater turtle populations have revealed a decrease in the abundance of species with a high reliance on aquatic respiration (*R. leukops and E. albagula*) while species with a lower reliance on aquatic respiration are flourishing (*Emydura signata*) (Tucker et al., 2001; Limpus et al., 2002; Arthington, 2003). These results suggest that species with a high reliance on aquatic respiration maybe more susceptible to changes in environmental conditions than species with a low reliance. This study has demonstrated that the diving behaviour and physiology of *E. macrurus* hatchlings is significantly affected by acute exposure to aquatic hypoxia and that these affects are exacerbated after long-term exposure. Diving behaviour in vertebrates is known to be closely linked with activities such as foraging and predator avoidance (Kramer et al., 1983; Dolphin, 1987; Kramer, 1988; Boyd, 1997; Mori, 1998; Thompson and Fedak, 2001; Acevedo-Gutierrez et al., 2002; Heithaus and Frid, 2003; Frid et al., 2007; Sparling et al., 2007). Any changes in diving behaviour that occurs in response to aquatic hypoxia may therefore influence these factors and potentially impact species survival. For example, a decrease in dive duration in aquatic hypoxia may cause a decrease in hatchling growth rate as a result of reduced time available for foraging. An increase in surfacing frequency also has the potential to influence survival by increasing the time that the hatchlings are exposed to predators within the water column and at the water surface. This is supported by a study on bimodally respiring fish species (Kramer et al., 1982) which showed that the fish experienced higher levels of predation when air-breathing at the water's surface. Findings from this study demonstrate the interaction between changes in environmental conditions (PO_2) and the physiological ecology of bimodally respiring turtles. The long-term consequences of these interactions need to be considered when addressing the impacts of river damming on freshwater turtle populations.

CHAPTER FIVE

THE USE OF AQUATIC RESPIRATION AS A PREDATOR AVOIDANCE STRATEGY IN BIMODALLY RESPIRING TURTLES

ABSTRACT

Risk of predation is known to influence the diving behaviour of aquatic, air-breathing vertebrates however very little is known about the impacts on bimodally respiring turtle species. The theory of optimal breathing predicts that in the presence of a predator, an individual will increase their reliance on aquatic respiration thereby reducing their surfacing frequency and hence their risk of predation**.** Hatchling turtles have extremely high predation rates and their ability to increase dive duration through the use of bimodal respiration suggests that aquatic respiration may be used as a predator avoidance strategy in this age class. The diving behaviour of two turtle species, *Elusor macrurus* and *Emydura signata* was examined under control conditions (no predator) and in the presence of a large piscine predator (barramundi - *Lates calcarifer*). We hypothesised that in the predator treatment, hatchling dive duration would increase while activity levels would decrease. In the presence of the predator, the mean dive duration of *E. macrurus* increased by 35% from 18 min to 25 min while maximum dive duration increased by 193% from 47 min to 139 min. The mean (11 min) and maximum (21 min) dive durations of *E. signata* were not influenced by the presence of the predator. Diving activity levels of both turtle species decreased in the presence of the predator indicating that the risk of predation was perceived to be greater during a dive than at the waters surface. The behavioural response differences between the turtle species may be a result of differences in reliance on aquatic respiration supporting our hypothesis that aquatic respiration may function as a predator avoidance strategy.

INTRODUCTION

Air-breathing aquatic vertebrates spend much of their time submerged underwater but must frequently return to the surface to exchange pulmonary gases. A theoretical estimate of the maximum time an individual can stay submerged underwater based on aerobic metabolism is known as the aerobic dive limit (ADL) (Kooyman, 1989). Diving studies have shown that animals regularly dive for periods both less and greater than their ADL and this is a result of the tradeoffs that occur between replenishing oxygen stores and diving activities such as foraging (Kramer, 1988; Houston and Carbone, 1992; Boyd, 1997; Mori, 1999; Williams et al., 2000; Costa et al., 2001; Croll et al., 2001; Sparling et al., 2007). Optimality models predict that divers will adjust diving behaviour in order to maximise resource gain during a dive. Factors such as prey abundance, likelihood of relocating prey and prey depth can all influence optimal allocation of time during a dive (Kooyman et al., 1980; Kramer, 1988; Thompson et al., 1991; Houston and Carbone, 1992; Chappell et al., 1993; Boyd, 1997; Mori, 1998; Mori, 1999; Costa et al., 2001; Croll et al., 2001; Sparling et al., 2007).

Predator avoidance has been suggested as an additional factor that is likely to influence diving behaviour (Heithaus and Frid, 2003; Frid et al., 2007). Diving vertebrates are at risk of predation from both aerial and aquatic predators and are most susceptible when at the water surface due to reduced escape ability and decreased visibility (Kramer et al., 1983; Heithaus et al., 2002; Heithaus and Frid, 2003). Optimal diving models predict that under the risk of predation, an air-breathing diver should either reduced surfacing duration or surfacing frequency depending upon the level of risk over time (Heithaus and Frid, 2003). For example, loggerhead turtles (*Caretta*

caretta) are known to reduce surface duration in response to a high abundance of tiger shakes (Heithaus and Frid, 2003).

For bimodally respiring animals (those that can exchange respiratory gases in both air and water), the influence of predation risk on diving behaviour is dictated by respiratory partitioning. The theory of optimal breathing (Kramer, 1988) states that reliance on aerial and aquatic respiration is dependent upon the costs associated with each form of respiration under specific conditions. That is, in the presence of a predator, an individual will increase their reliance on aquatic respiration thereby reducing their surfacing frequency and hence predation risk. This is demonstrated in the Florida gar, *Lepisosteus platyrhincus*, in which air-breathing frequency decreased from 18.4 breaths/h in control conditions to 8.4 breaths/h in the presence of a predator (Smith and Kramer, 1986). Predator exposure is known to decrease reliance upon aerial respiration in many bimodally respiring fish species as well as the African clawed frog, *Xenopus laevis* (Baird, 1983; Kramer, 1983; Kramer et al., 1983; Smith and Kramer, 1986; Wolf and Kramer, 1987; Herbet and Wells, 2001; Domenici et al., 2007).

Aquatic respiration is highly developed in some species of freshwater turtle (Gage and Gage, 1886; Smith and James, 1958; Girgis, 1961; Belkin, 1968; Stone et al., 1992a; King and Heatwole, 1994a) however its use as a predator avoidance strategy has yet to be investigated. Freshwater turtle hatchlings have high predation rates and only 1% of most turtle species are expected to survive to adulthood (Gibbons and Semlitsch, 1982; Cann, 1998). Freshwater turtles can use aquatic respiration to increase their ADL and reduce surfacing frequency (Belkin, 1968; Stone et al., 1992b; Bagatto et al., 1997; Bagatto and Henry, 1999; Prassack et al., 2001; Maina, 2002; Gordos et al., 2004b). A high reliance on aquatic respiration (63%) allows *Rheodytes leukops* hatchlings to dive for an average of 19 h, compared to only 11 min in *Emydura signata* which is only 29% reliant on aquatic respiration (Chapter Two). The ability of bimodally respiring turtles to increase dive duration by such lengths suggests that aquatic respiration may be used as a predator avoidance strategy.

The aim of this study was to determine if aquatic respiration could function as a predator avoidance strategy in bimodally respiring hatchling turtles. The diving behaviour of two turtle species, *Elusor macrurus* and *Emydura signata*, was examined under control conditions and in the presence of the piscine predator, *Lates calcarifer* (barramundi). We hypothesised that in the presence of an aquatic predator, hatchling dive duration would increase while activity levels would decrease. Previous diving behaviour studies (Chapter Two) suggest that *E. macrurus* has a higher reliance on aquatic respiration than *E. signata* and as a result diving behaviour may be used as an indirect measure of the use of aquatic respiration as a predator avoidance strategy. It was hypothesised that bimodally respiring turtles could use aquatic respiration as a predator avoidance strategy and as a result the observed increase in dive duration in response to a predator would be greater in species with a higher reliance on aquatic respiration.

MATERIALS AND METHODS

Turtle Collection and Husbandry

Eggs of *E. macrurus* and *E. signata* were collected from nesting sites along the Mary and Brisbane Rivers respectively. A minimum of four clutches were gathered for each species to ensure genetic variation. The eggs were incubated in river bank sand in a Styrofoam box that was kept at environmental temperature. Prior to experiments, the hatchlings were kept in sheltered outside holding tanks (1000 L) that contained basking platforms, habitat shelters and UV lights set on a 12:12 light:dark photoperiod. This research was approved by Queensland Parks and Wildlife Service (SPP-WISP-01477903) and supported by the University of Queensland Ethics Committee (AEC –ZOO/ENT/133/07/URG).

Diving Behaviour

The diving behaviour of hatchling *E. macrurus* (N= 16, 7.75g-15.23g) and *E. signata* (N=16, 5.86g-11.80g) was examined in the presence of a piscine predator (barramundi – *Lates calcarifer*) and under control conditions (no predator). Experiments were conducted in a large glass aquarium (150 x 60 x 65 cm (*l* x *w* x *h*)) that was divided length wise by a sheet of clear Perspex. Holes were drilled into the Perspex sheet to allow the exchange of water between the two sides of the tank. The purpose of dividing the tank was to give the turtles visual and chemical cues of the predator whilst ensuring no actual predation could occur. The front side of the tank was used as the turtle observation area and contained a pebble substrate and habitat shelters (PVC pipes, plants, rocks). The back side of the tank also contained a pebble substrate and housed the barramundi in the predator experiments. A light was positioned above the tank and was set at 12L:12D, with red lights used during the dark period to allow recording. Experiments were conducted in a controlled temperature room set at 25°C.

Four individual turtles of a single species were placed into the aquarium observation area and given 24 h to become accustomed to the new environment. After this period, either the barramundi (predator experiment) or a rock (control experiment) was added to the treatment area of the aquarium. In the control experiments, the addition of the rock to the aquarium accounted for the additive effect in the predator experiments allowing the two treatment groups to be compared. Diving behaviour was then recorded for 24 h using a networked video camera connected to a remote PC with Milestone Surveillance software. Turtles were allowed a minimum of one week rest before being tested at the second treatment.

Video files were analysed for resting dive durations which were defined as a dive in which the turtles sat still on the bottom of the tank for a period of greater than one minute. Mean and maximum dive durations for each turtle were then calculated over the 24 h period using a custom-written program (M.Gordos). Diel variations were investigated by partitioning dive durations into day (0600 h – 1800 h) and night (1800 h – 0600 h). Dive durations were also classified according to activity with an active dive being defined as a dive in which the turtles were stationary on the bottom of the tank for greater than a minute but the majority (> 50%) of the submergence time was spent being active. Inactive dives were therefore classified as a resting dive in which < 50% of the submergence time was spent being active. A surfacing period was defined as the time from which the turtle's nose broke the waters surface after a dive, to the time when the nose submerged at the beginning of the next dive. A custom-written program (M. Gordos) was used to determine the percent of active and inactive dives, and percent time spent diving and surfacing. Turtle diving behaviour was analysed using a two-way repeated measures analysis of variance (ANOVA) with turtle species and predator treatment as the two factors. Where normality or equal variance failed, data was transformed before analysis. An arcsin square root transformation was applied to percentage data before analysis. Significant differences were further analysed using a Tukeys post hoc test. All results are presented as mean ± standard error unless otherwise stated, with statistical significant differences considered at the level $P < 0.05$.

