The Journal of Experimental Biology 204, 2185–2195 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3293

EFFECTS OF TEMPERATURE AND AQUATIC P_{O_2} ON THE PHYSIOLOGY AND BEHAVIOUR OF *APALONE FEROX* AND *CHRYSEMYS PICTA*

S. L. PRASSACK, B. BAGATTO* AND R. P. HENRY

Department of Biological Sciences, Auburn University, Auburn, AL 36849, USA

*Author for correspondence and present address: Department of Biological Sciences, University of North Texas, Box 305220, Chestnut and Avenue C, Denton, TX 76203-5220, USA (e-mail: bagatto@unt.edu)

Accepted 30 March 2001

Summary

Softshell turtles overwinter in the same bodies of water as some emydids, but their reduced shell and increased non-pulmonary gas exchange may contribute to a different mechanism of overwintering. The dynamics of bimodal respiration, diving behaviour and blood acid–base status in *Apalone ferox* and *Chrysemys picta* were investigated under two different temperatures combined with three different aquatic P_{O_2} levels. Both species obtained oxygen through pulmonary and non-pulmonary routes. *Apalone ferox* obtained more oxygen through non-pulmonary routes and increased its non-pulmonary $\dot{V}_{O_2}$ in response to both higher aquatic P_{O_2} and lower temperatures. Both species increased pulmonary $\dot{V}_{O_2}$ in response to higher temperatures. As a consequence of the greater reliance of *A. ferox* on pulmonary $\dot{V}_{O_2}$, warmer temperatures caused plasma P_{CO_2} and $[HCO_3^-]$ values to increase significantly compared with *C. picta*. *Apalone ferox*, which is efficient at bimodal respiration, displayed a high degree of plasticity with respect to both its respiratory and acid–base profiles, behaving more like an aquatic air-breathing fish in bimodal respiration at low temperature and more like a terrestrial air-breather at high temperature. *Chrysemys picta*, which is poor at bimodal respiration, was highly dependent on aerial gas exchange at both temperatures. Aquatic P_{O_2} did not change any of the behavioural variables measured. At warm temperatures, *A. ferox* met O_2 demands by increasing the rate of lung ventilation, which resulted in a significantly greater number of breathing bouts per hour and breaths per emersion period. However, the number of breaths per bout was not affected by temperature. As temperatures decreased, *A. ferox* utilized its non-pulmonary respiration ability and significantly increased its dive duration. *Apalone ferox* became less active at colder temperatures by significantly increasing the duration of inactive periods (from 4 to 18 min) and by significantly decreasing the frequency of activity bursts. *Chrysemys picta* also met the higher gas-exchange demands associated with increased temperature by increasing the rate of lung ventilation; however, this increase was not as large as that measured in *A. ferox*. *Chrysemys picta* displayed multiple rhythmic breaths per bout. These results indicate that, unlike aquatic P_{O_2}, temperature is an important factor in the regulation of diving and ventilatory behaviour in turtles. The species responded to temperature in dissimilar ways because of differences in their bimodal respiration ability.

Key words: bimodal respiration, aquatic gas exchange, acid–base, softshell turtle, *Apalone ferox*, painted turtle, *Chrysemys picta*.

Introduction

Lower vertebrates first evolved mechanisms of gas exchange while existing in an aquatic environment. These mechanisms underwent considerable modification during the invasion of the terrestrial habitat in response to the enormously different physical properties of air and water as respiratory media (Dejours, 1976; Dejours, 1988; Dejours, 1994). In studies of bimodal respiration in fish and amphibians, the relative importance of aerial O_2 uptake can change as a function of various ambient factors such as temperature (Lenfant et al., 1970; Rahn et al., 1971; Burggren and Wood, 1981; Smatresk and Cameron, 1982b) and aquatic oxygen tension (Johansen et al., 1967; Randall et al., 1981; Smatresk and Cameron, 1982a). Unlike fishes and amphibians, turtles have re-invaded the aquatic habitat and, therefore, some species have re-adapted to the aquatic environment by independently evolving many of the mechanisms commonly found in primitive, bimodal-breathing animals. However, this characteristic is not distributed equally among turtle species.

It has been known for more than 100 years that aquatic, freely diving turtles utilize bimodal respiration, exchanging O_2 and CO_2 with both air and water (Gage and Gage, 1886). Except for periods of basking, most freshwater turtles spend the majority of their active time under water (Belkin, 1968a). In summer, these periods of submergence can last for minutes to hours, with turtles intermittently returning to the surface to breathe (Burggren and Shelton, 1979). Freshwater turtles are

known for their exceptional breath-holding ability (Belkin, 1963; Belkin 1968a; Robin et al., 1964). Voluntary dives are mainly aerobic in these animals during the summer (Burggren and Shelton, 1979; Gatten, 1984); however, in winter, turtles may remain continuously submerged for weeks or even months, often buried in the mud or some similar hypoxic environment (Carr, 1952; Ernst and Barbour, 1972). Little is known about the details of overwintering behaviour and/or physiology of any turtle species under natural conditions.

Turtles must be able to tolerate apnoea while continuing to meet metabolic demands during periods of submergence. They have been shown to do this in prolonged dives by depressing their metabolic rate (Robin et al., 1964; Jackson, 1968; Belkin, 1968a; Gatten, 1981; Caligiuri et al., 1981), by having organ systems that are tolerant to hypoxia (e.g. the central nervous system) (Belkin, 1968b; Lutz et al., 1980; Lutz et al., 1984) and by buffering their accumulated lactic acid (Jackson and Ultsch, 1982). Those turtles that utilize bimodal respiration can support their metabolism, at least in part, using O_2 obtained from the water *via* non-pulmonary routes (Gage and Gage, 1886; Root, 1949; Dunson, 1960; Girgis, 1961; Belkin, 1968b; Jackson et al., 1976; Gatten, 1980; Stone et al., 1992a) and they have been shown to do this even when access to atmospheric O_2 is available (Stone et al., 1992a; Bagatto and Henry, 1999).

During the winter in temperate climates, turtles spend long periods cut off from atmospheric O_2 sources. Turtles in the family Emydidae cope with these long periods of submergence by employing the first three mechanisms mentioned above. Softshell turtles, however, have an additional physiological mechanism in that they are able to transfer significant amounts of O_2 and CO_2 across their skin. Therefore, it is possible that they are able to support their metabolism by cutaneous O_2 uptake and/or offset an increased lactic acid load by increased CO_2 excretion into the water. This may be very important since the shells are extremely reduced in the family Trionychidae such that shell buffering may be significantly reduced. The present study focuses on the dynamics of aerial and aquatic oxygen uptake in *Apalone ferox* (efficient at bimodal respiration) and *Chrysemys picta* (poor at bimodal respiration), as influenced by temperature and aquatic P_{O_2}, to investigate the mechanisms employed by softshell turtles during cold-water submergence.

Materials and methods

Experimental animals and design

Nine Florida softshell turtles *Apalone ferox* (Schneider) (four females, five males; mean mass 1123.3 g; range 289.6–1638.0 g) and 11 painted turtles *Chrysemys picta* (Schneider) (all females; mean mass 295.4 g; range 153.0–374.6 g) were used in this study. Softshell turtles were collected from various localities in Florida, USA, whereas painted turtles were collected in Lee County, Alabama, USA, from an urban pond. Turtles were housed in a constant-temperature room at 25 °C and were maintained in aquaria with aerated tap water. Turtles were fed Reptomin turtle food *ad libitum* and were fasted for 1 week before experiments. The experiments were conducted in June, July and August 1993 with a 14 h:10 h L:D photoperiod. Algae covering the carapace and plastron of painted turtles were scrubbed off prior to the use of each animal. The integument of *Apalone ferox* was not covered with algae.

A 2×2×3 factorial design (species, temperature and aquatic P_{O_2}) was used to test the effects of temperature (15 and 25 °C) and aquatic P_{O_2} (<50, 100–150 and >200 mmHg; 1 mmHg=133.3 Pa) and their interactions on the breathing behaviour of freely diving, unrestrained turtles. These temperatures were chosen because they were within the thermal activity range of both genera (Plummer and Shirer, 1975; Ernst, 1972). All 25 °C experiments were conducted first; turtles were then gradually cold acclimated (<1 °C per day) for 14 days and maintained at 15 °C for the duration of the study.

During each experiment, turtles had access to normoxic air. In brief, each turtle was placed in a chamber and subjected to the experimental conditions for 4 h. This served two purposes: first, to acclimate the turtle to the experimental chamber and, second, to subject it to the environmental treatment for sufficient time to challenge the subject. During this period turtles had access to fresh air supplied by an air pump while aquatic P_{O_2} was held at experimental levels. Long-term (24 h) studies showed no further differences in behaviour and gas exchange after 4 h. After this acclimation phase, the aerial portion of the chamber was converted into a closed system by shutting the inflow and outflow ports. Each measurement period lasted 1 h.

