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Diving behaviour, aquatic respiration and blood respiratory properties: a comparison of hatchling and juvenile Australian turtles

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Abstract

Australia has a number of bimodally respiring freshwater turtle species that use aquatic respiration to extend their aerobic dive limit. While species variations in reliance on aquatic respiration are reflected in the diving behaviour and ecology of adults, it is unknown whether 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 *Els. albagula* and *Elu. macrurus* compared with *Em. signata* and *Els. latisternum*, there was no difference observed in per cent aquatic respiration or blood oxygen affinity between these species. When compared with adult individuals (data from previous studies), dive duration was positively correlated with body size in *Em. signata*, *Els. albagula* and *R. leukops*, but a negative relationship occurred in *Els. latisternum* and *Elu. 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 the cloacal bursae (Gage & Gage, 1886; Smith & James, 1958; Girgis, 1961; Belkin, 1968; Stone, Dobie & Henry, 1992; King & Heatwole, 1994*a*). In Australia, bimodal respiration occurs within several genera of freshwater turtles, with adult capabilities at temperatures above 20 °C ranging from a low reliance of 10% in *Emydura signata* (Priest, 1997; Priest & Franklin, 2002), through to medium capacities in *Elseya latisternum* (27%) (King & Heatwole, 1994*b*) and *Elseya albagula* (40%) (Mathie & Franklin, 2006) and up to >70% in the Fitzroy River turtle, *Rheodytes leukops* (Priest, 1997; Gordos, Franklin & Limpus, 2003).

The ability to supplement aerial respiration with aquatic oxygen allows these highly aquatic reptiles to extend their dive duration and reduce surfacing frequency (Bagatto & Henry, 1999; Gordos & Franklin, 2002). For example, aquatic respiration supports 10% of the total oxygen requirements in *Em. 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 & Franklin, 2002; Priest & Franklin, 2002; Gordos *et al.*, 2003). While species variations in reliance on aquatic respiration are reflected in the diving behaviour and ecology of adults, it is unknown whether 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. Owing 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 with adult turtles (Mathie & 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 (Hct) and haemoglobin (Hb) levels, would facilitate the uptake of oxygen from the aquatic environment and hence increase dive duration (Gordos *et al.*, 2004). P_{50} values range from 20.2 to 34.5 mmHg in adult bimodally respiring turtles (*Chrysemys picta*, *Trachemys scripta*, *R. leukops* and *Els. latisternum*) (Burggren, Hahn & Foex, 1977; Maginniss, Tapper & Miller, 1983; Gordos *et al.*, 2004); 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: *R. leukops*, *Elusor macrurus*, *Els. albagula*, *Els. latisternum* and *Em. 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 hypothesized 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 *R. leukops*, *Elu. macrurus*, *Els. albagula*, *Els. latisternum* and *Em. signata*. Eggs of the five species were collected from nests on the banks of the Mary (*Elu. macrurus*) and Fitzroy (*R. leukops*) Rivers, or from gravid females caught from the Brisbane (*Em. signata*), Burnett (*Els latisternum*) and Mary (*Els. albagula*) Rivers. A minimum of four clutches were gathered for each species to ensure genetic variation. The eggs were transported to The University of Queensland where they were incubated until hatching, whereupon the turtles were housed in 1000 L tanks that 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 (12L:12D) 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 the success of egg collection.

Diving behaviour

The diving behaviour of the five turtle species (refer to Table 1 for sample sizes and body masses) was examined in a large glass aquarium $150 \times 60 \times 65$ cm (l × w × d). The aquarium contained a pebble substrate and benthic shelters, with the water being constantly filtered and maintained at 23 °C (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 in 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 that were defined as a dive where the turtle sat still on the bottom of the tank for a period of >1 min. The mean and maximum resting dive durations were calculated for each turtle, along with the frequency of such dives using a custom-written program (M. A. Gordos). Dive durations were analysed using a generalized linear model with a gamma distribution and an inverse link function. After fitting the model, Tukey's *post hoc* test was used to determine between- and within-species comparisons ($P<0.05$). The dive duration frequency data were analysed using a two-way ANOVA with Tukey's *post hoc* test ($P<0.05$).