RESULTS

Turtle diving behaviour was affected by both turtle species (mean dive duration – $F_{[1,79]} = 11.35$, $P < 0.01$; maximum dive duration – $F_{[1,79]} = 11.36$, $P < 0.01$) and predator treatment (mean dive duration – $F_{[1,79]} = 3.65$, $P > 0.05$; maximum dive duration – $F_{[1,79]} = 6.26$, $P < 0.02$) (Figs. 5.1a & b). Within the control treatment, the mean dive durations of *E. macrurus* and *E. signata* were 18.3 ± 1.44 min and 11.1 ± 0.66 min while maximum dive durations reached 47.3 ± 6.78 min and 20.8 ± 1.43 min respectively. In the presence of the predator, *E. macrurus* significantly increased mean dive duration to 24.8 ± 3.65 min ($P < 0.05$) while maximum dive duration increased by 193% to 138.5 ± 40.6 min ($P < 0.001$) (Figs. 5.1a & b). The mean (11.7 ± 0.45 min) and maximum (24.2 ± 2.1 min) dive durations of *E. signata* were not however affected by the presence of the predator ($P > 0.05$). The dive durations of the two turtle species did not differ in the control treatment ($P > 0.05$) however, in the presence of the predator *E. macrurus* dived for significantly longer than *E. signata* ($P < 0.001$) (Figs. 5.1a & b).

Both turtle species exhibited a diel trend in mean dive duration within the control treatment ($F_{[1,79]} = 20.87$, $P < 0.001$) (Fig. 5.2a). During the daytime, the mean dive duration of *E. macrurus* was 15.3 ± 1.95 min whilst that of *E. signata* equaled 8.45 ± 0.53 min. These dive durations increased significantly at night to 21.7 ± 2.78 min ($P < 0.001$) and 12.53 ± 0.80 min ($P < 0.05$) in *E. macrurus* and *E. signata*, respectively. A diel trend was also observed in the maximum dive duration of *E. macrurus* with the night time dive duration (44.1 ± 8.18 min) being

37% longer than that of the day (32.2 ± 5.81 min) (P < 0.01) (Fig. 5.2b). No diel trends were observed in the maximum dive duration of *E. signata* under control conditions (P > 0.05) (Fig. 5.2b).

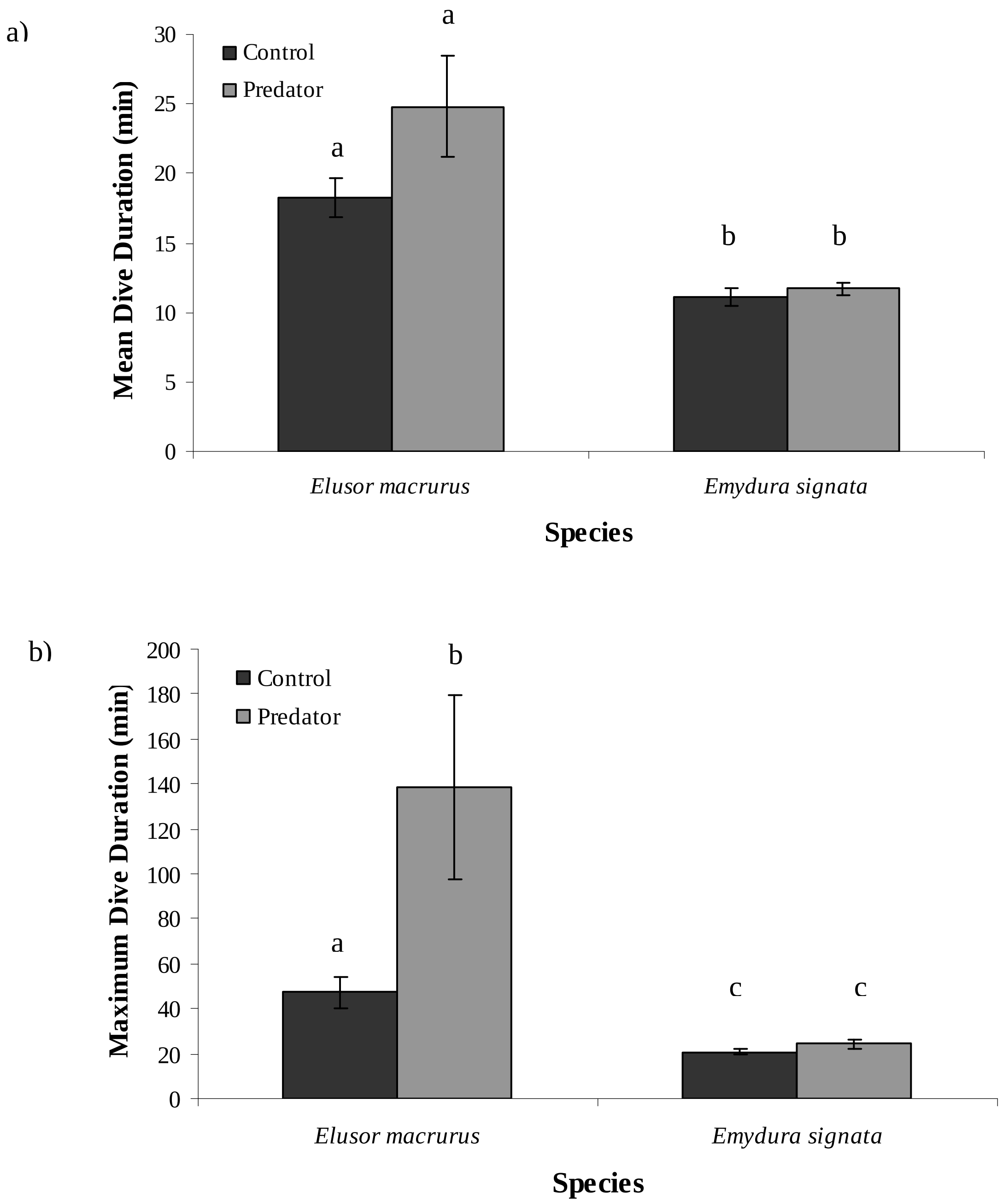

Figure 5.1 - The effect of a predator on the a) mean and b) maximum dive durations of *E. macrurus* and *E. signata.* Values represent means ± SEM. Letter differences indicate significant differences.

The presence of the predator had a significant effect on the mean diel trends of both turtle species ($F_{[1,79]} = 10.25$, $P < 0.01$) (Fig. 5.2a). Mean daytime dive duration did not differ between the control and predator treatments in either *E. macrurus* or *E. signata* ($P > 0.05$) however at night a predator affect was evident. In the presence of the predator, the mean dive duration of *E. macrurus* increased from 15.7 ± 2.29 min during the day to 40.6 ± 9.10 min at night ($P < 0.001$) which is a 87% longer night-time dive duration than that of the control treatment (see above) ($P < 0.01$). Night-time mean dive duration of *E. signata* did not differ between the control and predator treatments ($P > 0.05$) however the presence of the predator did remove the diel trends that were observed in the control treatment (Fig. 5.2a).

Predation risk also influenced the diel trends of maximum dive duration ($F_{[1,79]} = 6.05$, $P < 0.05$) (Fig. 5.2b). In the presence of the predator, maximum daytime dive duration increased to 45.5 ± 7.31 min in *E. macrurus* ($P < 0.05$), but no difference was recorded for *E. signata* ($P > 0.05$). During the night, the risk of predation caused *E. macrurus* to further increase dive duration to 134 min ± 41 min ($P < 0.01$), which is a five fold greater increase compared to that which occurred during day ($P < 0.01$). The presence of the predator did not effect the night-time maximum dive duration of *E. signata* ($P > 0.05$) and no dial trends were observed ($P > 0.05$) (Fig. 5.2b).

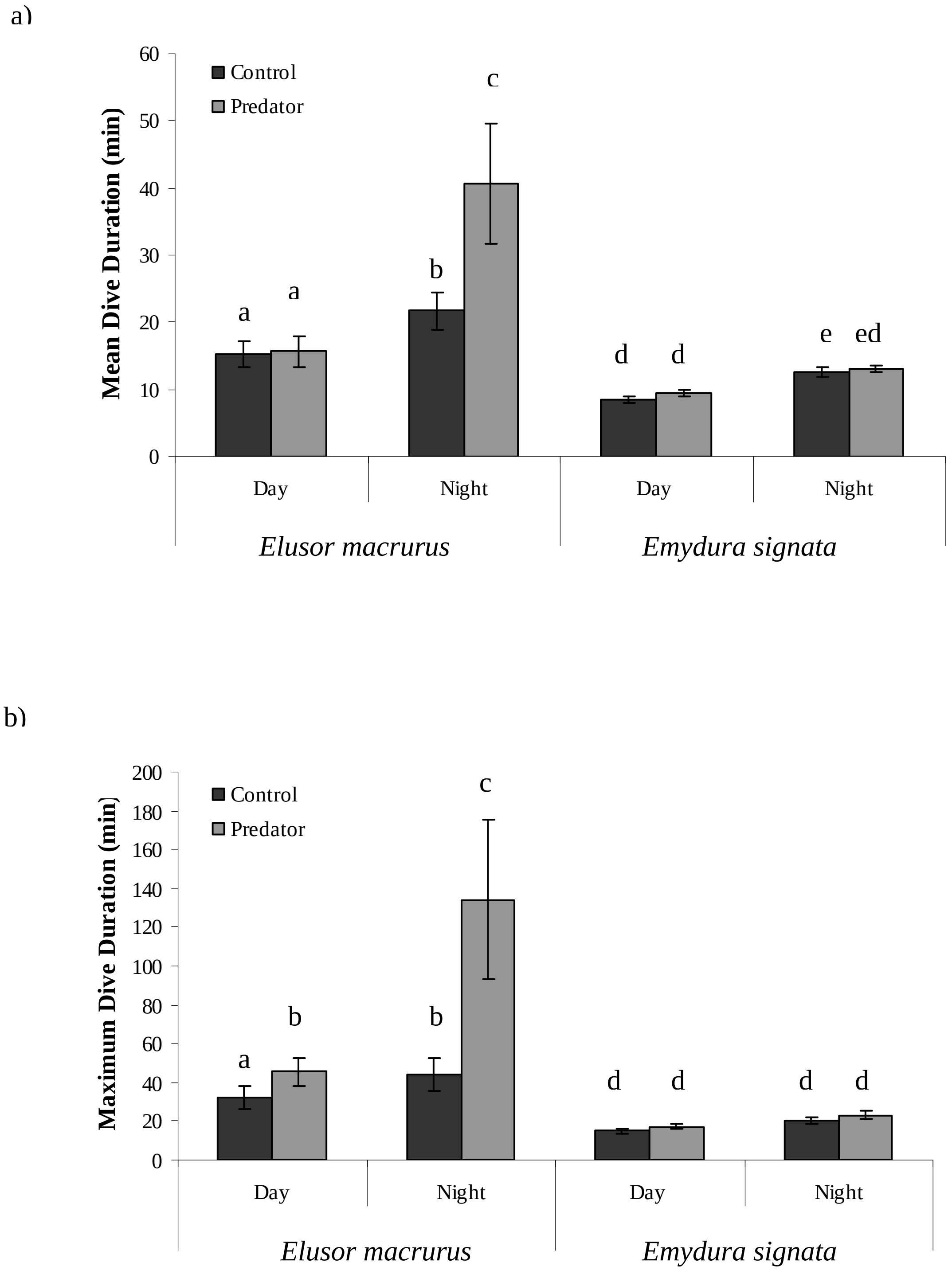

Figure 5.2 - Diel variation in the a) mean and b) maximum dive durations of *E. macrurus* and *E. signata* within the control and predator treatments. Values represent means ± SEM. Letter differences indicate significant differences.