Respiratory gas exchange

The $\dot{V}_{O_2}$ of unrestrained, freely diving and surfacing turtles was measured using a modification of methods previously described by Stone et al. (Stone et al., 1992a) and Bagatto and Henry (Bagatto and Henry, 1999). The methods allowed for measurement of O_2 uptake from a volume of water *via* non-pulmonary routes as well as from an air space above the water *via* the lungs. Thus, the relative importance of these two modes of gas exchange was ascertained. Turtles had access to normoxic air in all treatments. Normoxic water was obtained by running dechlorinated tap water through a gas equilibration column through which air was bubbled. Hyperoxic or hypoxic water were obtained by bubbling either pure O_2 or N_2, respectively, through the column.

Each turtle was placed individually in a chamber at least 4 h prior to a trial and immediately subjected to the experimental aquatic P_{O_2}. Fresh air was supplied to the aerial chamber throughout this period by means of an air pump. This flow-through system was converted into a closed system at the start of each experiment by closing all inflow and outflow ports. During experiments, the water was continuously stirred with a magnetic stir bar to prevent the establishment of O_2 or CO_2 gradients. The small surface area of the air/water interface tended to minimize the movement of gases between phases. Preliminary tests showed that O_2 diffusion across phases was

negligible when the water was made either hypoxic or hyperoxic even when the system was closed off for 12 h periods (Bagatto et al., 1997; Bagatto and Henry, 1999).

Oxygen uptake from both the air and water chambers was measured by calculating the difference between samples taken at the beginning and the end of 1 h trials and subtracting the mean background rate of oxygen consumption calculated during 'blank' runs. Oxygen tensions were measured with a Radiometer PHM73 blood/gas monitor equipped with an E5046 P_{O_2} electrode (accurate to 0.1 mmHg; range 0–250 mmHg), thermostatted to the experimental temperature. Oxygen tensions were converted into molar concentrations using solubility coefficients from Dejours (Dejours, 1975) at STDP. Aquatic, aerial and total $\dot{V}_{O_2}$ (based on the fresh mass of each turtle) and per cent aquatic $\dot{V}_{O_2}$ were calculated for each experiment.

Blood collection and analysis

A mixed pulmonary and systemic blood sample was obtained from all turtles by cardiac puncture. Although blood gas measurements obtained in this way are more variable than those from arterial catheterization (Kerr and Frankel, 1972), cardiac puncture was chosen because turtles demonstrate decreased viability (Ultsch and Jackson, 1982) and modified diving behaviour (Lutz et al., 1989) in response to catheterization. Because animals were used repeatedly and there was a behavioural component to this study, this sampling method was deemed necessary. Even though three samples were taken from a single turtle with at least 3 days between samples, no blood was observed in the pericardial cavity and there were no observable scars in the cardiac tissue upon dissection. A small hole was drilled into the plastron of each painted turtle and then covered with a rubber septum to allow repeated cardiac-puncture blood sampling. The plastron of softshell turtles was soft enough to allow repeated passage of a hypodermic needle so that drilling a hole was unnecessary. At each sampling time, a 0.5 ml sample of blood was withdrawn anaerobically using a 21 gauge needle and a heparinized 1 ml syringe. Whole-blood pH was measured immediately with a Radiometer PHM 72 acid–base analyser (G297-G2 glass and R407 reference electrodes). pH values for 15 °C animals were corrected for turtle body temperature using the techniques of Kelman and Numm (Kelman and Numm, 1966). Although these temperature-corrected data cannot be regarded as quantitatively exact, they do provide valid estimates of pH changes for comparative purposes (Kelman and Numm, 1966). Whole-blood P_{O_2} was measured immediately by a Radiometer PHM73 blood/gas monitor equipped with an E5046 P_{O_2} electrode. The remaining blood was centrifuged for 3 min at 10 000 revs min^{-1} (Fisher model 235B) to obtain a plasma sample. Total CO_2 was then measured on a 100 µl plasma sample with a Corning 965 CO_2 analyser. [HCO_3^-] and P_{CO_2} were calculated from pH and total CO_2 values using the Henderson–Hasselbalch equation with pK′ and CO_2 solubilities from Reeves (Reeves, 1976). For lactate determinations, 200 µl of plasma from the same sample was deproteinated by addition of an equal amount of chilled 8 % perchloric acid followed by centrifugation at 10 000 revs min^{-1} for 3 min; the resultant supernatant was stored frozen at −80 °C for later analysis. Plasma lactate levels were determined enzymatically using Sigma diagnostics procedure 826UV. To avoid bias associated with post-experimental stress, only blood samples taken within 30 s of an animal's removal from a chamber were used for analysis.

Ventilation and diving behaviour

The behaviour of each turtle was monitored with a Panasonic AG-160 video camera recording at 30 frames s^{-1}. Lung ventilations were verified by a Physiograph equipped with an RP-1500 pressure transducer, which measured changes in air pressure in the chamber. Behaviours associated with the Physiograph tracings were determined from videotapes viewed with a Sony VHS video recorder (SLV 373UC).

To characterize the discontinuous breathing patterns, the following criteria were used. Two breaths were considered part of the same bout if the apnoeic period between breaths was less than twice the average duration of individual breaths (Stone et al., 1992b). Variables measured included the duration of each immersion and emersion period, the duration of each period of emersion apnoea, the number of breaths per breathing bout and the number of breathing bouts per emersion period (Bagatto and Henry, 1999). Incomplete immersion or emersion bouts in progress at the beginning or end of an experiment were excluded from the data. A turtle was considered active if it moved one or more limbs. No attempt was made to quantify the intensity of these movements, so diving turtles were considered either active or inactive.

Statistical analyses

Means ± S.E.M. for each variable were compared by three-way analysis of variance (ANOVA) (2×2×3; species, temperature and aquatic O_2 tension, respectively) using repeated measures. In cases where an initial analysis of the main effect was significant but interaction effects were not significant, the sum of squares and degrees of freedom were added to the error term and thereafter treated as replications for the analysis of main effects (species, temperature or P_{O_2}) (Cochran and Cox, 1957). In cases where significant interactions were found, differences were determined by combining non-significant main effects and performing an ANOVA with Student–Newman–Keuls multiple comparisons when necessary. Percentage data were arcsine-square-root-transformed before analysis to meet the assumptions of normality (Snedecor and Cochran, 1980). Hypoxic plasma lactate values were non-normal even after attempted transformations. Duration measurements were square-root-transformed, whereas rate, frequency and count measurements were arsine-square-root-transformed. In all cases, transformations normalized the data. The *a priori* level of significance was P=0.05. All statistical analyses were performed using the statistical analysis system (SAS Institute, 1990).

Results

Respiratory gas exchange

Both species exhibited the ability to exchange O_2 with both air and water. Total mass-specific $\dot{V}_{O_2}$ did not differ between the species at any experimental P_{O_2} (Fig. 1) (F=2.64, P=0.10), and total $\dot{V}_{O_2}$ decreased significantly with temperature for both species, regardless of aquatic P_{O_2} (Fig. 1) (F=22.63, P>0.0001). The decrease in total $\dot{V}_{O_2}$ in *A. ferox* was 29 % (Q_{10}=1.727), but that in *C. picta* was only 14.8 % (Q_{10}=1.345). The significant decrease in total $\dot{V}_{O_2}$ with decreased temperature was due to a significant decrease in pulmonary $\dot{V}_{O_2}$ in both species (Fig. 1) (F=30.51, P<0.0001). However, no difference in pulmonary $\dot{V}_{O_2}$ was found between the species at either temperature (Fig. 1) (F=1.75, P=0.19) or at any of the aquatic oxygen tensions (F=2.42, P=0.09).

Mass-specific, non-pulmonary $\dot{V}_{O_2}$ was significantly greater in *A. ferox* (31 % of total $\dot{V}_{O_2}$) than in *C. picta* (8 %) regardless of temperature or aquatic P_{O_2} (Fig. 1) (F=86.52, P<0.0001). In addition, a significant species × oxygen interaction indicated that *A. ferox* increased non-pulmonary $\dot{V}_{O_2}$ with increasing aquatic P_{O_2} while *C. picta* did not (F=4.51, P=0.01). Unlike total $\dot{V}_{O_2}$, which decreased significantly with decreasing temperature, pulmonary $\dot{V}_{O_2}$ was not significantly affected by temperature (Fig. 1) (F=0.60, P=0.44), indicating that the proportion of total $\dot{V}_{O_2}$ due to non-pulmonary routes increased significantly with decreasing temperature in *A. ferox* only (25 °C, 17.4±1.6 %; 15 °C, 44.1±5.18 %; F=16.25, P<0.0001).