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 1 for sample sizes and body masses). Experiments were conducted at 23 °C and red lights were used to simulate darkness, which aids in reducing turtle

Table 1 Body masses (mean ± SEM, g) and samples sizes (*n*) of hatchling and juvenile turtles for studies on their diving behaviour, oxygen consumption and blood respiratory properties

	Diving behaviour		Oxygen consumption		Blood properties
Species	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)

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 allowed water and air sampling. Before the beginning of the experiments, the turtles were weighed and wiped down with a 70% ethanol solution to remove oxygen-consuming bacteria. Turtles were then placed in 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 (see Clark, Gordos & Franklin, 2008 for a complete description of the methods). Each turtle underwent a total of four 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 standardized to an average size turtle (12 g). Per cent 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 per cent aquatic respiration was determined using a two-way ANOVA (species and age as factors) with Tukey's *post hoc* comparison ($P<0.05$). Percentage data were transformed before analysis.

Blood respiratory properties

Blood respiratory properties of the five turtle species were determined using the juvenile turtles only as the small size of the hatchlings prevented blood sampling (refer to Table 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 G needle and a 1 mL syringe (Rogers & Booth, 2004). The needle tip and plunger of the syringe were dusted with sodium heparin to prevent coagulation. The blood sample was then transferred to a 0.5 mL Eppendorf tube where sub-samples were collected for analysis of Hb concentration, Hct and P_{50}, which was defined as the PO_2 at which 50% of the Hb was saturated.

The concentration of Hb was determining using a spectrophotometer (Beckman Coulter DU800 Spectrophotometer, Queensland, Australia). Five microlitres of blood was mixed with 1 mL of Drabkins solution and the absorbance was recorded at 540 nm. The Hb concentration was then determined from an average absorbance reading (Lewis, Bain & Bates, 2001). A sub-sample of blood collected in a capillary tube was centrifuged at 500 ***g*** for 3 min, with Hct determined as the per cent of red blood cells per sample volume. A Hemox analyser Model B (TCS Scientific Corp. New Hope, PA, USA) 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 an antifoaming agent. The blood sample was then added to the Hemox machine, where the deoxygenation and oxygenation curves were run at 23 °C, and the P_{50} values were recorded at 5% CO_2. Significant differences in Hb, Hct and P_{50} were determined using a one-way ANOVA with Tukey's *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 1 and 2). The 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 and 2288 ± 341 min, respectively) and the juveniles (839 ± 697 and 1565 ± 554 min, respectively) (Figs 1 and 2). Two hatchling *R. leukops* remained submerged throughout the entire experimental period (72 h), resulting in a maximum dive duration that was 17-fold longer than values recorded for the other turtle species. Within the hatchlings, the mean and maximum dive durations of *Els. albagula* were 47 ± 6 and 173 ± 38 min, respectively, which were significantly greater than that of *Elu. macrurus* (23 ± 2 and 98 ± 20 min, $P<0.05$), *Els. latisternum* (15 ± 0.5 and 26 ± 2 min, $P<0.001$) and *Em. signata* (11 ± 0.5 and 19 ± 1 min, $P<0.001$) (Figs 1 and 2). However, when comparing the

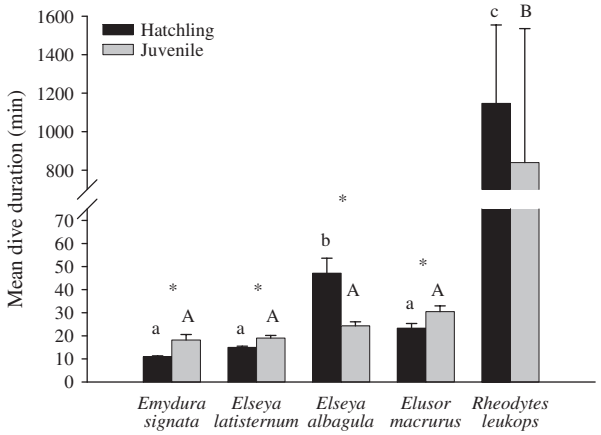

Figure 1 Mean dive duration (min) for five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant difference between juvenile species. Asterisks indicate significant differences between hatchling and juvenile turtles within a species.

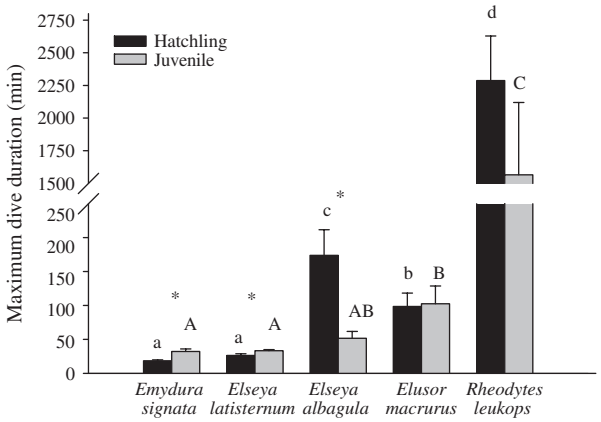

Figure 2 Maximum dive duration (min) for five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant differences between juvenile species. Asterisks indicate significant differences between hatchling and juvenile turtles within a species.