In the control treatment, *E. macrurus* hatchlings spent 39% of the time diving and 61% surfacing while *E. signata* hatchlings spent 48% of the time diving and 52% surfacing (Fig. 5.3). These percentages did not differ between the species and were not affected by the presence of the predator (P > 0.05). The predator treatment did have a significant effect on the diving activity of the hatchlings (Fig. 5.4). Active dives of *E. macrurus* decreased from 14% in the control treatment to 5% in the presence of the predator (P < 0.001) while that of *E. signata* decreased from 10% to 3% in the respective treatments (P < 0.001). The percentage of inactive and active dives did not differ between turtle species (P > 0.05).

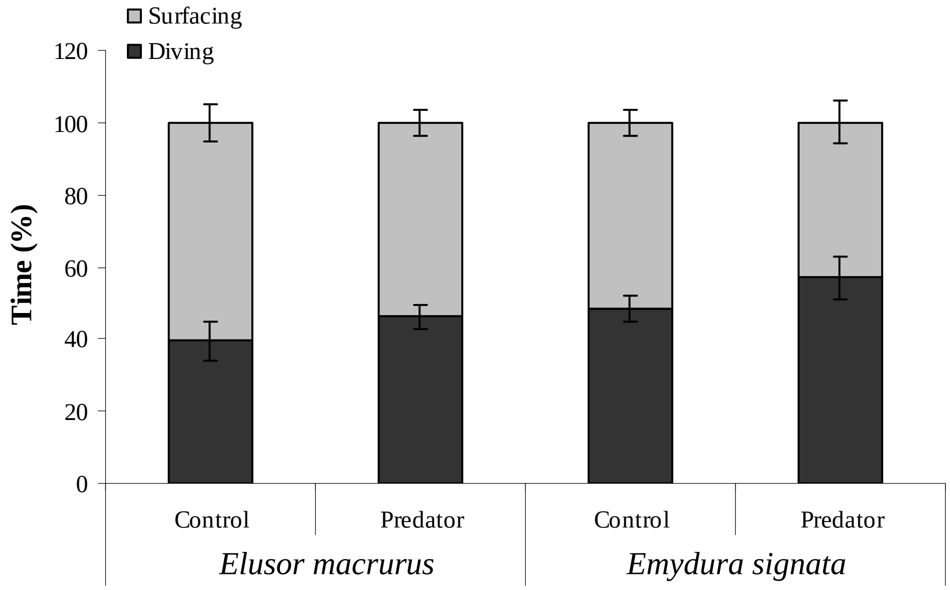

Figure 5.3 - The effect of a predator on the percentage of time spent diving and surfacing in *E. macrurus* and *E. signata* Values represent means ± SEM.

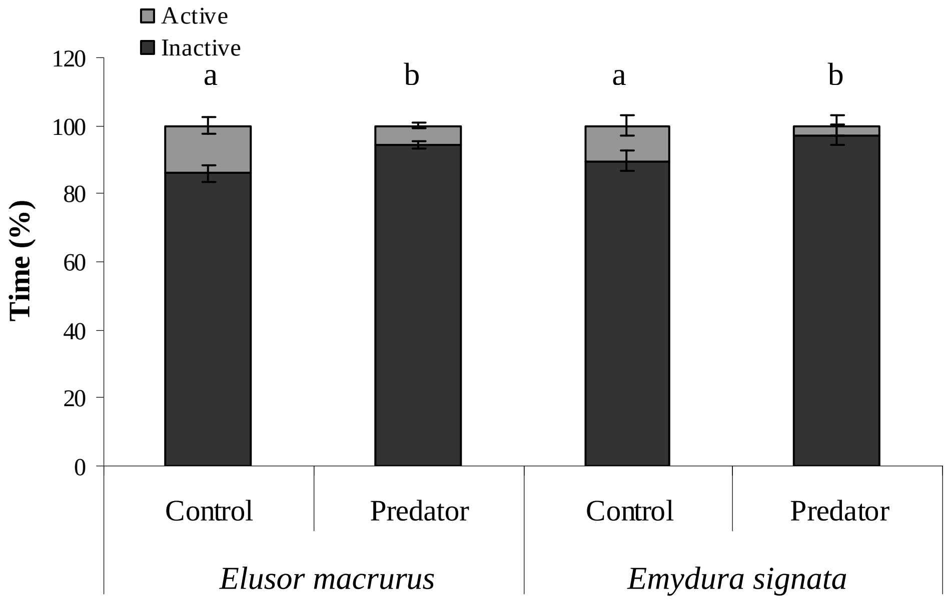

Figure 5.4 - The effect of a predator on diving activity in *E. macrurus* and *E. signata*. Values represent means ± SEM. Letter differences indicate significant differences between treatments.

DISCUSSION

The results of this study demonstrate that risk of predation is an important factor influencing the diving behaviour of the bimodally respiring hatchling turtles, *Elusor macrurus* and *Emydura signata*. The results of *E. macrurus* supported our hypothesis with mean and maximum dive duration increasing and diving activity decreasing in the presence of the predator. Diving activity also decreased in *E. signata* in the presence of the predator but no difference was observed for dive duration as expected. The behavioural response difference between the turtle species may be a result of differences in reliance on aquatic respiration which would support our hypothesis that aquatic respiration may function as a predator avoidance strategy.

For diving animals, time during a dive cycle must be shared between replenishing oxygen stores, travelling, other diving activities such as foraging and anti-predator behaviour (Kramer, 1988).

Divers must weigh up the costs and benefits of each of these activities under specific conditions to achieve the optimal allocation of time during a dive. Hatchling turtles are at risk of predation from a number of predators including fish, birds, crocodiles, water rats and snakes and are most at risk within the water column and at the waters surface (Worrell, 1966; Cann, 1998). Under these conditions it is therefore beneficial for hatchling turtles to increase dive duration thereby reducing the frequency and duration of high risk surfacing events. In this study, the mean and maximum dive duration of *E. macrurus* increased by 35% and 193% respectively in the presences of the predator. This finding suggests that the hatchlings were able to detect and respond to the predator by decreasing the probability of encountering or being captured by the barramundi. Green sea turtles (*Chelonia mydas*) are known to reduce the probability of shark predation at the waters surface by increasing dive duration and depth during migrations (Hays et al., 2001).

Analysis of the diel variation in diving behaviour of the hatchling turtles revealed that *E. macrurus* allocated more time to predator avoidance (increased dive duration) at night than during the day. The risk allocation hypothesis predicts that animals should allocate more effort to predator avoidance during high-risk situations and more effort to other activities such as foraging during low-risk situations (Lima and Bednekoff, 1999). This suggests that risk of predation in the hatchlings may be greater during the night-time period. As predator presence was kept constant during experiments, the observed diel response maybe associated with a reduced ability of the hatchlings to visually detect predators at night rather than a change in predator abundance or behaviour. Similar patterns of diving behaviour have been recorded in field experiments where turtles displayed an increase in activity and decrease in dive duration during the day (Chessman, 1988; Gordos and Franklin, 2002; Gordos et al., 2007). These periods of increased activity are specifically associated with dawn and dusk periods and are thought to indicate episodes of foraging (Chessman, 1986; Gordos and Franklin, 2002; Gordos et al., 2007). During the night-time periods, turtles have been recorded moving into shallower habitats which may also function as a predator avoidance strategy by reducing exposure to predators when travelling to the water's surface (Gordos et al., 2007).

Reducing activity levels is another anti-predator behaviour that animals can employ to reduce predator exposure (Lima and Dill, 1990; Lima, 1998). For example, in response to computer-generated visual stimuli, the blue gourami, *Trichogaster trichopterus*, reduced spontaneous activity by 50% (Herbet and Wells, 2001). In this study, both *E. macrurus* and *E. signata* reduced diving activity levels in the presence of the predator. Activity levels have been found to be directly related to predation risk in larval anurans where the higher the risk of predation the greater the decrease in activity was observed (Relyea, 2001). The reduction in diving activity of *E. macrurus* and *E. signata* therefore suggests that both species of turtle viewed the barramundi as a predation risk during this experiment. The behavioural responses of the turtles can also be used to gain insight into the spatial variation of predation risk. A reduction in diving activity as opposed to surfacing time suggests that the hatchlings determined the risk of predation to be greater during a dive than while surfacing or whilst at the waters surface. This response may be due to the behaviour of the barramundi which spent the majority of its time swimming in the bottom to middle section of the water column rather than at the waters surface. The spatial variation in predation risk is likely to change according to factors such as the predator species and habitat complexity (Brown and Kotler, 2004). Optimality models predict that when the risk of predation increases with time at the waters surface, divers should decrease surfacing duration rather than increase dive duration (Heithaus and Frid, 2003). It is predicted that an aerial predator would initiate a surfacing decrease response in hatchling turtles.

The diving behaviour responses to the presence of the predator differed between turtle species. *Elusor macrurus* exhibited a significant increase in dive duration in the predator treatment while no response occurred in *E. signata*. This dissimilarity may be attributed to differences in aquatic respiratory ability. Predator response differences have been attributed to variations in respiratory modes for bimodally respiring fish species where a higher reliance on aquatic respiration resulted in a increased dive duration and a lower rate of surface predation (Kramer, 1983; Kramer et al., 1983; Smith and Kramer, 1986; Wolf and Kramer, 1987; Herbet and Wells, 2001; Domenici et al., 2007). The results of Chapter Two suggest reliance on aquatic respiration does not differ between *E. macrurus* (24%) and *E. signata* (29%) however the accuracy of these results is questionable. Obtaining measures of maximum reliance on aquatic respiration is difficult due to the turtle's ability to voluntarily change their respiratory partitioning (Mathie and Franklin, 2006). It has been suggested in Chapter Two that reliance on aquatic respiration was reduced in this study due to the shallow water depth of the respiromety chambers. Reliance on aquatic respiration has been found to be positively correlated with dive duration in adult turtles (Belkin, 1968; Bagatto and Henry, 1999; Gordos and Franklin, 2002), suggesting dive duration may provide a more accurate indicator of a species reliance on aquatic respiration. During the diving behaviour trials of Chapter Two, the dive duration of *E. macrurus* was over five times longer than that of *E. signata* suggesting *E. macrurus* does have a higher reliance on aquatic respiration than *E. signata*. These findings are supported by the results of this study were in the control treatment, the mean and maximum dive durations of *E. macrurus* were 65% and 127% longer respectively than that of *E. signata*.

The theory of optimal breathing predicts that under the risk of predation bimodally respiring species should increase reliance on aquatic respiration and decrease aerial respiration (Kramer, 1988). Aquatic respiration is generally more energetically expensive than aerial respiration as water is 800 times denser, 60 times more viscous and has a lower oxygen capacitance than air (Dejours, 1994). Risk of predation however increases the costs associated with surfacing for aerial respiration thereby altering the cost-benefit analysis in favour of aquatic respiration (Kramer, 1988). In the presence of the predator *E. macrurus* increased maximum dive duration by almost 200% while the dive duration of *E. signata* remained the same. This result suggests that *E. macrurus* did increase reliance on aquatic respiration in the presence of the predator which supports our hypothesis that aquatic respiration can be used as a predator avoidance strategy.