Blood acid–base status

There were no significant species or treatment differences in whole-blood pH (Table 1). Blood oxygen tensions were significantly greater in *C. picta* than in *A. ferox* (F=15.51, P=0.0004), but blood P_{O_2} increased significantly in both species with increasing aquatic P_{O_2} (F=6.65, P=0.002). A significant species × temperature interaction indicated that

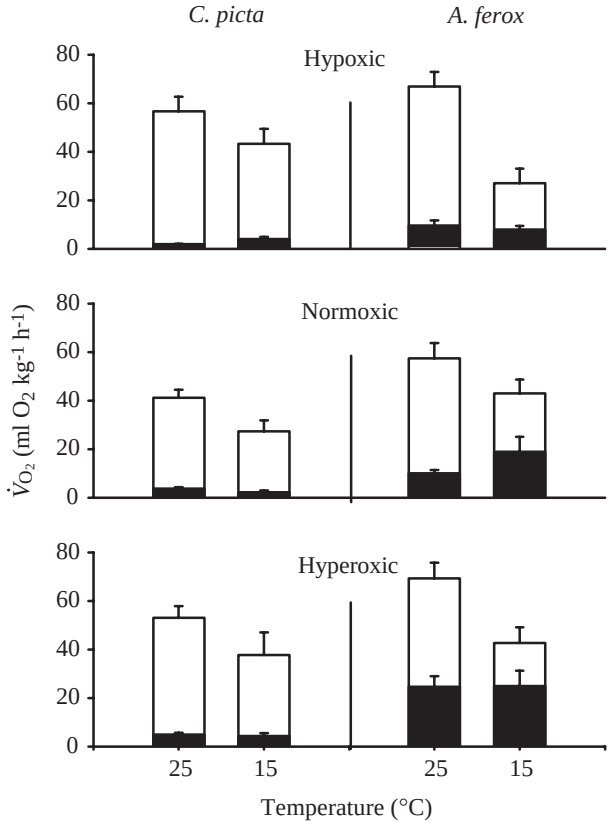

Fig. 1. $\dot{V}_{O_2}$ (ml kg^{-1} h^{-1}) at two temperatures for *Chrysemys picta* and *Apalone ferox*. Values represent means + S.E.M. For *C. picta*, N=11 at 25 °C and all P_{O_2} values, N=4 at 15 °C at normoxia, and N=5 at 15 °C for hypoxia and hyperoxia. For *A. ferox*, N=9 at 25 °C for normoxia and hypoxia and N=6 for hyperoxia; at 15 °C, N=6 for normoxia and hypoxia, and N=5 for hyperoxia. Vertical columns represent mass-specific total $\dot{V}_{O_2}$. The shaded area represents the non-pulmonary contribution and the unshaded area represents the pulmonary contribution. See text for statistical analysis.

Table 1. *Mean blood acid–base status in* Chrysemys picta *and* Apalone ferox *exposed to hypoxic, normoxic and hyperoxic water at 15 °C and 25 °C*

Variable	Temperature (°C)	*C. picta*			*A. ferox*		
		Hypoxic	Normoxic	Hyperoxic	Hypoxic	Normoxic	Hyperoxic
Plasma pH	15	7.71±0.07	7.72±0.12	7.66±0.06	7.60±0.06	7.60±0.07	7.72±0.05
	25	7.66±0.04	7.73±0.03	7.73±0.04	7.58±0.05	7.66±0.02	7.59±0.04
Blood P_{O_2} (mmHg)	15	56.8±19.5	81.3±15.1	84.3±29.8	23.7±5.3	41.3±15.5	41.4±6.3
	25	49.1±4.8	56.5±5.4	74.9±9.7	34.9±5.3	56.4±5.7	55.3±6.0
Plasma P_{CO_2} (mmHg)	15	15.1±3.0	15.0±3.5	17.3±4.7	14.4±1.2	21.0±2.4	14.6±1.7
	25	21.5±2.3	17.6±1.9	17.7±2.2	32.4±3.3	23.0±1.9	28.7±4.1
Plasma [HCO_3^-] (mequiv l^{-1})	15	25.3±1.4	26.0±0.7	26.3±4.1	20.1±2.6	28.7±1.8	26.9±4.0
	25	28.1±2.4	26.8±1.7	26.5±1.8	35.4±7.5	30.1±2.0	31.2±2.3
Plasma [lactate] (mequiv l^{-1})	15	1.6±0.5	0.7±0.1	6.9±3.3	4.2±1.7	0.8±0.3	5.1±1.4
	25	4.6±1.4	1.9±0.5	2.4±0.6	4.2±1.7	1.7±0.4	2.9±1.3

Sample sizes as in Fig. 1. Values are mean ± S.E.M.
1 mmHg=133.3 Pa.

blood P_{O_2} in *C. picta* increased with decreasing temperature, whereas it decreased in *A. ferox* (F=4.40, P=0.04).

Plasma P_{CO_2} was significantly lower in *C. picta* than in *A. ferox* (F=11.74, P=0.001). P_{CO_2} decreased with decreasing temperature in *A. ferox* (F=19.97, P<0.0001), and a significant species × temperature interaction was present (F=5.35, P=0.02). A significant temperature × oxygen effect indicated that plasma P_{CO_2} decreased more in hypoxic and hyperoxic water than in normoxic water (F=3.49, P=0.04). In *A. ferox*, plasma bicarbonate levels decreased significantly with decreasing temperature (F=9.38, P=0.003). A significant temperature × oxygen effect indicated that plasma bicarbonate levels decreased significantly in hypoxic water at 15 °C (F=3.33, P=0.04).

Plasma lactate levels did not differ between the species (F=0.01, P=0.862) or with temperature (F=0.11, P=0.27), but a significant aquatic P_{O_2} effect indicated that lactate levels were greater at hyperoxic and hypoxic than at normoxic aquatic P_{O_2} (F=5.54, P=0.01).

Ventilation and diving behaviour

Most of the individual dives for both species were of short duration. Of the 1159 dives recorded (combining species, temperatures and aquatic oxygen tensions), 81 % lasted less than 5 min, 12 % lasted between 5 and 10 min, and 7 % lasted more than 10 min. *A. ferox* spent significantly more total time diving than *C. picta* (F=14.55, P=0.0003, Table 2).

Ventilation and diving behaviour in both species were independent of aquatic P_{O_2}. In response to decreasing temperatures, both species increased their total diving time (F=4.46, P<0.038; Table 2). Specifically, the proportion of time *A. ferox* spent diving increased from 73 to 83 %, whereas in *C. picta* it increased from 50 to 66 %. As temperature decreased, *A. ferox* increased the proportion of dives lasting longer than 10 min (from 8 to 36 % of all dives), whereas *C. picta* showed a significantly greater frequency of short dives (92 % *versus* 85 %) with increasing temperature.

Activity for both species was characterized by swimming with occasional searching behaviour. Regardless of temperature, *C. picta* spent more time active during dives (23 %) than *A. ferox* (7 %) (F=15.24, P=0.0002), but both species decreased the frequency of activity periods at the lower temperature (F=6.27, P=0.01). In addition, regardless of temperature, *C. picta* had significantly more frequent activity periods than *A. ferox* (*C. picta* 10.45±1.5 bursts h^{-1}, *A. ferox* 4.45±0.65 bursts h^{-1}; F=10.53, P=0.002). *Apalone ferox* was inactive for longer periods than *C. picta* (F=38.90, P<0.0001), but both species increased their total inactive diving time when temperature was decreased (F=3.72, P=0.05).

Chrysemys picta spent significantly more time at the surface than *A. ferox* (43 % *versus* 23 %, respectively; F=15.77, P=0.0002; Table 2) and surfaced more frequently (*C. picta* 14.8±1.6 surfaces h^{-1}; *A. ferox* 9.5±1.03 surfaces h^{-1}; F=6.66, P=0.011). However, both species significantly decreased total surface time (F=4.97, P=0.03) and frequency of emersions at the lower temperature (F=16.38, P<0.0001).