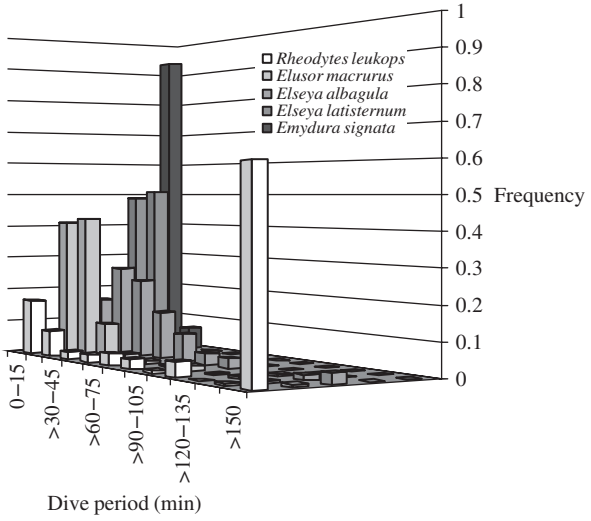

Figure 3 Frequency of dives that occurred within each 15 min dive period from 0 to >150 min for hatchlings of five species of freshwater turtle.

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 *Elu. macrurus* (103 ± 26 min), which was significantly longer than *Els. latisternum* (33 ± 2 min, $P<0.001$) and *Em. signata* (32 ± 4 min, $P<0.001$) (Figs 1 and 2). No statistically significant differences were observed when the dive duration frequency data were 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 3 and 4).

The relationships between hatchling and juvenile dive durations varied considerably between species. Juvenile

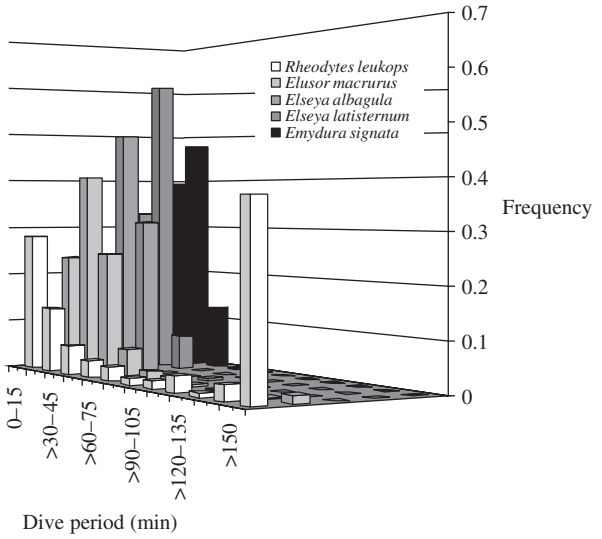

Figure 4 Frequency of dives that occurred within each 15 min dive period from 0 to >150 min for juveniles of five species of freshwater turtle.

mean and maximum dive durations were significantly greater than that of the hatchlings in *Elu. macrurus* ($P<0.01$, mean dive duration only), *Els. latisternum* ($P<0.01$) and *Em. signata* ($P<0.001$). Hatchling dive durations were, however, greater than for juveniles in *Els. albagula* ($P<0.001$) while no differences in age class were recorded for *R. leukops* (Figs 1 and 2). Seventy per cent of dives undertaken by both hatchling and juvenile turtles were short in duration (<30 min) for all species (Figs 3 and 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 the juveniles ($P<0.01$, except when compared with the 15–30 min dive period) (Fig. 3). Juvenile turtles also had significantly more dives occurring within the 15–30 min dive period than for the longer dive periods ($P<0.01$) (Fig. 4).

Oxygen consumption

Per cent aquatic respiration and aquatic oxygen consumption were significantly greater in *R. leukops* than in all other species ($P<0.001$) (Table 2 and Fig. 5). Aquatic respiration in *R. leukops* supported 63 ± 3 and 73 ± 10% of the total oxygen requirement in hatchling and juveniles, respectively. Per cent aquatic respiration did not differ between the remaining species in either the hatchling or the juvenile age class (Fig. 5). The aquatic oxygen consumption of *Elu. macrurus* ($0.109 \pm 0.008\ mL\ O_2\ h^{-1}$) was greater than *Els. latisternum* ($0.088 \pm 0.011\ mL\ O_2\ h^{-1}$, $P<0.05$) and *Els. albagula* ($0.068 \pm 0.007\ mL\ O_2\ h^{-1}$, $P<0.05$) while that of *Em. signata* ($0.122 \pm 0.017\ mL\ O_2\ h^{-1}$) was also higher than *Els. latisternum* ($P<0.05$) (Table 2). Overall, aquatic oxygen consumption and per cent aquatic respiration did not significantly differ between age classes; however, the aquatic oxygen consumption of hatchling *R. leukops* (0.447 ±