This study used species differences in aquatic respiration combined with diving behaviour to determine the use of aquatic respiration as a predator avoidance strategy. Further research is required into the direct effects of a predator on the respiratory partitioning of bimodally respiring turtles. The observed increase in dive duration of *E. macrurus* may not have been a result of an increased reliance on aquatic respiration but rather a result of metabolic depression or anaerobic metabolism. Dive durations are thought to be predominately supported by aerobic metabolism however diving vertebrates can also use anaerobic metabolism to extend the duration of a dive (Kooyman, 1989; Carbone and Houston, 1996; Carbone et al., 1996; Boyd, 1997; Mori, 1999). Anaerobic metabolism is less energy efficient than aerobic metabolism and produces lactate as an end-product (Hochachka and Mommsen, 1983). Some species of freshwater turtles are highly tolerant to metabolic acidosis especially at cold temperatures (Belkin, 1968; Jackson and Silverblatt, 1974; Ultsch et al., 1984; Ultsch and Jackson, 1982; Herbert and Jackson, 1985b; Jackson, 2000; Jackson, 2004), however it is unlikely that the prolonged dive duration seen in *E. macrurus* was support by anaerobic metabolism as bimodally respiring turtles are known to use aquatic respiration to specifically avoid metabolic acidosis (Gordos et al., 2004b; Jackson, 2004). If anaerobic metabolism was used as a predator avoidance strategy then an extended dive duration should have also been seen in *E. signata* as the capacity for anaerobic metabolism should be the same for both species of turtle. Additionally, dives supported by anaerobic metabolism are generally followed by a long surfacing period during which the animal oxidises the lactate that has built up during the dive (Kooyman et al., 1980; Kooyman et al., 1983; Kramer, 1988). Whilst the surfacing periods of *E. macrurus* were long they were not affected by the presence of the predator suggesting that the turtles did not require long surfacing periods to recover from anaerobic metabolism.

This study suggests that *E. macrurus* utilises aquatic respiration to increase dive duration as a predator avoidance strategy. Variations in aquatic respiration and diving behaviour therefore have the potential to directly impact hatchling predation rates. For example, aquatic respiration and diving behaviour in bimodally respiring turtles are known to be influenced by environmental conditions such as aquatic temperature and oxygen levels (Herbert and Jackson, 1985b; Stone et al., 1992b; Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2003a,b; Mathie and Franklin, 2006). Changes in environmental conditions therefore may influence hatchling predation rates and ultimately may effect species survival.

CHAPTER SIX

GENERAL DISCUSSION

Bimodally respiring turtles utilise aquatic respiration as a mechanism to extend dive duration (Belkin, 1968; Stone et al., 1992b; Prassack et al., 2001; Gordos and Franklin, 2002; Mathie and Franklin, 2006). Turtle diving physiology and behaviour are closely related to ecological activities such as foraging, reproduction and predator avoidance (Kramer, 1988). Variations in diving physiology and behaviour therefore have the potential to influence individual fitness and survival. The physiological ecology of bimodally respiring turtles has primarily been investigated in adults, yet Mathie and Franklin (2006) demonstrated that there are significant differences in reliance on aquatic respiration between hatchling and adult turtles. Due to the high reliance of hatchling turtles on aquatic respiration, hatchling diving ecology is expected to be highly influenced by variations in respiratory physiology.

Turtle habitat throughout the world is being fragmented and transformed as a result of river damming (Ward and Stanford, 1989; Reese and Welsh, 1998; Tucker, 1999; Arthington, 2003). The environmental impacts of river damming include reduced water flow, decreased temperature, increased water depth and decreased aquatic oxygen levels (Ward and Stanford, 1989; Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001). Many of these environmental factors are known to influence the diving physiology and behaviour of adult turtles (Prassack et al., 2001; Priest and Franklin, 2002; Gordos et al., 2004a) and the high reliance of hatchlings on aquatic respiration suggests that this life history stage is likely to be exceedingly sensitive to these changes. Understanding the relationships between hatchling turtles and their environment is therefore critical to the future conservation and management of turtle species (Wikelski and Cooke, 2006).

In the present study, I examined the relationships between hatchling diving physiology and behaviour and the influence of variations in environmental conditions. Here I discuss our advanced understanding of hatchling diving physiological ecology and through the use of physiological techniques, examine the potential impacts of long-term environmental change on species conservation.

PHYSIOLOGICAL ECOLOGY OF HATCHLING TURTLES

Dive duration in air-breathing vertebrates is positively correlated with body size due to the allometric scaling of metabolic rate and oxygen stores (Schreer and Kovacs, 1997). Larger animals have a lower mass-specific metabolic rate and proportionally higher oxygen storage capacity allowing them to dive deeper and for longer than smaller animals (Kleiber, 1961; Bartels, 1982; Butler and Jones, 1982; Schmidt-Nielsen, 1984; Kooyman, 1989; Schreer and Kovacs, 1997; Kooyman and Ponganis, 1998). This relationship between dive duration and body size in bimodally respiring turtles is further complicated by variations in reliance on aquatic respiration (Mathie and Franklin, 2006). Hatchling turtles have a higher surface-area-to-volume ratio than adults which allows them to extract a relatively larger amount of oxygen from the aquatic environment. A higher reliance on aquatic respiration allows hatchlings to dive for durations equal to or greater than the larger adults (Mathie and Franklin, 2006). In this study, the relationship between dive duration and body size did not conform to the positive correlation seen in air-breathing divers. The dive durations of hatchling *Emydura signata*, *Elseya albagula* and *Rheodytes leukops*, were longer than those previously recorded for adults leading to a negative relationship (Chapter Two). Reliance on aquatic respiration in these species was higher in the hatchlings than the adults (Chapter Two), supporting the hypothesis that the higher mass specific surface area of hatchlings allows them to extract a relatively larger amount of oxygen from the water than adults (Mathie and Franklin, 2006). The negative relationship between dive duration and body size was not however evident in all species. The dives of hatchling *Elseya latisternum* and *Elusor macrurus* were shorter in duration than those previously recorded for adults (Chapter Two). Reliance on aquatic respiration is not only influence by surface area but other factors such as the perfusion and ventilation of the aquatic respiratory organs may also affect respiratory ability. Future investigations into how these factors scale allometrically may explain why the dive durations of these bimodally respiring turtle species did not also correlate negatively with body size.

The relationships between diving physiology and behaviour in hatchling turtles may not simply be a by-product of turtle morphology but may also have an ecological influence. Bimodally respiring fish species are known to use aquatic respiration as a predator avoidance strategy. In the presence of a predator, these fish species increase their reliance on aquatic respiration thereby reducing predator exposure at the water surface (Kramer, 1983; Kramer et al., 1983; Smith and Kramer, 1986; Wolf and Kramer, 1987; Herbet and Wells, 2001; Domenici et al., 2007). Hatchling turtles have extremely high predation rates and typically less than 1% are expected to survive to adulthood (Gibbons and Semlitsch, 1982; Cann, 1998). The ability to reduce surface frequency and hence risk of predation through the use of aquatic respiration may benefit hatchling survival. The diving behaviour of *E. macrurus* and *E. signata* in the presence of a predator suggests that aquatic respiration in bimodally respiring turtles may function as a predator avoidance strategy. In the presence of a large piscine predator (barramundi - *Lates calcarifer*), the maximum dive duration of *E. macrurus* increased by ~200% while no difference in dive duration was recorded for *E. signata* (Chapter Five). Diving behaviour studies suggest that *E. macrurus* has a higher reliance on aquatic respiration than *E. signata* (Chapter Two; Chapter Five). The predator response differences between the turtle species may therefore be a result of differences in reliance on aquatic respiration suggesting aquatic respiration may function as a predator avoidance strategy in hatchling turtles.

Diving physiology and behaviour in hatchling turtles may also be influenced by species ecology. Aquatic respiration in *R. leukops* was approximately four times greater than that of the other Australian hatchlings and allowed this species to remain underwater for > 72 h without surfacing (Chapter Two). The remarkable ability of *R. leukops* to extend dive duration through the use of aquatic respiration is thought be a key factor in the ability of this species to inhabit fast-flowing riffle zones (Gordos, 2004; Gordos et al., 2004a). A high reliance on aquatic respiration decreases the frequency and therefore the costs associated with surfacing in a high velocity environment (Gordos, 2004). Within riffle zones, *R. leukops* has reduced competition from other turtle species for food resources as well as reduced predator exposure (Gordos, 2004). The maximum dive durations of *E. macrurus* and *E. albagula* (4 h) were eight times longer than that of *E. signata* and *E. latisternum* (30 min) suggesting they too may use an increased reliance on aquatic respiration to further exploit the aquatic environment (Chapter Two). Future investigations into hatchling physiological ecology in the natural environment is however required to confirm these suspicions as reliance on aquatic respiration and blood oxygen affinity did not differ between these four species within the laboratory environment (Chapter Two).

Within the natural environment, habitat selection is an important aspect of hatchling ecology. Environmental conditions such as temperature and oxygen levels are known to vary on temporal and spatial scales and factors such as water depth, water velocity, vegetation density and turbidity can influence the conditions experienced by hatchling turtles (Gordos and Franklin, 2002; Wu, 2002; Gordos et al., 2003b). In the laboratory, as temperature increased from 17ºC to 28ºC, the metabolic rate of *E. macrurus* hatchlings increased by two fold (Chapter Three). This increase in metabolic rate could not be sustained via aquatic respiration alone and as a result aerial respiration increased and dive duration decreased (Chapter Three). Hatchling turtles inhabiting the warm surface layers of a river will therefore have a reduced reliance on aquatic respiration and shorter dive duration. Under these conditions, hatchling turtles may select microhabitats with a high degree of shelter such as reed beds, to protect them from the increased predator exposure experienced as a result of the increased reliance on aerial respiration at these high temperatures. Hatchling turtles that choose to inhabit the cool deep sections of the river benefit from a reduced metabolic rate (Chapter Four) and increase in the oxygen capacitance of water (Dejours, 1994). Aquatic PO_2 levels can also vary diurnally, as a result of plant photosynthesis and respiration and spatially with factors such as water flow and water depth. High levels of aquatic PO_2 enhance diffusion of respiratory gases across the aquatic respiratory organs thereby increasing reliance on aquatic respiration and dive duration. In hypoxic conditions, *E. macrurus* hatchlings lost oxygen from their respiratory organs to the aquatic environment leading to a significant reduction in dive duration (Chapter Four). The susceptibility of bimodally respiring hatchling turtles to acute changes in environmental conditions raises concerns about the impacts of long-term environmental change on species survival.

CONSERVATION CONSIDERATIONS

Changes in environmental conditions as a result of anthropogenic influences are becoming increasing common in today's society. Understanding how animals interact with their environment is therefore critical to the management and conservation of species (Wikelski and Cooke, 2006). Conservation physiology is a relatively new field of scientific research which through the use of physiological disciplines such as endocrinology, biochemistry, immunology and toxicology, aims to provided a mechanistic understanding of the underlying causes of conservation problems (Wikelski and Cooke, 2006). For example, analysis of nutritional physiology in the Sonoran Desert tortoise, *Gopherus agassizii*, revealed population declines were a direct result of high potassium levels in food plants. This physiological information was then use to design a conservation management plan that focussed on increasing the presence of low potassium plant species within the environment (Devender, 2002). In the present study, the use of physiological techniques has proved a vital tool for understanding how bimodally respiring turtles respond to long-term changes in environmental conditions as a result of river damming.

Almost half of the world's river systems have been dammed to meet the energy and water requirements of the human population (World Commission on Dams, 2000). River dams fragment and transform natural river systems and therefore disrupt important ecosystem processes in both the terrestrial and aquatic environments. Specifically, within the aquatic environment river damming converts lotic to lentic water, eliminates pool-riffle sequences, decreases water temperature and velocity, increases water depth and decreases aquatic oxygen levels (Walker, 1985; Ligon et al., 1995; Reese and Welsh, 1998; Bodie, 2001; Arthington, 2003; Snoussi et al., 2007). The impacts of river damming have largely been investigated in fish populations (Antonio et al., 2007; Clarke et al., 2007; Fukushima et al., 2007; Xie et al., 2007) with very few studies considering the effects on freshwater turtles.