Regardless of temperature, *C. picta* had a significantly faster ventilatory rate than *A. ferox* (F=229.74, P<0.0001). Ventilatory rate decreased significantly with temperature in both species (F=38.49, P<0.0001), but a significant species × temperature interaction indicated that a greater decrease occurred in *A. ferox* (61 % decrease) than in *C. picta* (47 % decrease; F=8.38, P<0.005). *Apalone ferox* had significantly fewer breaths per emersion period than *C. picta* (F=78.53, P<0.0001). Both species significantly decreased the number of breaths per emersion period in response to decreased temperature (F=4.61, P=0.04; Table 2).

Table 2. *Mean durations and frequencies of ventilation behaviours in* Chrysemys picta *and* Apalone ferox *exposed to water at 15 °C and 25 °C*

	C. picta		*A. ferox*	
	15 °C	25 °C	15 °C	25 °C
Dive duration (min)	5.7±0.9	3.9±0.8	18.6±3.9	5.4±1.0
Emersion duration (min h^{-1})	20.3±5.1	29.2±2.6	10.5±2.7	16.6±2.5
Emersion apnoea (min)	1.33±0.48	0.36±0.04	0.71±0.14	0.54±0.08
Number of bouts per emersion	2.9±3.1	7.8±3.3	2.8±0.5	4.2±1.3
Number of breaths per bout	11.0±1.7	9.3±3.7	1.6±0.3	1.3±0.1
Number of breaths per emersion	28.8±5.5	55.3±11.7	3.9±0.8	7.8±2.3

Ventilation and diving behaviour was independent of P_{O_2}, so data shown are for all P_{O_2} categories combined.
N=11 for *C. picta* and N=9 for *A. ferox*.
Values are mean ± S.E.M.

The number of breathing bouts per hour was significantly greater in *C. picta* (F=29.28, P<0.0001), and this variable decreased significantly with temperature in both species (F=51.59, P<0.0001). However, there was no significant species, temperature or aquatic P_{O_2} effect on the number of bouts per emersion period (F=1.09, P=0.38; Table 2). Breathing bouts for *A. ferox* contained significantly fewer breaths than those for *C. picta* (F=66.06, P<0.0001); 74.4 % of *A. ferox* bouts contained one breath, whereas 98 % of *C. picta* bouts consisted of four or more breaths. The number of breaths per bout was not affected by temperature in either species (F=0.95, P=0.42).

Both species had periods of emersion apnoea that occurred between breathing bouts. Emersion apnoea was more frequent in *C. picta* than in *A. ferox* (F=16.10, P<0.0001; Table 2) because of more frequent breathing bouts. With decreasing temperature, both species significantly decreased the frequency (F=29.37, P<0.0001), but increased the duration (F=10.06, P=0.002), of apnoeic periods.

Discussion

Normoxic measurements at 25 °C

Oxygen uptake and measured blood values of *C. picta* resemble values reported in the literature for this species and

for other emydid turtles that dwell in similar habitats (Jackson et al., 1974; Glass et al., 1983; Herbert and Jackson, 1985). The measured $\dot{V}_{O_2}$ falls at the high end of the range of values reported for resting animals (26–44 ml kg^{-1} h^{-1}) at temperatures between 20 and 28 °C (Rapatz and Musacchia, 1957; Seidel, 1977; Stockard and Gatten, 1983). A $\dot{V}_{O_2}$ of 74.6 ml kg^{-1} h^{-1} was reported for active *C. picta* (Lowell, 1990), whereas values greater than 200 ml kg^{-1} h^{-1} were measured in vigorously exercised individuals (Stockard and Gatten, 1983; Lowell, 1990). The somewhat high values reported here may be due in part to some motor movement by the turtles because activity levels were moderate, with less than 25 % of diving time spent swimming.

This is the second study to report oxygen uptake and blood gas values for *A. ferox*. Control $\dot{V}_{O_2}$ values and whole-blood pH values at 25 °C in the present study closely match those of Bagatto and Henry (Bagatto and Henry, 1999). In addition, four studies have reported aquatic $\dot{V}_{O_2}$ in other species of softshell turtle (Gage and Gage, 1886; Dunson, 1960; Girgis, 1961; Stone et al., 1992a), and the results of the present study are comparable. Another study that investigated the partitioning of $\dot{V}_{O_2}$ found that *Apalone spiniferus*, at 22–24 °C, acquires 37.5 % of its total $\dot{V}_{O_2}$ through the aquatic medium (Stone et al., 1992a). This is similar to the 31 % obtained by *A. ferox*.

In reptiles, routine activity of moderate intensity appears to be supported by aerobic metabolism, whereas short bursts of intense exercise depend mostly on energy provided by anaerobic pathways (Bennett, 1980). Levels of lactate, the major end-product of anaerobiosis in turtles (Hochachka et al., 1975), were relatively low and typical of reptiles engaged in moderate aerobic activity (Bennett, 1980), and all lactate levels were much lower than those reported in the literature for turtles following intense activity or forced dives (Robin et al., 1964; Ultsch and Jackson, 1982; Stockard and Gatten, 1983; Bagatto and Henry, 1999). This indicates that anaerobic respiration did not play a significant role in the overall metabolism at any treatment level. Similarly, voluntary dives by pond sliders (*Trachemys scripta*), snapping turtles (*Chelydra serpentina*) and loggerhead musk turtles (*Sternotherus minor*) appear to be mostly aerobic (Gatten, 1980; Gatten, 1984). In another study, *Chrysemys picta* accumulated high levels of lactate during voluntary dives at hibernation temperatures (3.5 °C; Gatten, 1981), but not at warm temperatures (25 °C). Regardless of temperature, they accumulated high lactate levels either during forced dives or when threatened but not forcibly immersed (Gatten, 1981). Similarly, loggerhead musk turtles (*Kinosternon minor*) generate lactate rapidly during vigorous swimming but do not accumulate lactate during long periods of voluntary diving (Gatten, 1984).

The ventilation patterns recorded in the present study are very similar to patterns in spiny softshells, *A. spinifera* (Stone et al., 1992b; Bagatto and Henry, 1999), and pond sliders, *Trachemys scripta* (Bagatto and Henry, 1999). In *A. spinifera*, 99 % of breathing bouts consisted of one breath (Stone et al., 1992b), which was consistent with the present findings.

Effects of aquatic oxygen tension

Among fishes that utilize bimodal respiration, pulmonary oxygen uptake increases in facultative air-breathers as aquatic oxygen availability decreases, to fulfil metabolic demand (Johansen et al., 1967; Randall et al., 1981; Smatresk and Cameron, 1982a). This is not the case, however, for obligate air-breathing fish, in which pulmonary $\dot{V}_{O_2}$ remains constant irrespective of aquatic P_{O_2} (Johansen et al., 1968; Johansen and Lenfant, 1968; Glass et al., 1986). *Apalone ferox* resemble the obligate air-breathing fish, because changes in aquatic oxygen tension do not change the amount of pulmonary respiration in either group (Fig. 1).

The respiratory responses of *A. ferox* to changes in aquatic P_{O_2} also set it apart from other aquatic turtles. Bagatto and Henry (Bagatto and Henry, 1999) showed that *A. ferox* is able to increase its non-pulmonary gas exchange when submerged for prolonged periods. *Chrysemys picta*, like most other turtle species, does not increase non-pulmonary $\dot{V}_{O_2}$ with increasing aquatic P_{O_2}. This effect of oxygen tension on the partitioning of $\dot{V}_{O_2}$ has also been reported in *Anabas testudineus*, an obligate air-breathing fish. *Anabas testudineus* consumes more O_2 from air (54 %) than from water (46 %) in normoxic water but reverses this pattern in hyperoxic water (Hughes and Singh, 1970a). *Anabas tesdudineus*, like *Apalone ferox*, shows a nearly constant level of aquatic O_2 uptake over the normal range of P_{O_2} in natural waters inhabited by both species (between 100 and 60 mmHg). In addition, these species maintain constant blood P_{O_2} levels irrespective of aquatic P_{O_2}. This effect of ambient oxygen tension on non-pulmonary $\dot{V}_{O_2}$ was not observed in *C. picta*, again indicating that *A. ferox* has diverged physiologically from other aquatic turtles and is more similar physiologically to primitive fish that use bimodal respiration.

The transition from water- to air-breathing also affects blood gas variables and, consequently, extracellular acid–base status. Again, as a result of the differences in solubility of O_2 and CO_2 in air and water, and the different demands placed on ventilation and diffusion, air-breathers tend to have a higher blood P_{CO_2} and HCO_3^- concentration but a lower blood pH (Dejours, 1976; Dejours, 1978; Dejours, 1981; Piiper, 1982; Truchot, 1987).

