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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

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Summary

The dynamics of bimodal respiration, diving behaviour and blood acid–base status in the softshell turtle *Trachemys scripta* and the pond slider *Apalone ferox* were investigated at rest and under conditions of stress induced by exercise and forced submergence. During periods of forced submergence, only *A. ferox* doubled its aquatic gas exchange rate. Both *A. ferox* and *T. scripta* increased their aerial gas exchange profoundly following exercise and forced submergence, a pattern indicative of increased anaerobic respiration. Emersion duration increased significantly in *A. ferox* following forced submergence, and mean apnoeic time decreased significantly in *A. ferox* following exercise, indicating that a larger proportion of time at the surface was spent ventilating. Also, *A. ferox* maintained a one-breath breathing bout regardless of treatment. Submergence produced a respiratory acidosis in the plasma of approximately 0.2 pH units in magnitude in *T. scripta* and a mixed respiratory/metabolic acidosis of 0.4 pH units in *A. ferox*. Exercise induced an acidosis of 0.2 pH units of primarily metabolic origin in both species. Intra-erythrocyte pH was also reduced in both species in response to submergence and exercise. Both intracellular and extracellular acidoses were more severe and longer lasting in *A. ferox* after each treatment. Plasma [HCO_3^-] decreased by 25 % in both species following exercise, but only in *A. ferox* following submergence. Plasma lactate concentrations increased by equal amounts in each species following exercise; however, they returned to resting concentrations sooner in *T. scripta* than in *A. ferox*. *A. ferox* had significantly higher lactate levels than *T. scripta* following forced submergence as well as a slower recovery time. *A. ferox*, which is normally a good bimodal gas exchanger at rest, utilizes aerial respiration to a greater extent when under respiratory and/or metabolic stress. *T. scripta*, although almost entirely dependent on aerial respiration, is physiologically better able to deal with the respiratory and metabolic stresses associated with both forced submergence and exercise.

Key words: turtle, *Trachemys scripta*, softshell, *Apalone ferox*, bimodal breathing, aquatic gas exchange, exercise, forced submergence, blood, acid–base.

Introduction

Testudines use water as a respiratory medium, exchanging O_2 and CO_2 across nonpulmonary surfaces (Belkin, 1968; Jackson et al., 1976; Gatten, 1980, 1984; Stone et al., 1992a). As such, many turtle species are bimodal gas exchangers, exchanging respiratory gases with both air and water. While the relative importance of aquatic gas exchange has been studied in only a handful of species, it has been documented that the utilization of water as a respiratory medium varies widely (Jackson et al., 1976; Stone et al., 1992a). For example, in members of the softshell family (Trionychidae), aquatic gas exchange accounts for approximately 38 % of the total $\dot{V}_{O_2}$ and 85 % of the total $\dot{V}_{CO_2}$ (Gage and Gage, 1886; Stone et al., 1992a), whereas in members of the Emydidae, aquatic $\dot{V}_{O_2}$ values range from 4 % (Belkin, 1968) to 11 % (Jackson et al., 1976). Additionally, the ability of these bimodal gas exchangers to increase their reliance on aquatic gas exchange during periods of stress or increased metabolic rate has never been studied systematically.

One important physiological stressor is exercise, which can be utilized as a physiological correlate of survival (Bennett and Huey, 1990). Reptiles frequently undergo brief but sometimes intense periods of burst exercise in escaping from predators and/or capturing prey. During periods of increased anaerobic activity, an animal must cope with increased rates of CO_2 production and the accumulation of lactic acid. The resultant respiratory and/or metabolic acidosis can be further complicated if exercise takes place while the animal is submerged and therefore unable to utilize aerial gas exchange. Exercise has been studied in sea turtles (*Chelonia mydas*), painted turtles (*Chrysemys picta*), pond sliders (*Trachemys*

scripta) and snapping turtles (*Chelydra serpentina*); however, these studies have focused on the metabolic costs of and factors affecting aerobic exercise (Gatten, 1974, 1988; Butler et al., 1984; Lowell, 1990; Jackson and Prange, 1979; Zani and Claussen, 1994). The acid–base status during the recovery from exhaustive exercise has been well documented in fish (Wood and Perry, 1983; Boutilier et al., 1993) and in a few terrestrial reptiles (Seymour et al., 1985; Gleeson and Dalessio, 1989), but acid–base status and the way in which turtles deal with strenuous exercise have not yet been investigated in turtles, especially in those species that are aquatic and have varying abilities as bimodal gas exchangers.

Another significant physiological stressor is any period of submergence that is extended beyond a 'routine' dive duration. Turtles may extend dives beyond their aerobic capacity during predator/prey interactions and certainly during hibernation (Ultsch et al., 1985). Whether members of the genus *Apalone* are able to increase aquatic respiration significantly in response to prolonged submersion is not known.