Population monitoring results reveal within impoundments the abundance of turtle species with a high reliance on aquatic respiration (*R. leukops and E. albagula*) is decreasing while species with a lower reliance on aquatic respiration are increasing (*E. signata*) (Tucker et al., 2001; Limpus et al., 2002; Arthington, 2003). The differences observed in the sensitivity of these species to environmental change maybe attributed to their variations in respiratory physiologies. Specialist species with a higher reliance on aquatic respiration are likely to be impacted by river damming to a greater extent than generalists (Moll, 1997; Tucker et al., 2001). For example, the high reliance of *R. leukops* on aquatic respiration allows this species to inhabit fast-flowing riffle zones from which air-breathing species are excluded (Cann, 1998; Gordos et al., 2004a). The loss of riffle zones due to the construction of dams and weirs may influence the distribution and abundance of predator and prey species thereby manipulating the growth and survival rates of this specialist species (Gordos, 2004; Gordos et al., 2004a). A decrease in water velocity also reduces aquatic oxygen levels thereby increasing turtle surfacing frequency and reducing the time available for activities such as foraging (Gordos et al., 2004a).

The environmental change that occurs as a result of river damming is a long-term change which raises question about the ability of bimodally respiring turtles to acclimatise to the new conditions. Many animals can compensate for long-term environmental fluctuations through the process of acclimation (laboratory based) or acclimatisation (field based), which is the reversible change in physiological processes in response to environmental variability (Prosser, 1991; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). The beneficial acclimation hypothesis suggests that these acclimatory responses benefit the organism by increasing individual fitness (Leroi et al., 1994; Wilson and Franklin, 2002b; Seebacher, 2005; Angilletta et al., 2006). For example, the long-necked turtle, *Chelodina longicollis*, increases metabolic enzyme activity during winter in order to maintain metabolic potential in cold conditions (Seebacher et al., 2004). Acclimation has been reported in all major reptilian groups and may occur at all levels of the organisms from gene expression through to whole animal performance (Seebacher, 2005). Investigations into the diving physiological ecology of *E. macrurus* hatchlings revealed that bimodally respiring turtle species have very little ability to thermally acclimate. After long-term exposure to 28ºC, *E. macrurus* hatchlings were unable to negate the acute effect of increasing temperature on metabolic rate, aquatic respiration, dive duration or burst swimming speed (Chapter Three). Hatchlings acclimated to the cold temperature (17ºC) were able to increase dive duration at 17ºC however this response was only possible in waters with sufficient levels of dissolved oxygen. River dams are however characterised by benthic hypoxic and anoxic conditions which after long-term exposure, have proven to be detrimental to *E. macrurus* hatchlings (Chapter Four).

Knowledge of the relationships between animals and their environment is essential for understanding the conservational implications of long-term environmental change (Wikelski and Cooke, 2006). This study has demonstrated that the diving physiological ecology of hatchling turtles is directly influenced by long-term changes in environmental temperature and oxygen levels. Diving physiology and behaviour in bimodally respiring turtles is directly related to ecological activities such as foraging, reproduction and predator exposure (Kramer, 1988).

Variations in diving ecology as a result of changes in these environmental conditions therefore has the potential to seriously influence species survival. As turtle habitat within our natural river systems continues to be degraded as a result of human influence, understanding how bimodally respiring turtles interact with their environment is essential for predicting the consequences of long-term environmental change and for the successful conservation and management of these unique and wonderful reptiles.

FUTURE DIRECTIONS

The influence of body size on diving physiology and behaviour has in the past been overlooked in bimodally respiring turtle species. Studies have primarily focused on adults and conclusions generalised based solely on these results. This study has demonstrated that body size is an important aspect of turtle diving physiology and behaviour however this research was limited to a small selection of diving parameters and body sizes. Comprehensive studies that focus on the influence of body size on a range of diving physiological and behavioural parameters (eg, diving heart rate, acid-base balance, foraging behaviour) would substantially enhance our knowledge of the fundamental diving processes and their relationships.

Research into the diving physiology and behaviour of bimodally respiring turtles is generally centred around reliance on aquatic respiration. Measures of aquatic respiration however vary substantially both between and within studies and this has been attributed to the difficultly in obtaining accurate measures of a species' maximum ability to respire aquatically. Closed-box respirometry experiments potentially underestimate reliance on aquatic respiration due to shallow water depth (Mathie and Franklin, 2006). New technology that employs the use to fiber-optic microsensors may provide a more accurate measure of aquatic respiration by recording the amount of oxygen extracted from the water for each ventilation of the cloacal bursae (Gatti et al., 2002; Glazer et al., 2004; Kocinocova et al., 2007). Research into the differences in cloacal bursae morphology, perfusion and ventilation between turtle species and age classes would also provide further insight into aquatic respiration variations.

In the present study, the use of aquatic respiration as a predator avoidance strategy was examined as an initial investigation into the ecological consequences of environmental change. This study used measures of diving behaviour as an indicator of reliance on aquatic respiration and concluded that hatchling turtles were able to use aquatic respiration as a predator avoidance strategy. This result suggests that long-term changes in environmental conditions are likely to influence the survival of hatchling turtles however, further research is required into the direct effects of a predator on turtle respiratory partitioning in order to confirm this conclusion.

In this thesis, the use of physiological techniques has proved a vital tool for understanding how bimodally respiring turtles respond to long-term changes in environmental conditions as a result of river damming. The study of animal physiology is becoming increasing popular in the field of conservation science due to its ability to provide a detailed mechanistic understanding of the cause of conservation issues (Wikelski and Cooke, 2006). In today’s society where changes in environmental conditions as a result of anthropogenic influences are becoming increasingly more common, understanding how animals interact with their environment is an essential aspect of species’ conservation and management (Wikelski and Cooke, 2006). This investigation discovered that unlike many other ectotherms (Seebacher, 2005), bimodally respiring turtles display low acclimation ability. Turtle diving physiology and behaviour are therefore highly susceptible to changes in environmental conditions. Research in this study was however limited to a small number of physiological and behavioural parameters and as a result the long-term ecological or fitness consequences of these changes remain unknown. Through the use of remote data loggers and transmitters, research into the ecophysiology of populations in natural and dammed environments could provide further information on species' natural response to acute and long-term changes in environmental conditions. Additionally, investigations into how long-term changes in environmental conditions influence turtle reproduction (eg. endocrinology, physiological genomics) and survival (eg. evolutionary physiology, toxicology) would enhance our understanding of the ecological consequences of environmental change and will contribute to conservation and management of species with a dammed environment.

REFERENCES

Acevedo-Gutierrez, A., Croll, D. A. and Tershy, B. R. (2002). High feeding costs limit dive time in the largest whales. *Journal of Experimental Biology* **205**: 1747-1753.

Alton, L. A., White, C. R. and Seymour, R. S. (2007). Effect of aerial O_2 partial pressure on bimodal gas exchange and air-breathing behaviour in *Trichogaster leeri*. *Journal of Experimental Biology* **210**: 2311-2319.

Angilletta, M. J., Bennett, A. F., Guderley, H., Navas, C. A., Seebacher, F. and Wilson, R. S. (2006). Coadaptation: A unifying principle in evolutionary thermal biology. *Physiological and Biochemical Zoology* **79**: 282-294.

Angilletta, M. J., Niewiarowski, P. H. and Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. *Journal of Thermal Biology* **27**: 249-268.

Antonio, R. R., Agostinho, A. A., Pelicice, F. M., Bailly, D., Okada, E. K. and Dias, J. H. P. (2007). Blockage of migration routes by dam construction: can migratory fish find alternative routes? *Neotropical Ichthyology* **5**: 177-184.

Arthington, A. H. (2003). Burnet Basin WAMP - Current environmental condition and impacts of existing water resource development - Appendix H. Queensland Department of Natural Resources and Mines.

Bagatto, B., Guyer, C., Hauge, B. and Henry, R. P. (1997). Bimodal respiration in two species of central American turtles. *Copeia* **1997**: 834-839.

Bagatto, B. and Henry, R. P. (1999). Exercise and forced submergence in the pond slider (*Trachemys scripta*) and softshell turtle (*Apalone ferox*): influence on bimodal gas exchange, diving behaviour and blood acid-base status. *Journal of Experimental Biology* **202**: 267-278.

Baird, T. A. (1983). Influence of social and predatory stimuli on the air-breathing behavior of the African clawed frog, *Xenopus laevis*. *Copeia* **1983:** 411-420.

Barrell, J. (1916). The influence of Silurian-Devonian climates on the rise of air-breathing vertebrates. *Proceedings of the National Academy of Science of the United States of America* **2:** 499-504.

Bartels, H. (1982). Metabolic rate of mammals equals the 0.75 power of their body weight. *Experimental Biology and Medicine* **7**: 1-11.

Belkin, D. A. (1968). Aquatic respiration and underwater survival of two freshwater turtle species. *Respiration Physiology* **4**: 1-14.

Bennett, A. F. (1990). Thermal-dependence of locomotor capacity. *American Journal of Physiology* **259**: R253-R258.

Bennett, A. F. and Huey, R. B. (1990). Studying the evolution of physiological performance. *Oxford Surveys in Evolutionary Biology* **7**: 251-284.

Bevan, D. J. and Kramer, D. L. (1986). The effect of swimming depth on respiratory behavior of the honey gourami, *Colisa Chuna* (Pisces, Belontiidae). *Canadian Journal of Zoology* **64**: 1893-1896.

Bickler, P. E. and Buck, L. T. (2007). Hypoxia tolerance in reptiles, amphibians, and fishes: life with variable oxygen availability. *Annual Review of Physiology* **69**: 145-170.

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

Boutilier, R. G. (1990). Control and co-ordination of gas exchange in bimodal breathers. *Advances in Comparative and Environmental Physiology* **6**: 279-345.

Boyd, I. L. (1997). The behavioural and physiological ecology of diving. *Trends in Ecology & Evolution* **12**: 213-217.

Brauner, C. J., Ballantyne, C. L., Randall, D. J. and Val, A. L. (1995). Air-breathing in the armored catfish (*Hoplosternum littorale*) as an adaptation to hypoxic, acidic, and hydrogen-sulfide rich waters. *Canadian Journal of Zoology* **73**: 739-744.

Bray, A. A. (1985). The evolution of the terrestrial vertebrates: environmental and physiological considerations. *Philosophical Transactions of the Royal Society of London B* **309**: 289-322.

Brown, J. S. and Kotler, B. P. (2004). Hazardous duty pay and the foraging cost of predation. *Ecology Letters* **7**: 999-1014.

Burggren, W., Hahn, C. E. W. and Foex, P. (1977). Properties of blood oxygen transport in the turtle *Pseudemys scripta* and the tortoise *Testudp graeca*: effects of temperature, CO_2 and pH. *Respiration Physiology* **31**: 39-50.

Butler, P. J. and Jones, D. R. (1982). The comparative physiology of diving in vertebrates. In *Advances in Comparative Physiology and Biochemistry*, (ed. O. Lowenstein), pp. 179-364. Academic Press. New York.

Butler, P. J. and Jones, D. R. (1997). Physiology of diving birds and mammals. *Physiological Reviews* **77**: 837-899.

Cann, J. (1998). Australian Freshwater Turtles. 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.

Carbone, C. and Houston, A. I. (1996). The optimal allocation of time over the dive cycle: An approach based on aerobic and anaerobic respiration. *Animal Behaviour* **51**: 1247-1255.

Carbone, C., Leeuw, J. J. D. and Houston, A. I. (1996). Adjustments in the diving time budgets of tufted duck and pochard: is there evidence for a mix of metabolic pathways? *Animal Behaviour* **51**: 1257-1268.

Carter, A. J. and Wilson, R. S. (2006). Improving sneaky-sex in a low oxygen environment: reproductive and physiological responses of male mosquito fish to chronic hypoxia. *Journal of Experimental Biology* **209**: 4878-4884.