Not surprisingly, *A. ferox* exhibited a higher blood P_{CO_2} and plasma [HCO_3^-] at 25 °C than at 15 °C, reflecting its greater reliance on pulmonary respiration at higher temperatures. In effect, at 25 °C, *A. ferox* displays the same type of blood gas shift that is seen during respiration in air compared with water. This trend has also been reported in the lungfish (*Neoceratodus forsteri*) and the mud puppy (*Necturus maculosus*) as well as in obligate air-breathing fishes (*Electrophorus electricus* and *Protopterus aethiopicus*) (Lenfant et al., 1967; Lenfant and Johansen, 1967; Delaney et al., 1977). In addition, this trend can be seen in the developmental stage of the salamander *Ambystoma mexicanum* when the adult becomes more dependent on air-breathing for CO_2 elimination (Burggren and Wood, 1981). *C. picta*, in contrast, did not display this pattern with respect to either its respiratory or acid–base profiles.

Many lung-breathing amphibians and reptiles remove CO_2 cutaneously both at rest and while diving (Gage and Gage, 1886; Jackson et al., 1976; Shelton and Boutilier, 1982; Stone et al., 1992a; Bagatto and Henry, 1999). Compared with emydids, softshell turtles are more proficient at non-pulmonary CO_2 excretion. *Apalone mutica* and *A. spinifera* excrete 64 % and 85 % of their total CO_2, respectively, into the aquatic habitat; this is more than twice as much as *Pseudemys scripta* and six times more than *Trachemys scripta elegans*, two emydid species (Jackson et al., 1976; Stone et al., 1992a; Bagatto and Henry, 1999). The high percentage aquatic rate of CO_2 excretion in softshell turtles is similar to that reported for air-breathing fish (70–81 %) (Lenfant and Johansen, 1968; Farber and Rahn, 1970). This ability may enable an animal to increase its dive duration. At 15 °C, softshell turtles significantly increased their dive duration despite the fact that, during these apnoeic periods, oxygen levels were progressively depleted. However, increased lactate and long-term apnoea (two conditions not found in this study) have been shown to facilitate non-pulmonary CO_2 excretion in *Trachemys scripta elegans*, indicating that this ability may be important during the winter months in emydids (Ultsch and Jackson, 1982).

Another way to demonstrate the influence of the partitioning of gas exchange on extracellular P_{CO_2} is to utilize a P_{CO_2} *versus* P_{O_2} diagram (Rahn and Howell, 1976; Dejours, 1981; Dejours, 1988; Truchot, 1987) (Fig. 2). Decreased pulmonary gas exchange and increased cutaneous gas exchange shift P_{CO_2} values of species using bimodal respiration to various levels intermediate between those of strict water- and air-breathers. Within this large intermediate range, the actual level may determine the relative importance of the lungs and skin as organs of CO_2 exchange (Truchot, 1987). Even though O_2 and CO_2 exchanges in the lung and gills are very complex (Piiper and Scheid, 1975), postbranchial and postpulmonary blood P_{O_2} and P_{CO_2} are not very different from their values in gill water or in the gas located in the alveolus (Dejours, 1988). Therefore, blood gas values can be superimposed on the P_{CO_2} *versus* P_{O_2} diagram. For a given value of respiratory quotient (RQ), the relationship between (P_{extO_2}–P_{intO_2}, where P_{extO_2} is water P_{O_2} and P_{intO_2} is blood P_{O_2}) and P_{intCO_2} is a straight line originating at the abscissa P_{extO_2} (Dejours, 1981). P_{CO_2} values were lower in softshell turtles at cold temperatures indicating that, during the longer dives at cold temperatures, these animals rely on non-pulmonary gas exchange and thus become superior non-pulmonary respirers. At warm temperatures, values for softshell turtles move closer to the air-breathing line. Their blood gas data support the suggestion that they are physiologically switching from water-breathing to air-breathing. In contrast, *C. picta* blood gas data reflect their high dependence on aerial oxygen uptake regardless of environmental temperature.

Exposure to hypoxic water did not have a significant effect on either the diving or the breathing patterns of *A. ferox* because these behaviours were independent of aquatic P_{O_2}. This differs from the spiny softshell, *A. spinifera*, which decreases mean dive duration in hypoxia (Stone et al., 1992b). This difference in behavioural response is probably due to the difference in habitats of the two species. Spiny softshells are found in free-flowing creeks and rivers, habitats that rarely become hypoxic, whereas Florida softshells inhabit sluggish streams, lakes and ponds (Mount, 1975), which may be subject to greater daily fluctuations in oxygen tension. The habitat of the Florida softshell is more similar to that of obligate air-breathing fishes which, like *A. ferox*, do not display changes in diving or ventilatory behaviour in response to fluctuations in aquatic oxygen tension (Johansen et al., 1968; Johansen and Lenfant, 1968; Glass et al., 1986). They neither increase lung ventilation in response to hypoxic water nor change the number of breaths per bout. In fact, some obligate air-breathers surface for air even in normoxic and hyperoxic water (Munshi and Singh, 1968; Hughes and Singh, 1970b). Air-breathing fish and *A. ferox* benefit from aquatic respiration, yet being able to meet metabolic demands without having to make behavioural adjustments may be further beneficial to these species. In a sense, they have become independent of environmental deoxygenation stress (Johansen, 1970).

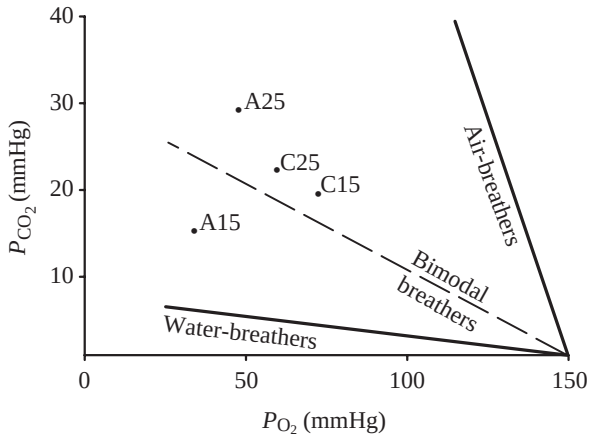

Fig. 2. P_{CO_2}/P_{O_2} diagram. Diagram of gas exchange by diffusion in gas and water phases (modified from Dejours, 1981). For a given value of respiratory quotient (RQ), the relationship between (P_{extO_2}–P_{intO_2}, where P_{extO_2} is water P_{O_2} and P_{intO_2} is blood P_{O_2}) and P_{intCO_2} is a straight line originating at the abscissa P_{extO_2}. Increases in RQ (non-pulmonary) and decreases in RQ (pulmonary) shift P_{CO_2} values of bimodal breathers to various levels between those for strict water- and air-breathers. A25, A15, *A. ferox* at 25 and 15 °C; C25, C15, *C. picta* at 25 and 15 °C. 1 mmHg=0.133 kPa.

Effects of temperature

For poikilothermic bimodal respirers, changes in temperature cause a variety of physiological and behavioural changes that have been reported in obligate and facultative air-breathing fish and in amphibians (Johansen et al., 1970; Lenfant et al., 1970; Rahn et al., 1971; Burggren and Wood, 1981; Smatresk and Cameron, 1982b). Principal among these effects is a decrease in O_2 availability in water and an increase in metabolic rate with increasing temperature, both of which can necessitate greater pulmonary uptake to meet increased O_2 demand (Burggren et al., 1983; Dejours, 1994). *Apalone ferox* obtained a substantial percentage of oxygen *via* non-

pulmonary respiration but increased the proportion of oxygen uptake from pulmonary routes at the higher temperature. This trend of increased reliance on aerial gas exchange in response to increasing temperatures has been reported in both fish and amphibians (Johansen et al., 1970; Lenfant et al., 1970; Rahn et al., 1971; Burggren and Wood, 1981; Smatresk and Cameron, 1982b). The $\dot{V}_{O_2}$ through the skin of frogs is nearly constant throughout the year; increases in $\dot{V}_{O_2}$ during the summer are met by greatly increased lung ventilation (Hutchison et al., 1968). The gar (*Lepisosteus* osseus), which also relies more heavily on aerial respiration in warmer waters, is a facultative air-breather at low temperatures but becomes an obligate air-breather when the $\dot{V}_{O_2}$ increases at high temperatures (Rahn et al., 1971). Therefore, respiratory characteristics documented in the softshell *A. ferox*, as affected by temperature, appear to have converged with those reported in obligate air-breathing fish and bimodal-breathing amphibians.