Table 2 Aerial and aquatic oxygen consumption (mL O_2 h^{-1}) in hatchlings and juveniles of five species of freshwater turtle

	Aquatic oxygen consumption (mL O_2 h^{-1})		Aerial oxygen consumption (mL O_2 h^{-1})	
Species	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

VO_2 aerial and VO_2 aquatic were scaled to 0.75 then standardized to a 12 g turtle. Values represent means ± SEM.

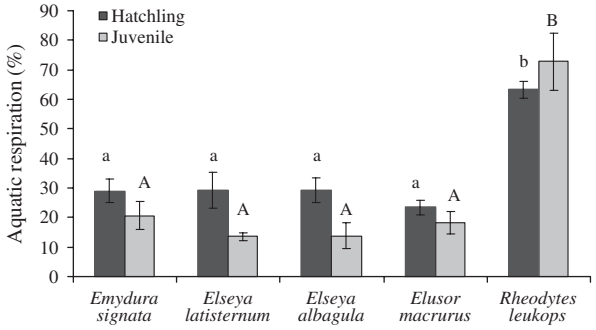

Figure 5 Aquatic respiration for five species of hatchling and juvenile turtles. Values represent means ± SEM. Lowercase letter differences indicate significant differences between hatchling species. Uppercase letter differences indicate significant differences between juvenile species.

0.014 mL O_2 h^{-1}) was greater than that of the juveniles (0.259 ± 0.037 mL O_2 h^{-1}) ($P<0.001$) (Table 2 and Fig. 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 *Els. latisternum* (0.512 ± 0.033 mL O_2 h^{-1}, $P<0.01$), *Els. albagula* (0.789 ± 0.054 mL O_2 h^{-1}, $P<0.001$) and *Elu. macrurus* (0.926 ± 0.188 mL O_2 h^{-1}, $P<0.001$) (Table 2). Aerial oxygen consumption in hatchling turtles was generally less than that of the juveniles; however, this was only significant in *Els. albagula* ($P<0.01$) and *Elu. macrurus* ($P<0.05$) (Table 2).

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 ($P<0.001$) (Fig. 6a and b). The Hb levels of *Elu. macrurus* (1.6 ± 0.1 mmol L^{-1}) were also significantly higher than *Em. signata* (1.0 ± 0.1 mmol L^{-1}, $P<0.05$) and *Els. albagula* (1.0 ± 0.1 mmol L^{-1}, $P<0.05$) (Fig. 6b). The P_{50} values of the turtles varied significantly among species ($P<0.001$) (Fig. 6c). *Rheodytes leukops* recorded the lowest P_{50} of 17.24 ± 0.45 mmHg, which was significantly different from all the other species ($P<0.05$), except *Els. albagula*, which recorded a value of 21.81 ± 1.56 mmHg. The P_{50} of *Els. albagula* was lower than