Chapman, L. J., Galis, F. and Shinn, J. (2000). Phenotypic plasticity and the possible role of genetic assimilation: hypoxia-induced trade-offs in the morphological traits of an African cichlid. *Ecology Letters* **3:** 387-393.

Chapman, L. J. and Hulen, K. G. (2001). Implications of hypoxia for the brain size and gill morphometry of mormyrid fishes. *Journal of Zoology* **254**: 461-472.

Chappell, M. A., Shoemaker, V. H., James, D. N., Bucher, T. L. and Maloney, S. K. (1993). Diving behavior during foraging in breeding adelie penguins. *Ecology* **74**: 1204-1215.

Chessman, B. C. (1986). Diet of the Murray turtle, *Emydura macquarii* (Gray) (Testudines: Chelidae). *Australian Wildlife Research* **13:** 65-69.

Chessman, B. C. (1988). Habitat preferences of freshwater turtles in the Murray Valley, Victoria and New South Wales. *Australian Wildlife Research* **15:** 485-494.

Clark, T. D., Seymour, R. S., Christian, K., Wells, R. M. G., Baldwin, J. and Farrell, A. P. (2007). Changes in cardiac output during swimming and aquatic hypoxia in the air-breathing Pacific tarpon. *Comparative Biochemistry and Physiology A* **148**: 562-571.

Clarke, A. D., Telmer, K. H. and Shrimpton, J. M. (2007). Habitat use and movement patterns for a fluvial species, the Arctic grayling, in a watershed impacted by a large reservoir: evidence from otolith microchemistry. *Journal of Applied Ecology* **44**: 1156-1165.

Cogger, H., Cameron, E., Sadlier, R. and Eggler, P. (1993). The Action Plan for Australian Reptiles. Australian Nature Conservation Agency. Canberra.

Costa, D. P., Gales, N. J. and Goebel, M. E. (2001). Aerobic dive limit: how often does it occur in nature? *Comparative Biochemistry and Physiology A* **129**: 771-783.

Costanzo, J. P., Jones, E. E. and Lee, R. E. (2001). Physiological responses to supercooling and hypoxia in the hatchling painted turtle, *Chrysemys picta. Journal of Comparative Physiology B* **171**: 335-340.

Crocker, C. E., Ultsch, G. R. and Jackson, D. C. (1999). The physiology of diving in a north-temperate and three tropical turtle species. *Journal of Comparative Physiology B* **169**: 249-255.

Croll, D. A., Acevedo-Gutierrez, A., Tershy, B. R. and Urban-Ramirez, J. (2001). The diving behavior of blue and fin whales: is dive duration shorter than expected based on oxygen stores? *Comparative Biochemistry and Physiology A* **129**: 797-809.

Davis, B. J. (1991). Developmental changes in the blood oxygen transport system of Kemp's ridley sea turtle, *Lepidochelys kempi*. *Canadian Journal of Zoology* **69**: 2660-2666.

Dejours, P. (1994). Environmental-factors as determinants in bimodal breathing - an introductory overview. *American Zoologist* **34**: 178-183.

Department of Natural Resources, Integrated Catchment Management. (1993). The condition of river catchments in Queensland: A broad overview of catchment management issues. Queensland Government. Brisbane.

Devender, T. R. V. (2002). The Sonoran Desert Tortoise. University of Arizona Press. Arizona.

Dolphin, W. F. (1987). Ventilation and dive patterns of humpback whales, *Megaptera novaeangliae*, on their Alaskan feeding grounds. *Canadian Journal of Zoology* **65**: 83-90.

Domenici, P. and Blake, R. W. (1997). The kinematics and performance of fish fast-start swimming. *Journal of Experimental Biology* **200**: 1165-1178.

Domenici, P., Lefrancois, C. and Shingles, A. (2007). Hypoxia and the antipredator behaviours of fishes. *Philosophical Transactions of the Royal Society B* **362**: 2105-2121.

Evans, D. O. (1990). Metabolic thermal compensation by rainbow-trout - effects on standard metabolic-rate and potential usable power. *Transactions of the American Fisheries Society* **119**: 585-600.

Flakus, S. (2002). The ecology of the Mary River turtle. Masters Thesis, The University of Queensland, Brisbane.

Frid, A., Heithaus, M. R. and Dill, L. M. (2007). Dangerous dive cycles and the proverbial ostrich. *Oikos* **116**: 893-902.

Fry, F. E. J. and Hart, J. S. (1948). Cruising speed of goldfish in relation to water temperature. *Journal of Fisheries Research Board of Canada* **7**: 169-175.

Fukushima, M., Kameyama, S., Kaneko, M., Nakao, K. and Steel, E. A. (2007). Modelling the effects of dams on freshwater fish distributions in Hokkaido, Japan. *Freshwater Biology* **52**: 1511-1524.

Fuster, J. F., Pages, T. and Palacios, L. (1997). Effect of temperature on oxygen stores during aerobic diving in the freshwater turtle *Mauremys caspica leprosa*. *Physiological Zoology* **70**: 7-18.

Gage, S. H. and Gage, S. P. (1886). Aquatic respiration in soft-shelled turtles: a contribution to the physiology of respiration in vertebrates. *The American Naturalist* **20**: 233-236.

Gans, C. (1971). Strategy and sequency in the evolution of the external gas exchanges of ectothermal vertebrates. *Form and Function* **3**: 61-104.

Gatten, R. E. (1978). Aerobic metabolism in snapping turtles, *Chelydra serpentina*, after thermal acclimation. *Comparative Biochemistry and Physiology A* **61**: 325-337.

Gatten, R. E. (1980). Aerial and aquatic oxygen-uptake by freely-diving snapping turtles (*Chelydra serpentina*). *Oecologia* **46**: 266-271.

Gatti, S., Brey, T., Muller, W. E. G., Heilmayer, O. and Holst, G. (2002). Oxygen mircrooptodes: a new tool for oxygen measurements in aquatic animal ecology. *Marine Biology* **140**: 1075-1085.

Gauthier, J. A., Kluge, A. G. and Rowe, T. (1988). The early evolution of the Amniota. In *The Phylogeny and Classification of the Tetrapods*, special volume No 35A (ed. M. J. Benton), pp. 103-155. Clarendon Press. Oxford.

Geiger, S. P., Torres, J. J. and Crabtree, R. E. (2000). Air breathing and gill ventilation frequencies in juvenile tarpon, *Megalops atlanticus*: responses to changes in dissolved oxygen, temperature, hydrogen sulfide, and pH. *Environmental Biology of Fishes* **59:** 181-190.

Georges, A. and Adams, M. (1992). A phylogeny for Australian chelid turtles based on allozyme electrophoresis. *Australian Journal of Zoology* **40**: 453-476.

Georges, A., Birrell, J., Saint, K. M., McCord, W. and Donnellan, S. C. (1998). A phylogeny for side-necked turtles (Chelonia : Pleurodira) based on mitochondrial and nuclear gene sequence variation. *Biological Journal of the Linnean Society* **67**: 213-246.

Georges, A. and Thomson, S. (2006) Evolution and zoogeography of Australian freshwater turtles, In *Evolution and Biogeography of Australian Vertebrates*. (eds. J.R. Merrick, M. Archer, G.M. Hickey, M.S.Y. Less), pp. 291-308. Auscipub Pty Ltd. Oatlands.

Gibbons, J. W. and Semlitsch, R. D. (1982). Survivorship and longevity of a long-lived vertebrate species: how long do turtles live? *Journal of Animal Ecology* **51**: 523-527.

Girgis, S. (1961). Aquatic respiration in the common Nile turtle, *Trionyx triunguis* (Forskal). *Comparative Biochemistry and Physiology* **3**: 206-217.

Glanville, E. J. and Seebacher, F. (2006). Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *Journal of Experimental Biology* **209**: 4869-4877.

Glazer, B. T., Marsh, A. G., Stierhoff, K. and Luther, G. W. (2004). The dynamic response of optical oxygen sensors and voltammetric electrodes to temporal changes in dissolved oxygen concentrations. *Analytica Chimica Acta* **518**: 93-100.

Gordos, M. A. (2004). Diving physiological ecology of the bimodally respiring freshwater turtles, *Rheodytes leukops*. PhD Thesis, The University of Queensland. Brisbane.

Gordos, M. and Franklin, C. E. (2002). Diving behaviour of two Australian bimodally respiring turtles, *Rheodytes leukops* and *Emydura macquarii*, in a natural setting. *Journal of Zoology* **258**: 335-342.

Gordos, M. A., Franklin, C. E. and Limpus, C. J. (2003a). Seasonal changes in the diel surfacing behaviour of the bimodally respiring turtle *Rheodytes leukops*. *Canadian Journal of Zoology* **81**: 1614-1622.

Gordos, M. A., Franklin, C. E. and Limpus, C. J. (2003b). Seasonal changes in the diving performance of the bimodally respiring freshwater turtle *Rheodytes leukops* in a natural setting. *Canadian Journal of Zoology* **81**: 617-625.

Gordos, M. A., Franklin, C. E. and Limpus, C. J. (2004a). Effect of water depth and water velocity upon the surfacing frequency of the bimodally respiring freshwater turtle, *Rheodytes leukops*. *Journal of Experimental Biology* **207**: 3099-3107.

Gordos, M. A., Franklin, C. E., Limpus, C. J. and Wilson, G. (2004b). Blood-respiratory and acid-base changes during extended diving in the bimodally respiring freshwater turtle *Rheodytes leukops*. *Journal of Comparative Physiology B* **174**: 347-354.

Gordos, M. A., Hamann, M., Schauble, C. S., Limpus, C. J. and Franklin, C. E. (2007). Diving behaviour of *Elseya albagula* from a naturally flowing and hydrologically altered habitat. *Journal of Zoology* **272**: 458-469.

Gordos, M. A., Limpus, C. J. and Franklin, C. E. (2006). Response of heart rate and cloacal ventilation in the bimodally respiring freshwater turtle, *Rheodytes leukops* to experimental changes in aquatic $PO_{2.}$ *Journal of Comparative Physiology B* **176**: 65-73.

Graham, J. B. (1994). An evolutionary perspective for bimodal respiration: a biological synthesis of fish air breathing. *American Zoologist* **34**: 229-237.

Graham, J. B., Rosenblatt, R. H. and Gans, C. (1978). Vertebrate air-breathing arose in fresh waters and not in the ocean. *Evolution* **32**: 459-463.

Grigg, G. C. (1969). Temperature induced changes in the oxygen equilibrium curve of the blood of the brown bullhead *Ictaluras nebulosus*. *Compariative Biochemistry and Physiology* **28**: 1203-1223.

Guppy, M. and Withers, P. (1999). Metabolic depression in animals: physiological perspectives and biochemical generalizations. *Biological Reviews* **74**: 1-40.

Hamann, M., Schauble, C. S., Limpus, D. J., Emerick, S. P. and Limpus, C. J. (2007). Management plan for the conservation of *Elseya sp* (Burnett River) in the Burnett River catchment: Queensland Environmental Protection Agency. Queensland.

Haynie, D. (2001). Biological Thermodynamics. Cambridge University Press. Cambridge.

Hays, G. C., Akesson, S., Broderick, A. C., Glen, F., Godley, B. J., Luschi, P., Martin, C., Metcalfe, J. D. and Papi, F. (2001). The diving behaviour of green turtles undertaking oceanic migration to and from Ascension Island: dive durations, dive profiles and depth distribution. *Journal of Experimental Biology* **204**: 4093-4098.

Heithaus, M. R., Dill, L. M., Marshall, G. J. and Buhleier, B. M. (2002). Habitat use and foraging behavior of tiger sharks (*Galeocerdo cuvier*) in a seagrass ecosystem. *Marine Biology* **140**: 237-248.