Chrysemys picta, behaving more as a typical air-breather, did not increase its percentage $\dot{V}_{O_2}$ from pulmonary routes as temperatures increased from 15 to 25 °C. Ultsch et al. (Ultsch et al., 1984) found that *C. picta* fared significantly better in normoxic water than in anoxic water during long-term forced submersion at 10 °C, suggesting that non-pulmonary uptake might be more important at very cold temperatures when metabolic requirements are very low or when turtles are unable to surface. At these temperatures, the non-pulmonary route may increase in importance during the winter months by increasing the length of the apnoeic periods and permitting some gas exchange, but this is not apparent during the time of year when the animal is active. Although *C. picta* live primarily in the aquatic environment, they have employed a variety of strategies other than bimodal respiration that set them apart physiologically and behaviourally from air-breathing fish, amphibians and other turtles.

Both *C. picta* and *A. ferox* supply the higher O_2 demands associated with increased temperature by increasing the rate of lung ventilation. The number of breaths per bout was not affected by temperature in either species. The basic pattern of a single breath separated by variable-length periods of apnoea in *A. ferox* was also shown (Bagatto and Henry, 1999) to remain unaltered during recovery from exhaustive exercise and from forced submergence. *C. picta*, in contrast, had breathing bouts characterized by multiple rhythmic breaths. Both species, however, increased the number of bouts per hour and, therefore, the number of breaths per emersion period at the higher temperature. Overall ventilatory rate increased more in *A. ferox* (61 %) than in *C. picta* (47 %). Total rate of oxygen uptake in both species was similar regardless of temperature, but temperature affected the partitioning of gas exchange differently. *Chrysemys picta* did not change the proportion of non-pulmonary (i.e. cutaneous) $\dot{V}_{O_2}$ with a change in temperature. In contrast, *A. ferox* decreased the proportion of cutaneous $\dot{V}_{O_2}$ from 44 to 17 % with increasing temperature, indicating a greater reliance on pulmonary gas exchange at higher temperatures. Therefore, ventilatory frequency increased more in *A. ferox* because its reliance on pulmonary oxygen uptake increased more in response to higher temperatures. A similar pattern has been observed in many bimodal-breathing fishes and amphibians (Johansen et al., 1970; Lenfant et al., 1970; Rahn et al., 1971; Burggren and Wood, 1981; Smatresk and Cameron, 1982b).

The relatively higher, and less temperature-sensitive, ventilation rate in *C. picta* is probably more a result of its pattern of CO_2 excretion. Air-breathing fish and softshell turtles excrete the majority of metabolically produced CO_2 into the aquatic medium *via* non-pulmonary routes (e.g. 80 and 64 % for *A. spinifera* and *A. mutica*, respectively; Jackson et al., 1976; Stone et al., 1992a; Bagatto and Henry, 1999). In contrast, *Pseudemys scripta* remove only 10.5 % of CO_2 cutaneously (Jackson et al., 1976; Bagatto and Henry, 1999). *Chrysemys picta* probably do not excrete a large proportion of their CO_2 through non-pulmonary sources and, therefore, it is possible that they must maintain high ventilatory rates (regardless of temperature) to remove CO_2 through the lungs to avoid respiratory acidosis.

As temperature decreased, *A. ferox* greatly increased their diving duration compared with *C. picta*. Dives of longer duration at colder temperatures indicate that *A. ferox* takes advantage of its ability to exchange both O_2 and CO_2 through non-pulmonary sources. This trait allows the lungs to be ventilated less frequently, requiring only infrequent and brief excursions to the air–water interface. At higher temperatures, when metabolic demand is greater and their reliance on pulmonary O_2 uptake increases, they surface more frequently, dive more frequently and make dives of much shorter duration.

In *C. picta*, longer emersion apnoea periods may represent surface basking; they are known to bask at 15 °C in the field (Ernst, 1972). Alternatively, because *C. picta* excrete only small amounts of CO_2 through non-pulmonary sources, at warmer temperatures they may need to make more frequent ventilations to maintain resting acid–base status. For this reason, multiple breath bouts may be more important in *C. picta*. In addition, *C. picta* may have retained the multiple-breath pattern effectively to eliminate the CO_2 that has accumulated during periods of apnoea. A related species, *Pseudemys scripta*, stores approximately 85 % of its total internal CO_2 in blood and tissues during periods of apnoea. Only 10 % of the total O_2 uptake in *P. scripta* is stored in the red blood cells, while 90 % is stored in the lungs (Burggren and Shelton, 1979). During apnoea, the blood functions to transport oxygen from pulmonary stores. When the turtle returns to the surface, ventilation causes a large gas gradient, which favours CO_2 removal through the lungs, while the pulmonary, tissue and blood O_2 stores are replenished (Burggren and Shelton, 1979). *Apalone ferox*, in contrast, may never accumulate enough CO_2 in its blood during periods of resting apnoea to cause a significant change in ventilation pattern.

The pattern and temperature-sensitivity of diving in *Apalone ferox* are similar to those of other bimodal breathers. The spotted gar *Lepisosteus osseus* is considered a facultative air-

breather in cold water and an obligate air-breather in warm water (Rahn et al., 1971). Similarly, survival and diving behaviour in marine snakes are dependent on temperature and the associated O_2 content of the water, with survival and dive duration being reduced in hypoxia and at high temperature (Graham, 1974; Heatwole, 1975). *Apalone ferox* became less active at colder temperatures by greatly increasing the duration of inactive periods (from 4 to 18 min) and by decreasing the frequency of activity bursts. Similarly, the bowfin *Amia calva*, an air-breathing fish, decreases activity at low temperatures when it relies almost exclusively on water-breathing. Although activity patterns in *C. picta* were affected by temperature, this species was always significantly more active than *A. ferox*.

It may be advantageous for *A. ferox* to be less active than *C. picta*. *Apalone ferox*, which feed on a variety of free-swimming animals including frogs, fish and crayfish, are ambush predators that are dependent on remaining unobtrusive. *Chrysemys picta*, however, are wide foragers, feeding on molluscs, arthropods, vegetation and carrion (Mount, 1975); an ability to remain unobtrusive while feeding is thus less important. Conversely, several studies have shown that anti-predator factors are also influential in the regulation of air-breathing in bimodal-breathing species (Kramer and Graham, 1976; Gee, 1980; Drummond, 1980). The shell of the turtle is a structural adaptation against predators derived from a semiaquatic ancestor (Carr, 1952; Pritchard, 1979). In softshells, the epidermal shields of the shell are lost and the thecal dermal portions reduced (Zangerl, 1969). The resulting exposed epidermis of the skin may place softshells at greater risk of predation or injury than *C. picta*. Lower activity levels (as well as fewer migrations to the surface) could be more advantageous to *A. ferox* in this regard.

In addition to ecological correlates to the breathing physiology of *C. picta* and *A. ferox*, patterns exhibited by these two species may illuminate events in the evolution of bimodal respiration. The pattern of lung ventilation is periodic in most reptiles. Many species exhibit episodic bouts of continuous breathing separated by a highly variable period of breath-holding (emersion apnoea or diving), while others take only single breaths, each ending with a breath-hold. The single-breath pattern has been noted in spiny softshells (*Apalone spinifera*; Stone et al., 1992b; Bagatto and Henry, 1999), loggerhead sea turtles (*Caretta caretta*; Milsom and Johansen, 1975; Lutcavage, 1989), green sea turtles (*Chelonia mydas*; Jackson et al., 1979) and marine snakes (Heatwole, 1975), all of which are highly aquatic. In contrast, other freshwater chelonians, crocodiles and freshwater snakes (*Acrochorous javanicus*) show breathing patterns that consist of episodic bouts of multiple breaths (McCutcheon, 1943; Belkin, 1964; Jackson, 1971; Glass et al., 1978).

In air-breathing fishes and larval amphibians, solitary breaths are taken at irregular intervals. This is presumed to be the primitive condition for air-breathing vertebrates (Smatresk, 1994). *A. ferox* and the solitary-breath reptiles mentioned above have a breathing pattern more similar to the primitive condition. These species of turtle and snake are capable of rhythmic breathing but tend not to show multiple breath bouts during voluntary diving. For example, marine snakes show one exhalation followed by an inhalation and breath-holding in the field, but multiple breath bouts in the laboratory (Heatwole and Seymour, 1975). Also, sea turtles tend to revert to rhythmic breathing while on the shore laying eggs (Jackson and Prange, 1979). The pattern of single-breath bouts separated by variable periods of apnoea may be an adaptation particularly suited to life at the air–water interface. Softshells, a more highly derived species, may have evolved this trait, which parallels the pattern displayed by air-breathing fish, during their transition from a terrestrial to an amphibious mode of life.

We would like to thank Craig Guyer for his help and guidance with the statistical analysis of the data and Jennifer Shelby for assisting in scoring the videotapes. This work was supported by NSF IBN 97-27835 to R.P.H.