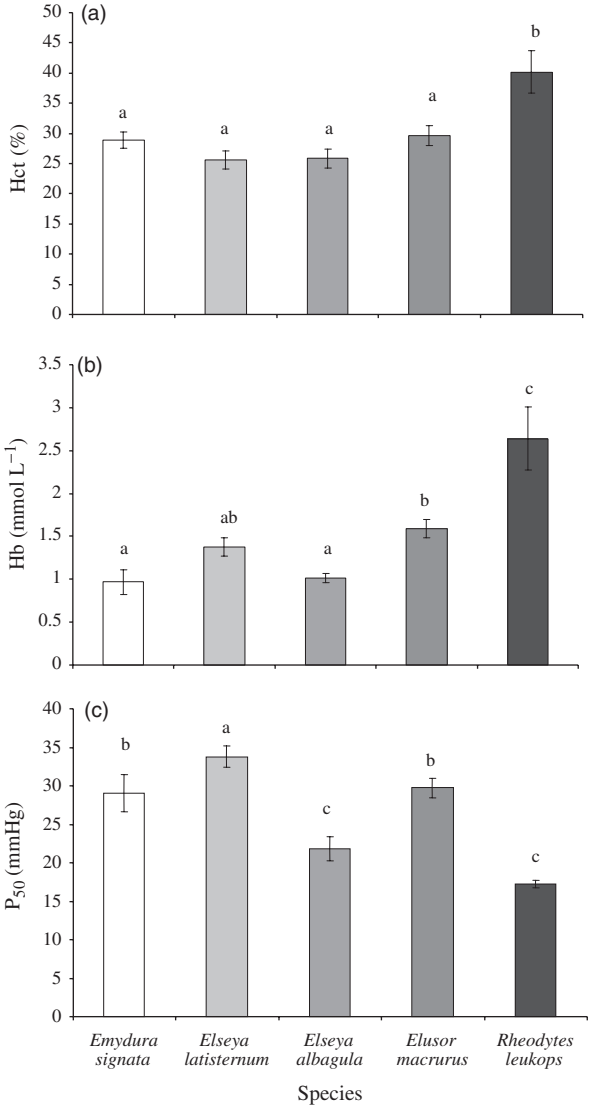

Figure 6 Blood respiratory properties of juvenile freshwater turtles. (a) Per cent haematocrit, (b) haemoglobin and (c) P_{50}. Values represent means ± SEM. Letter differences indicate significant differences.

that of *Em. signata* (29.03 ± 2.44 mmHg, $P<0.01$) and *Elu. macrurus* (29.71 ± 1.30 mmHg, $P<0.01$), in which the P_{50} values did not differ ($P>0.05$), and *Els. Latisternum*, which had the highest P_{50} value of 33.79 ± 1.42 mmHg ($P<0.001$).

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 *Els. albagula* and *Elu. macrurus* compared with *Em. signata* and *Els. latisternum*, there was no difference observed in per cent 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 dividing 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*., 1992; King & Heatwole, 1994*a*,*b*; Bagatto *et al*., 1997; Bagatto & Henry, 1999; Prassack, Bagatto & Henry, 2001; Maina, 2002; Gordos *et al*., 2004). Per cent aquatic respiration in *R. leukops* reached a mean value of 67%, which was approximately four 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 on 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 turtles' ability to voluntarily change their reliance on aerial and aquatic oxygen consumption (Mathie & Franklin, 2006). Owing 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 possessed cloacal bursae which are dorso-lateral diverticula of the cloaca. The degree of morphological development in this respiratory organ differs dramatically between species (Legler, 1987; Legler & Georges, 1987). The cloacal bursae of adult *Em. signata*, *Els. latisternum* and *Elu. macrurus* are the least developed, with the bursal lining only partly covered by papillae (Legler & Georges, 1987; Cann & Legler, 1994; King & Heatwole, 1994*a*). The cloacal bursae of adult *Els. albagula* are completely covered in branched but flattened papillae, while that of adult *R. leukops* are the most specialized, with the papillae being highly vascularized and multi-branching (Legler & Cann, 1980; Legler, 1987; 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*., 2004). A study by Gordos *et al*. (2004), 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 observed between this study and that of Gordos *et al*. (2004) may be due to differences in turtle body size and species developmental rates. Hb 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 of age in green sea turtle *Chelonia mydas, mydas* hatchlings (Isaacks, Harkness & Whitham, 1978) but, between 4 and 7 months of age 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 *Elu. macrurus* and *Els. albagula* (4 h) are eight times longer than that of *Em. signata* and *Els. latisternum* (30 min), suggesting they too may use an increased reliance on aquatic respiration to further exploit the aquatic environment.

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 & Jones, 1982; Schmidt-Nielsen, 1984; Kooyman, 1989; Schreer & Kovacs, 1997; Kooyman & Ponganis, 1998). Dive duration, however, is predicted to correlate negatively with body mass in bimodally respiring turtles as small turtles have a relatively higher reliance on aquatic respiration and higher predation

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

	Mean aquatic respiration (%)			Mean dive duration (min)			
Species	Hatchlings	Juveniles	Adults	Hatchlings	Juveniles	Adults	Citation
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	~25.1	15.0 ± 0.5	19.0 ± 1.1	32.9 ± 11.5	This study; King & Heatwole (1994*b*), 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)

pressures (Stone *et al*., 1992; Bagatto *et al*., 1997; Heithaus & Frid, 2003; Mathie & 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 3 reports the mean aquatic respiration and mean dive duration for hatchlings, juveniles and adults recorded in captivity between 23 and 25 °C. Aquatic respiration was higher in hatchlings than adults for *Em. signata*, *Els. albagula* and *R. leukops*. Consequently, the dive durations of these three species were longer in the hatchlings. This trend was not supported for all species, however, with the dive durations of hatchlings *Els. latisternum* and *Elu. 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 (e.g., 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 are required to understand the among-species differences that occur (Georges *et al*., 1998).

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