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

Herbert, C. V. and Jackson, D. C. (1985a). Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta belli*. I. blood acid-base and ionic changes during and following anoxic submergence. *Physiological Zoology* **58**: 665-669.

Herbert, C. V. and Jackson, D. C. (1985b). Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta belli*. II. metabolic rate, blood acid-base and ionic changes and cardiovascular function in aerated and anoxic water. *Physiological Zoology* **58**: 670-681.

Herbet, N. A. and Wells, R. M. G. (2001). The aerobic physiology of the air-breathing blue gourami, *Trichogaster trichopterus*, necessitates behavioural regulation of breath-hold limits during hypoxic stress and predatory challenge. *Journal of Comparative Physiology B* **171**: 603-612.

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

Hochachka, P. W. and Mommsen, T. P. (1983). Protons and anaerobiosis. *Science* **219**: 1391-1397.

Houston, A. I. and Carbone, C. (1992). The optimal allocation of time during the diving cycle. *Behavioral Ecology* **3**: 255-265.

Huey, R. B. (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia*, vol. 12 (eds. C. Gans and F. H. Pough), pp. 25-74. Academic Press. New York.

Huey, R. B., Berrigan, D., Gilchrist, G. W. and Herron, J. C. (1999). Testing the adaptive significance of acclimation: a strong inference approach. *American Zoologist* **29**: 323-336.

Irving, L. (1939). Respiration in diving mammals. *Physiological Reviews* **19**: 112-134.

Isaacks, R. R., Harkness, D. R. and Whitham, P. R. (1978). Relationship between the major phosphorylated metabolic intermediates and oxygen affinity of whole blood in the loggerhead (*Caretta caretta*) and the green sea turtle (*Chelonia mydas mydas*) during development. *Developmental Biology* **62**: 344-353.

Jackson, D. C. (1968). Metabolic depression and oxygen depletion in the diving turtle. *Journal of Applied Physiology* **24**: 503-509.

Jackson, D. C. (1976). Non-pulmonary CO_2 loss during diving in turtle, *Pseudemys scripta elegans*. *Comparative Biochemistry and Physiology A* **55:** 237-241.

Jackson, D. C. (2000). Living without oxygen: lessons from the freshwater turtle. *Comparative Biochemistry and Physiology A* **125**: 299-315.

Jackson, D. C. (2004). Acid-base balance during hypoxic hypometabolism: selected vertebrate strategies. *Respiratory Physiology and Neurobiology* **141**: 273-283.

Jackson, D. C. (2007). Temperature and hypoxia in ectothermic tetrapods. *Journal of Thermal Biology* **32**: 125-133.

Jackson, D. C., Allen, J. and Strupp, P. K. (1976). Contribution of non-pulmonary surfaces to CO_2 loss in 6 species of turtles at 20ºC. *Comparative Biochemistry and Physiology A* **55**: 243-246.

Jackson, D. C. and Silverblatt, H. (1974). Respiration and acid-base status of turtles following experimental dives. *American Journal of Physiology* **226**: 903-909.

Jensen, F. B. and Weber, R. E. (1982). Respiratory properties of tench blood and haemoglobin adaptation to hypoxic-hypercapnic water. *Molecular Physiology* **2**: 235-250.

Johnson, T. P. and Bennett, A. F. (1995). The thermal-acclimation of burst escape performance in fish - an integrated study of molecular and cellular physiology and organismal performance. *Journal of Experimental Biology* **198**: 2165-2175.

Johnston, I. A. and Temple, G. K. (2002). Thermal plasticity of skeletal muscle phenotype in ectothermic vertebrates and its significance for locomotory behaviour. *Journal of Experimental Biology* **205**: 2305-2322.

Kayes, S (2005). The significance of a diving bradycardia during voluntary dives in two species of freshwater turtle, *Emydura signata* and *Elseya latisternum.* BSc. Honours Thesis, The University of Queensland, Brisbane.

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

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

Kleiber, M. (1961). The fire of life. An Introduction to Animal Energetics. John Wiley and Sons. New York.

Kocinocova, A. S., Borisov, S. M., Krause, C. and Wolfbeis, O. S. (2007). Fiber-optic microsensors for simultaneous sensing of oxygen and pH, and of oxygen and temperature. *Analytical Chemistry* **79**: 8486-8493.

Kooyman, G. L. (1989). Diverse Divers. Springer-Verlag. London.

Kooyman, G. L., Castellini, M. A., Davis, R. W. and Maue, R. A. (1983). Aerobic dive limits of immature Weddell seals. *Journal of Comparative Physiology B* **151**: 171-174.

Kooyman, G. L. and Ponganis, P. J. (1998). The physiological basis of diving to depth: birds and mammals. *Annual Review of Physiology* **60**: 19-32.

Kooyman, G. L., Wahrenbrock, E. A., Castellini, M. A., Davis, R. W. and Sinnett, E. E. (1980). Aerobic and anaerobic metabolism during voluntary diving in Weddell seals: evidence of preferred pathways from blood chemistry and behavior. *Journal of Comparative Physiology* **138**: 335-346.

Kramer, D. L. (1983). The evolutionary ecology of respiratory mode in fishes: an analysis based on the costs of breathing. *Environmental Biology of Fishes* **9**: 145-158.

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

Kramer, D. L., Manley, D. and Bourgeois, R. (1983). The effect of respiratory mode and oxygen concentration on the risk of aerial predation in fishes. *Canadian Journal of Zoology* **61**: 653-665.

Legler, J. M. (1993). Morphology and physiology of the Chelonia. In *Fauna of Australia*, vol. 2A (eds. C. J. Glasby G. J. B. Ross and P. L. Beesley), pp. 108-119. Australian Government Publishing Service. Canberra.

Legler, J. M. and Cann, J. (1980). A new genus and species of Chelid turtle from Queensland, Australia. *Contributions to the Science and Natural History Museum Los Angeles Country* **324**: 1-18.

Legler, J. M. and Georges, A. (1993). Family Chelidae. In *Fauna of Australia*, vol. 2A (eds. C. J. Glasby G. J. B. Ross and P. L. Beesley), pp. 142-152. Australian Government Publishing Service. Canberra.

Leroi, A. M., Bennett, A. F. and Lenski, R. E. (1994). Temperature acclimation and competitive fitness: an experimental test of the beneficial acclimation assumption. *Proceedings of the Natural Academe of Science United States of America* **91**: 1917-1921.

Lewis, S. M., Bain, B. J. and Bates, I. (2001). Dacie and Lewis Practical Haemotology. Harcourt Publishers. London.

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

Lima, S. L. (1998). Stress and decision making under risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Advances in the Study of Behavior* **27**: 215-290.

Lima, S. L. and Bednekoff, P. A. (1999). Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. *The American Naturalist* **153**: 649-659.

Lima, S. L. and Dill, L. M. (1990). Behavioural decisions made under the risk of predation. *Canadian Journal of Zoology* **68**: 619-640.

Limpus, C. J., Limpus, D. J. and Hamann, M. (2002). Freshwater turtle populations in the area to be flooded by the Walla Weir, Burnett River, Queensland: baseline study. *Memoirs of the Queensland Museum* **48:**155-168.

Loeschcke, V. and Hoffmann, A. A. (2002). The detrimental acclimation hypothesis. *Trends in Ecology and Evolution* **17**: 407-408.

Lomholt, J. P. and Johansen, K. (1979). Hypoxia acclimation in the carp - how it affects O_2 uptake, ventilation and O_2 extraction from the water. *Physiological Zoology* **52**: 38-49.

Maginniss, L. A., Tapper, S. S. and Miller, L. S. (1983). Effects of chronic cold and submergence on blood oxygen transport in the turtle *Chrysemys picta*. *Respiration Physiology* **53**: 15-29.

Maina, J. N. (2002). Structure, function and evolution of the gas exchangers: comparative perspectives. *Journal of Anatomy* **201**: 281-304.

Manwell, C. (1960). Comparative physiology - blood pigments. *Annual Review of Physiology* **22**: 191-244.

Mathie, N. J. and Franklin, C. E. (2006). The influence of body size on the diving behaviour and physiology of the bimodally respiring turtle *Elseya albagula*. *Journal of Comparative Physiology B* **176**: 739-747.

Mattias, A. T., Rantin, F. T. and Fernandes, M. N. (1998). Gill respiratory parameters during progressive hypoxia in the facultative air-breathing fish, *Hypostomus regani* (Loricariidae). *Comparative Biochemistry and Physiology A* **120**: 311-315.

Megirian, D. and Murray, P. (1999). Chelid turtles (Pleurodira, Chelidae) from the Miocene Camfield Beds, Northern Territory of Australia, with a description of a new genus and species. *The Beagle (Records of the Museums and Art Galleries of the Northern Territory)* **15**: 75-130.

Milton, S. L. and Prentice, H. M. (2007). Beyond anoxia: the physiology of metabolic downregulation and recovery in the anoxia-tolerant turtle. *Comparative Biochemistry and Physiology A* **147**: 277-290.

Moll, E. O. (1997). Effects of habitat alteration on river turtles of tropical Asia with emphasis on sand mining and dams. In *Proceedings: Conservation, Restoration, and Management of Tortoises and Turtles - An International Conference*, pp. 37-41. New York Turtle and Tortoises Society. New York.

Mori, Y. (1998). Optimal choice of foraging depth in divers. *Journal of Zoology* **245**: 279-283.

Mori, Y. (1999). The optimal allocation of time and respiratory metabolism over the dive cycle. *Behavioral Ecology* **10**: 155-160.

O'Steen, S. and Bennett, A. F. (2003). Thermal acclimation effects differ between voluntary, maximum, and critical swimming velocities in two cyprinid fishes. *Physiological and Biochemical Zoology* **76**: 484-496.

Packard, M. J. and Seymour, R. S. (1997). Evolution of the amniote egg. In *Amniote Origins: Completing The Transition To Land*, (ed. S. S. Sumida), pp. 53-69. Academic Press. San Diego.

Park, Y., Chand, J., Lek, S., Cao, W. and Brosse, S. (2003). Conservation strategies for endemic fish species threatened by the Three Gorges dam. *Conservation Biology* **17**: 1748-1758.

Prassack, S. L., Bagatto, B. and Henry, R. P. (2001). Effects of temperature and aquatic PO_2 on the physiology and behaviour of *Apalone ferox* and *Chrysemys picta*. *Journal of Experimental Biology* **204:** 2185-2195.

Priest, T. (1997). Bimodal respiration and diving behaviour of the Fitzroy river turtle *Rheodytes leukops*. BSc Honours Thesis, The University of Queensland. Brisbane.

Priest, T. E. and Franklin, C. E. (2002). Effect of water temperature and oxygen levels on the diving behavior of two freshwater turtles: *Rheodytes leukops* and *Emydura macquarii*. *Journal of Herpetology* **36**: 555-561.

Prosser, C. L. (1991). Environmental and Metabolic Animal Physiology: Comparative Animal Physiology 4th ed.Wiley. Liss.

Pusey, B. J., Arthington, A. H. and Read, M. G. (1993). Spatial and temporal variation in fish assemblage structure in the Mary River, south-eastern Queensland - the influence of habitat structure. *Environmental Biology of Fishes* **37**: 355-380.

Randle, A. M. and Chapman, L. J. (2005). Air-breathing behaviour of the African anabantoid fish *Ctenopoma muriei*. *Journal of Fish Biology* **67**: 292-298.

Reese, D. A. and Welsh, H. H. (1998). Comparative demography of *Clemmys marmorata* populations in the Trinity River of California in the context of dam-induced alterations. *Journal of Herpetology* **32**: 505-515.

Relyea, R. A. (2001). The relationship between predation risk and antipredator responses in larval anurans. *Ecology* **82**: 541-554.