References

Bagatto, B., Guyer, C., Hauge, B. and Henry, R. P. (1997). Bimodal respiration in two species of Central American turtles, *Staurotypus triporcatus* and *Kinosternon leucostomum*. *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. *J. Exp. Biol.* **202**, 267–278.

Belkin, D. A. (1963). Anoxia: tolerance in reptiles. *Science* **139**, 492–493.

Belkin, D. A. (1964). Variations in heart rate during voluntary diving in the turtle *Pseudemys concinna*. *Copeia* **2**, 321–330.

Belkin, D. A. (1968a). Aquatic respiration and underwater survival of two freshwater turtle species. *Respir. Physiol.* **4**, 1–14.

Belkin, D. A. (1968b). Anaerobic brain function: effects of stagnant and anoxia on persistence of breathing in reptiles. *Science* **162**, 1017–1018.

Bennett, A. F. (1980). The metabolic foundations of vertebrate behavior. *Biol. Sci.* **30**, 452–456.

Burggren, W. W., Feder, M. E. and Pinder, A. W. (1983). Temperature and the balance between aerial and aquatic respiration in larvae of *Rana berlandieri* and *Rana catesbeiana*. *Physiol. Zool.* **56**, 263–273.

Burggren, W. W. and Shelton, G. (1979). Gas exchange and transport during intermittent breathing in chelonian reptiles. *J. Exp. Biol.* **82**, 75–92.

Burggren, W. W. and Wood, S. C. (1981). Respiration and acid–base balance in the salamander, *Ambystoma tigrinum*: Influence of temperature acclimation and metamorphosis. *J. Comp. Physiol.* **144**, 241–246.

Caligiuri, M., Robin, E. D., Hance, A. J., Robin, D. A., Lewiston, N. and Theodore, J. (1981). Prolonged diving and recovery in the freshwater turtle, *Pseudemys scripta*. II. Magnitude of depression of O_2 requirements and the relation to body O_2 stores. *Comp. Biochem. Physiol.* **70**A, 365–369.

Carr, A. (1952). *Handbook of Turtles*. Ithaca, NY: Cornell University Press. 217pp.

Cochran, W. G. and Cox, G. M. (1957). *Experimental Designs.* Second edition. New York: John Wiley & Sons, Inc. 617pp.

Dejours, P. (1975). *Principles of Comparative Respiratory Physiology.* Amsterdam: North-Holland Publishing Company. 253pp.

Dejours, P. (1976). Water *versus* air as the respiratory media. In *Respiration of Amphibious Vertebrates* (ed. G. M. Hughes), pp. 1–15. London, New York, San Francisco: Academic Press.

Dejours, P. (1978). Carbon dioxide in water- and air-breathers. *Respir. Physiol.* **33**, 121–128.

Dejours, P. (1981). *Principles of Comparative Respiratory Physiology.* Second edition. Amsterdam: Elsevier/North Holland Biomedical Press. 265pp.

Dejours, P. (1988). *Respiration in Water and Air. Adaptations – Regulation – Evolution.* Elsevier: Amsterdam. 179pp.

Dejours, P. (1994). Environmental factors as determinants in bimodal breathing: An introductory overview. *Am. Zool.* **34**, 178–183.

Delaney, R. G., Lahiri, S., Hamilton, R. and Fishman, R. P. (1977).

Acid–base balance and plasma composition in the aestivating lungfish (*Protopterus*). *Am. J. Physiol.* **232**, R10–R17.

Drummond, H. M. (1980). Aquatic foraging in some new world natricine snakes: generalists and specialists and their behavioral evolution. PhD dissertation, University of Tennessee, Knoxville, USA.

Dunson, W. A. (1960). Aquatic respiration in *Trionyx spinifer asper*. *Herpetologica* **16**, 227–283.

Ernst, C. H. (1972). Temperature–activity relationships in the painted turtle, *Chrysemys picta*. *Copeia* **1972**, 217–222.

Ernst, C. H. and Barbour, R. W. (1972). *Turtles of the United States*. Lexington: University Press of Kentucky.

Farber, J. and Rahn, H. (1970). Gas exchange between air and water and the ventilation pattern in the electric eel. *Respir. Physiol.* **9**, 151–161.

Gage, S. H. and Gage, S. P. (1886). Aquatic respiration in soft-shelled turtles: a contribution to the physiology of respiration in vertebrates. *Am. Nat.* **20**, 223–236.

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

Gatten, R. E., Jr (1981). Anaerobic metabolism in freely diving painted turtles (*Chrysemys picta*). *J. Exp. Zool.* **216**, 377–385.

Gatten, R. E., Jr (1984). Aerobic and anaerobic metabolism of freely-diving loggerhead musk turtles (*Sternotherus minor*). *Herpetologia* **40**, 1–7.

Gee, J. H. (1980). Respiratory patterns and antipredator responses in the central mudminnow, *Umbra limi*, a continuous, facultative, air-breathing fish. *Can. J. Zool.* **58**, 819–827.

Girgis, S. (1961). Aquatic respiration in the common Nile turtle, *Trionyx triunguis* (Forskål). *Comp. Biochem. Physiol.* **3**, 206–217.

Glass, M. L., Boutilier, R. G. and Heisler, N. (1983). Ventilatory control of arterial P_{O_2} in the turtle *Chrysemys picta bellii*: effects of temperature and hypoxia. *J. Comp. Physiol.* B **151**, 145–153.

Glass, M., Burggren, W. W. and Johansen, K. (1978). Ventilation in an aquatic and a terrestrial chelonian reptile. *J. Exp. Biol.* **72**, 165–179.

Glass, M. L., Ishimatsu, A. and Johansen, K. (1986). Responses of aerial ventilation to hypoxia and hypercapnia in *Channa argus*, an air-breathing fish. *J. Comp. Physiol.* B **156**, 425–430.

Graham, J. B. (1974). Aquatic respiration in the sea snake *Pelamis platurus*. *Respir. Physiol.* **21**, 1–7.

Heatwole, H. (1975). Voluntary submergence times of marine snakes. *Mar. Biol.* **32**, 205–213.

Heatwole, H. and Seymour, R. (1975). Pulmonary and cutaneous oxygen uptake in sea snakes and a file snake. *Comp. Biochem. Physiol.* **50A**, 399–405.

Herbert, C. V. and Jackson, D. C. (1985). Temperature effects on the responses to prolonged submergence in the turtle *Chrysemys picta bellii*. I. Blood acid–base and ionic changes during and following anoxic submergence. *Physiol. Zool.* **58**, 655–669.

Hochachka, P. W., Owen, T. G., Allen, J. F. and Whittow, G. C. (1975). Multiple end products of anaerobiosis in diving vertebrates. *Comp. Biochem. Physiol.* **50**B, 17–22.

Hughes, G. M. and Singh, B. N. (1970a). Respiration in an air-breathing fish, the climbing perch *Anabas testudineus* Block. I. Oxygen uptake and carbon dioxide release into air and water. *J. Exp. Biol.* **53**, 265–280.

Hughes, G. M. and Singh, B. N. (1970b). Respiration in an air-breathing fish, the climbing perch *Anabas testudineus* Block. II. Respiratory patterns and the control of breathing. *J. Exp. Biol.* **53**, 281–298.

Hutchison, V. H., Whitford, W. G. and Kohl, M. (1968). Relation of body size and surface area to gas exchange in anurans. *Physiol. Zool.* **41**, 65–85.

Jackson, D. C. (1968). Metabolic depression and oxygen depletion in the diving turtle. *J. Appl. Physiol.* **24**, 503–509.

Jackson, D. C. (1971). The effect of temperature on ventilation in the turtle, *Pseudemys scripta elegans*. *Respir. Physiol.* **12**, 131–140.

Jackson, D. C., Allen, J. and Strupp, P. K. (1976). The contribution of non-pulmonary surfaces to CO_2 loss in 6 species of turtles at 20 °C. *Comp. Biochem. Physiol.* **55**A, 243–246.

Jackson, D. C., Kraus, D. R. and Prange, H. D. (1979). Ventilatory response to inspired CO_2 in the sea turtle: Effects of body size and temperature. *Respir. Physiol.* **38**, 71–81.

Jackson, D. C., Palmer, S. E. and Meadow, W. L. (1974). The effects of temperature and carbon dioxide breathing on ventilation and acid–base status of turtles. *Respir. Physiol.* **20**, 131–146.

Jackson, D. C. and Prange, H. D. (1979). Ventilation and gas exchange during rest and exercise in adult green sea turtles. *J. Comp. Physiol.* **134**, 315–319.

Jackson, D. C. and Ultsch, G. R. (1982). Long-term submergence at 3 °C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. II. Extracellular ionic responses to extreme lactic acidosis. *J. Exp. Biol.* **96**, 29–43.

Johansen, K. (1970). Air breathing in fishes. In *Fish Physiology*, vol. 4L (ed. W. S. Hoar and D. J. Randall), pp. 361–411. London, New York: Academic Press.

Johansen, K., Hanson, D. and Lenfant, C. (1970). Respiration in a primitive air breather, *Amia calva*. *Respir. Physiol.* **9**, 162–174.

Johansen, K. and Lenfant, C. (1968). Respiration in the African lungfish *Protopterus aethiopicus*. II. Control of breathing. *J. Exp. Biol.* **49**, 453–468.

Johansen, K., Lenfant, C. and Grigg, G. C. (1967). Respiratory control in the lungfish, *Neoceratodus forsteri* (Krefft). *Comp. Biochem. Physiol.* **20**, 835–854.

Johansen, K., Lenfant, C., Schmidt-Nielsen, K. and Petersen, J. A. (1968). Gas exchange and control of breathing in the electric eel, *Electrophorus electricus*. *Z. Vergl. Physiol.* **61**, 137–163.

Kelman, G. R. and Numm, J. F. (1966). Nomograms for correction of blood P_{O_2} and P_{CO_2}, pH and base excess for time and temperature. *J. Appl. Physiol.* **21**, 1484–1490.

Kerr, J. and Frankel, H. M. (1972). Inadequacy of blood drawn by cardiac puncture as a source for respiratory gas measurements in turtle *Pseudemys scripta*. *Comp. Biochem. Physiol.* **41**A, 913–915.

Kramer, D. L. and Graham, J. F. (1976). Synchronous air breathing, a social component of respiration in fishes. *Copeia* **1976**, 689–697.

Lenfant, C. and Johansen, K. (1967). Respiratory adaptations in selected amphibians. *Respir. Physiol.* **2**, 247–260.

Lenfant, C. and Johansen, K. (1968). Respiration in the African lungfish, *Protopterus aethiopicus*. *J. Exp. Biol.* **49**, 437–452.

Lenfant, C., Johansen, K. and Grigg, G. C. (1967). Respiratory properties of blood and pattern of gas exchange in the lungfish *Neoceratodus fosteri* (Krefft). *Respir. Physiol.* **2**, 1–21.

Lenfant, C., Johansen, K. and Hanson, D. (1970). Bimodal gas exchange and ventilation–perfusion relationships in lower vertebrates. *Fedn. Proc.* **29**, 1124–1129.

Lowell, W. R. (1990). Aerobic metabolism and swimming energetic of the painted turtle, *Chrysemys picta*. *Exp. Biol.* **48**, 349–355.

Lutcavage, M. E., Lutz, P. L. and Baier, H. (1989). Respiratory mechanics of the loggerhead sea turtle *Caretta caretta*. *Respir. Physiol.* **76**, 13–24.

Lutz, P. L., Bergey, A. and Bergey, M. (1989). Effects of temperature on gas exchange and acid–base balance in the sea turtle *Caretta caretta* at rest and during routine activity. *J. Exp. Biol.* **144**, 155–160.

Lutz, P. L., LaManna, J. C., Adams, M. R. and Rosenthal, M. (1980). Cerebral resistance to anoxia in the marine turtle. *Respir. Physiol.* **41**, 241–251.

Lutz, P. L., McMahon, P., Rosenthal, M. and Sick, T. J. (1984). Relationships between aerobic and anaerobic energy production in turtle brain *in situ*. *Am. Physiol. Soc.* **247**, 740–744.

McCutcheon, F. H. (1943). The respiratory mechanism in turtles. *Physiol. Zool.* **16**, 255–269.

Milsom, W. K. and Johansen, K. (1975). The effect of buoyancy induced lung volume changes on respiratory frequency in a Chelonian (*Caretta caretta*). *J. Comp. Physiol.* **98**, 157–160.

Mount, R. H. (1975). *The Reptiles and Amphibians of Alabama.* Auburn, AL: Auburn University, Agricultural Experiment Station. 347pp.

Munshi, J. S. D. and Singh, B. N. (1968). On the respiratory organs of *Amphipnous cuchia* (Ham. Buch.). *J. Morph.* **124**, 423–444.

Piiper, J. (1982). Respiratory gas exchange at lungs, gills and tissues: mechanisms and adjustments. *J. Exp. Biol.* **100**, 5–22.

Piiper, J. and Scheid, P. (1975). Gas transport efficacy of gills, lungs and skin: Theory and experimental data. *Respir. Physiol.* **23**, 209–221.

Plummer, M. V. and Shirer, H. W. (1975). Movement patterns in a river population of the softshelled turtles, *Trionyx muticus.* Occasional Papers. *Mus. Nat. Hist. Univ. Kans.* **43**, 1–26.

Pritchard, P. C. (1979). *Encyclopedia of Turtles.* New Jersey: T. F. H. Publications. 895pp.

Rahn, H. and Howell, B. J. (1976). Bimodal gas exchange. In *Respiration of Amphibious Vertebrates* (ed. G. M. Hughes), pp. 271–285. London, New York, San Francisco: Academic Press.

Rahn, H., Rahn, K. B., Howell, B. J., Gans, C. and Tenney, S. M. (1971). Air-breathing in the garfish, *Lepisosteus osseus*. *Respir. Physiol.* **11**, 443–466.

Randall, D. J., Cameron, J. N., Daxboeck, C. and Smatresk, N. (1981). Aspects of bimodal gas exchange in the bowfin, *Amia calva* L. (Actinopterygii: Amiformes). *Respir. Physiol.* **43**, 339–348.

Rapatz, G. L. and Musacchia, X. L. (1957). Metabolism of *Chrysemys picta* during fasting and during cold torpor. *Am. J. Physiol.* **188**, 456–460.

Reeves, R. B. (1976). Temperature induced changes in blood acid–base status: pH and P_{CO_2} in a binary buffer. *J. Appl. Physiol.* **40**, 752–761.

Robin, E. D., Vester, J. W., Murdaugh, H. V. and Millen, J. E. (1964). Prolonged anaerobiosis in a vertebrate: anaerobic metabolism in the freshwater turtle. *J. Cell. Comp. Physiol.* **63**, 287–297.

Root, R. W. (1949). Aquatic respiration in the musk turtle. *Physiol. Zool.* **22**, 172–178.

Sas Institute (1990). SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA. All rights reserved.

Seidel, M. E. (1977). Respiratory metabolism of temperate and tropical American turtles (Genus *Chrysemys*). *Comp. Biochem. Physiol.* **57**A, 297–298.

Shelton, G. and Boutilier, R. G. (1982). Apnoea in amphibians and reptiles. *J. Exp. Biol.* **100**, 245–273.

Smatresk, N. J. (1994). Respiratory control in the transition from water to air breathing in vertebrates. *Am. Zool.* **34**, 264–279.

Smatresk, N. J. and Cameron, J. N. (1982a). Respiration and acid–base physiology of the spotted gar, a bimodal breather. I. Normal values and response to severe hypoxia. *J. Exp. Biol.* **96**, 263–280.

Smatresk, N. J. and Cameron, J. N. (1982b). Respiration and acid–base physiology of the spotted gar, a bimodal breather. II. Responses to temperature change and hypercapnia. *J. Exp. Biol.* **96**, 281–293.

Snedecor, G. W. and Cochran, W. G. (1980). *Statistical Methods*. Seventh edition. Ames, IA: Iowa State University Press. 507pp.

Stockard, M. E. and Gatten, R. E., Jr (1983). Activity metabolism of painted turtles (*Chrysemys picta*). *Copeia* **1983**, 214–221.

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*). *Physiol. Zool.* **65**, 311–330.

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

Truchot, J. P. (1987). *Comparative Aspects of Extracellular Acid–Base Balance*. Heidelberg: Springer-Verlag. 248pp.

Ultsch, G. R., Herbert, C. V. and Jackson, D. C. (1984). The comparative physiology of diving in North American freshwater turtles. I. Submergence tolerance, gas exchange and acid–base balance. *Physiol. Zool.* **57**, 620–631.

Ultsch, G. R. and Jackson, D. C. (1982). Long-term submergence at 3 °C of the turtle *Chrysemys picta bellii*, in normoxic and severely hypoxic water. I. Survival, gas exchange and acid–base status. *J. Exp. Biol.* **96**, 11–28.

Zangerl, R. (1969). The turtle shell. In *Biology of the Reptilia*, vol. 1 (ed. C. Gans), pp. 311–339. New York: Academic Press.