Rogers, K. D. and Booth, D. T. (2004). A method of sampling blood from Australian freshwater turtles. *Wildlife Research* **31**: 93-95.

Romer, A. S. (1956). The early evolution of land vertebrates. *Proceedings of the American Philosophical Society* **100**: 157-167.

Root, R. W. (1949). Aquatic respiration in the musk turtle. *Physiological Zoology* **22**: 172-181.

Sandjian, B (2007). Effect of water velocity and exhaustive exercise upon the diving behaviour of the bimodally respiring freshwater turtle *Elusor macrurus.* BSc. Honours Thesis, The University of Queensland, Brisbane.

Schaack, S. and Chapman, L. J. (2003). Interdemic variation in the African cyprinid *Barbus neumayeri*: correlations among hypoxia, morphology, and feeding performance. *Canadian Journal of Zoology* **81**: 430-440.

Schmalhausen, I. I. (1968). The Origin Of Terrestrial Vertebrates. Academic Press. London.

Schmidt-Nielsen, K. (1984). Scaling, Why Is Animal Size So Important? Cambridge University Press. Melbourne.

Schreer, J. F. and Kovacs, K. M. (1997). Allometry of diving capacity in air-breathing vertebrates. *Canadian Journal of Zoology* **75**: 339-358.

Seddon, J. M., Georges, A., Baverstock, P. R. and McCord, W. (1997). Phylogenetic relationships of Chelid turtles (Pleurodira: Chelidae) based on mitochondrial 12S rRNA gene sequence variation. *Molecular Phylogenetics and Evolution* **7**: 55-61.

Seebacher, F. (2005). A review of thermoregulation and physiological performance in reptiles: what is the role of phenotypic flexibility? *Journal of Comparative Physiology B* **175**: 453-461.

Seebacher, F., Guderley, H., Elsey, R. M. and Trosclair, P. L. (2003). Seasonal acclimatisation of muscle metabolic enzymes in a reptile (*Alligator mississippiensis*). *Journal of Experimental Biology* **206**: 1193-1200.

Seebacher, F., Sparrow, J. and Thompson, M. B. (2004). Turtles (*Chelodina longicollis)* regulate muscle metabolic enzyme activity in response to seasonal variation in body temperature. *Journal of Comparative Physiology B* **174**: 205-210.

Seymour, R. S. (1982). Physiological adaptations to aquatic life. In *Biology of the Reptilia*, (ed. C. Gans), pp. 1-51. Academic Press. London.

Seymour, R. S., Christian, K., Bennett, M. B., Baldwin, J., Wells, R. M. G. and Baudinette, R. V. (2004). Partitioning of respiration between the gills and air-breathing organ in response to aquatic hypoxia and exercise in the Pacific tarpon, *Megalops cyprinoides*. *Physiological and Biochemical Zoology* **77**: 760-767.

Seymour, R. S., Farrell, A. P., Christian, K., Clark, T. D., Bennett, M. B., Wells, R. M. G. and Baldwin, J. (2007). Continuous measurement of oxygen tensions in the air-breathing organ of Pacific tarpon (*Megalops cyprinoides*) in relation to aquatic hypoxia and exercise. *Journal of Comparative Physiology and Biochemistry* **177**: 579-587.

Smith, H. M. and James, L. F. (1958). The taxonomic significance of cloacal bursae in turtles. *Transactions of the Kansas Academy of Science* **61**: 86-96.

Smith, R. S. and Kramer, D. L. (1986). The effect of predation risk on the respiratory behaviour of the Florida gar (*Lepisosteus platyrhincus*). *Canadian Journal of Zoology* **64**: 2133-2136.

Snoussi, M., Kitheka, J., Shaghude, T., Kane, A., Arthurton, R., Tissier, M. L. and Virji, H. (2007). Downstream and coastal impacts of damming and water abstraction in Africa. *Environmental Management* **39**: 587-600.

Soivio, A., Nikinmaa, M. and Westmas, K. (1980). The blood oxygen binding properties of hypoxic *Salmo gairdneri*. *Comparative Physiology B* **136**: 83-87.

Sollid, J., De Angelis, P., Gundersen, K. and Nilsson, G. E. (2003). Hypoxia induces adaptive and reversible gross morphological changes in crucian carp gills. *Journal of Experimental Biology* **206**: 3667-3673.

Sparling, C. E., Georges, J., Gallon, S. L., Fedak, M. and Thompson, D. (2007). How long does a dive last? Foraging decisions by breath-hold divers in a patchy environment: a test of a simple model. *Animal Behaviour* **74**: 207-218.

Stecyk, J. A. W. and Farrell, A. P. (2007). Effects of extracellular changes on spontaneous heart rate of normoxia- and anoxia-acclimated turtles (*Trachemys scripta*). *The Journal of Experimental Biology* **210**: 421-431.

Stone, P. A., Dobie, J. L. and Henry, R. P. (1992a). Cutaneous surface-area and bimodal respiration in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). *Physiological Zoology* **65**: 311-330.

Stone, P. A., Dobie, J. L. and Henry, R. P. (1992b). The effect of aquatic O_2 levels on diving and ventilatory behavior in soft-shelled (*Trionyx spiniferus*), stinkpot (*Sternotherus odoratus*), and mud turtles (*Kinosternon subrubrum*). *Physiological Zoology* **65**: 331-345.

Storey, K. B. (1996). Metabolic adaptations supporting anoxia tolerance in reptiles: recent advances. *Comparative Biochemistry and Physiology B* **113**: 23-35.

Storey, K. B. (2007). Anoxia tolerance in turtles: metabolic regulation and gene expression. *Comparative Biochemistry and Physiology A* **147**: 263-276.

Thompson, D. and Fedak, M. A. (2001). How long should a dive last? A simple model of foraging decisions by breath-hold divers in a patchy environment. *Animal Behaviour* **61**: 287-296.

Thompson, D., Hammond, P. S., Nicholas, K. S. and Fedak, M. A. (1991). Movements, diving and foraging behaviour of grey seals *Halichoerus grypus. Journal of Zoology* **224**: 223-232.

Thomson, K. S. (1993). The origin of tetrapods. *American Journal of Science* **293A**: 33-62.

Thomson, S., Georges, A. and Limpus, C., J. (2006). A new species of freshwater turtle in the genus *Elseya* (Testudines: Chelidae) from central coastal Queensland, Australian. *Chelonian Conservation Biology* **5**: 74-86.

Tucker, A. D. (1999). Cumulative effects of dams and weirs on freshwater turtle populations: Fitzroy, Kolan, Burnett and Mary River catchments: Queensland Parks and Wildlife Service, Queensland Department of Natural Resources. Queensland.

Tucker, A. D., Limpus, C. J., Priest, T. E., Cay, J., Glen, C. and Guarino, E. (2001). Home ranges of Fitzroy River turtle (*Rheodytes leukops*) overlap riffle zones: potential concerns related to river regulation. *Biological Conservation* **102**: 171-181.

Turner, L. and Erskine, W. D. (2005). Variability in the development, persistence and breakdown of thermal, oxygen and salt stratification on regulated rivers of southeastern Australia. *River Research and Applications* **21**: 151-168.

Ultsch, G. R. (1985). The viability of nearctic fresh-water turtles submerged in anoxia and normoxia at 3ºC and 10ºC. *Comparative Biochemistry and Physiology A* **81**: 607-611.

Ultsch, G. R., Herbert, C. V. and Jackson, D. C. (1984). The comparative physiology of diving in North-American fresh-water turtles.1. submergence tolerance, gas-exchange, and acid-base-balance. *Physiological Zoology* **57**: 620-631.

Ultsch, G. R. and Jackson, D. C. (1982). Long-term submergence at 3ºC of the turtle *Chrysemys picta belli*, in normoxic and severely hypoxic water. *Journal of Experimental Biology* **96**: 11-28.

Val, A. L., Silva, M. N. P. and Almeida-Val, V. M. F. (1998). Hypoxia adaptation in fish of the Amazon: a never-ending task. *South African Journal of Zoology* **33**: 107-114.

Vleck, D. (1987). Measurement of O_2 consumption, CO_2 production, and water vapor production in a closed system. *Journal of Applied Physiology* **65**: 2103-2106.

Walker, K. F. (1985). A review of the ecological effects of river regulation in Australia. *Hydrobiologia* **125**: 111-129.

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

Wells, R. M. G., Grigg, G. C., Beard, L. A. and Summers, G. (1989). Hypoxic responses in a fish from a stable environment: blood oxygen transport in the Antarctic fish *Pagothenia borchgrevinki*. *Journal of Experimental Biology* **141**: 97-111.

Westoll, T. S. (1938). Ancestory of the tetrapods. *Nature* **141**: 127.

Westoll, T. S. (1943). The origin of the tetrapods. *Biological Reveiws* **18**: 78-98.

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

Williams, T. M., Davis, R. W., Fuiman, L. A., Francis, J., Le Boeuf, B. L., Horning, M., Calambokidis, J. and Croll, D. A. (2000). Sink or swim: strategies for cost-efficient diving by marine mammals. *Science* **288**: 133-136.

Wilson, R. S. (2005). Temperature influences the coercive mating and swimming performance of male eastern mosquitofish. *Animal Behaviour* **70**: 1387-1394.

Wilson, R. S. and Franklin, C. E. (1999). Thermal acclimation of locomotor performance in tadpoles of the frog *Limnodynastes peronii*. *Journal of Comparative Physiology B* **169**: 445-451.

Wilson, R. S. and Franklin, C. E. (2000). Inability of adult *Limnodynastes peronii* (Amphibia : Anura) to thermally acclimate locomotor performance. *Comparative Biochemistry and Physiology A* **127:** 21-28.

Wilson, R. S. and Franklin, C. E. (2002a). The detrimental acclimation hypothesis - response. *Trends in Ecology & Evolution* **17**: 408-408.

Wilson, R. S. and Franklin, C. E. (2002b). Testing the beneficial acclimation hypothesis. *Trends in ecology and evolution* **17**: 66-70.

Wolf, N. G. and Kramer, D. L. (1987). Use of cover and the need to breathe: the effects of hypoxia on vulnerability of dwarf gouramis to predatory snakeheads. *Oecologia* **73**: 127-132.

Wood, S. C. and Johansen, K. (1972). Adaptations to hypoxia by increasing HbO_2 affinity and decreasing red cell ATP concentration. *Nature* **237**: 278-279.

Wood, S. C., Lykkeboe, G., Johansen, K., Weber, R. E. and Maloiy, G. M. O. (1978). Temperature-acclimation in the pancake tortoise, *Malacochersus tornieri* - metabolic-rate, blood-Ph, oxygen-affinity and red-cell organic-phosphates. *Comparative Biochemistry and Physiology A* **59**: 155-160.

Woods, H. A. and Harrison, J. F. (2002). Interpreting rejections of the beneficial acclimation hypothesis: When is physiological plasticity adaptive? *Evolution* **56**: 1863-1866.

World Commission on Dams (2000). Dams and Development: A New Famework for Decision-Making. Earthscan Publications Ltd. London.

Worrell, E. (1966). Reptiles of Australia. Angus and Robertson. Sydney.

Wu, R. S. S. (2002). Hypoxia: from molecular responses to ecosystem responses. *Marine Pollution Bulletin* **45**: 35-45.

Xie, S. G., Li, Z. J., Liu, J. S., Xie, S. Q., Wang, H. Z. and Murphy, B. R. (2007). Fisheries of the Yangtze river show immediate impacts of the three gorges dam. *Fisheries* **32**: 343-344.

Yu, K. L. and Woo, N. Y. S. (1985). Effects of ambient oxygen-tension and temperature on the bimodal respiration of an air-breathing Teleost, *Channa maculata*. *Physiological Zoology* **58**: 181-189.