Species within the Trionychidae are so highly aquatic that some have been reported to support resting metabolism solely *via* the aquatic medium (Dunson, 1960; Girgis, 1961). However, it is not known whether resting aquatic gas exchange is indicative of the maximal ability of the Trionychidae to respire aquatically. It is possible that members of this family could increase aquatic $\dot{V}_{O_2}/\dot{V}_{CO_2}$ during periods in which the animal is effectively cut off from aerial respiration or when an increase in metabolic rate places a higher demand on total $\dot{V}_{O_2}$. Additionally, a comparison between Trionychidae and Emydidae, a family containing characteristically poor bimodal gas exchangers, will aid in determining the comparative importance of aquatic respiration under various physiologically stressful conditions. Pond sliders (*Trachemys scripta*), Florida softshells (*Apalone ferox*) and spiny softshells (*Apalone spinifera*) were used in a comparison of resting aquatic respiration to investigate inter-family differences as well as intra-genus differences. *T. scripta* and *A. ferox* were used to compare resting aquatic respiration *versus* maximal aquatic respiration to elucidate the degree of reliance on aerial *versus* aquatic gas exchange in freshwater turtles.

Materials and methods

Collection and maintenance of animals

Specimens of *Apalone spinifera* (Le Sueur) (*N*=7; 3 male, 4 female; mean mass 2196±616 g; range 237–4225 g), *Apalone ferox* (Schneider) (*N*=18; 17 male, 1 female; mean mass 1024±352 g; range 409–5900 g) and *Trachemys scripta* (Schoepff) (*N*=28; 15 male, 13 female; mean mass 987±53 g; range 545–1785 g) (means ± S.E.M.) were trapped from the Tallapoosa drainage in Alabama, USA, using baited hoop nets or purchased through a commercial supplier. All animals were housed in laboratory aquaria and were fed a combination of trout pellets and ReptoMin *ad libitum*. *A. ferox* and *A. spinifera* were housed individually, and *T. scripta* were housed in groups of a maximum of five for at least 2 weeks prior to experimentation. Housing containers for both species were 4 m^2 in surface area and 1 m in height. Food was withheld for at least 3 days prior to experimentation. Temperature remained between 22 and 25 °C, and the photoperiod was approximately 14 h:10 h L:D. All experiments began between 06:00 and 10:00 h and ended between 10:00 and 14:00 h.

Respiratory gas exchange

Turtles were placed in a Plexiglas chamber made up of a water-filled compartment (45 cm×45 cm×30 cm) and a smaller air-filled breathing hood (13 cm×13 cm×15 cm) built into the chamber lid. For *T. scripta*, the larger water compartment was reduced in size (25 cm×25 cm×25 cm) to measure changes in aquatic gas partial pressures more easily. The chamber structure was such that the turtle remained submerged in the water compartment but could raise its head into the air-breathing hood for pulmonary ventilation. Both compartments were fitted with ports from which gas and water samples were collected (Stone et al., 1992a) and subsequently analyzed for O_2 partial pressure and CO_2 concentration. To avoid behavioural acclimation (Stone et al., 1992b), each turtle was placed in the chamber for only one experiment.

Each turtle was maintained in the chamber for at least 12 h prior to an experiment. Air-equilibrated water and fresh air were supplied *via* a gas equilibrium column and air pump, respectively. This flow-through system was closed prior to the beginning of each experiment. Water was continuously stirred during the experiment by means of magnetic stir bars to prevent the occurrence of O_2 and CO_2 gradients. The small surface area of the air–water interface relative to the volume of water minimized movement of gases between phases. The negligible movement of gases was confirmed over 8 h periods. Either hypercapnic (10 % CO_2) water or normoxic water was tested, each with normoxic air, hypercapnic air or anoxic air. Each air and water combination was replicated four times. There were no significant differences in either the air or water composition after the 8 h period.

Resting gas exchange for the control treatment was monitored for a period of 4 h. Air samples were taken at 0.5 h intervals, while water samples were taken at 2.0 h intervals. The breathing hood was flushed with atmospheric air for 30 s after each air sample to minimize the build-up of hypoxic or hypercapnic air. Before the treatments of forced submergence or exercise, the resting rate of gas exchange was measured in an identical manner. Turtles were submerged by filling the breathing hood with aerated water and then sealing this chamber. For *T. scripta*, experimental subjects were maintained in the sealed, flooded chamber for 1.0 h before samples were collected. For *A. ferox*, aerated water was pumped into the breathing hood to forcibly submerge the animal; however, it was not immediately sealed. To prevent aquatic hypoxia or hypercapnia, water was continuously pumped through the breathing hood for 30 min, after which this chamber was sealed. Samples were collected 30 min after the breathing hood had been sealed (a total of 1 h of forced submergence). During recovery, the air compartment was

refilled with air, and samples were withdrawn more frequently to prevent excessive O_2 depletion and CO_2 build-up in the air chamber.

The exercise treatments utilized a similar protocol. In this case, the turtle was removed from the chamber following the rest period and exercised in a large plastic pool *via* caudal stimulation with tongs. A plastic screen was held over the anterior portion of the turtle to ensure that no air was inspired during the exercise period. Exercise proceeded to exhaustion, which was defined as the point at which a turtle no longer responded vigorously to caudal stimulation (i.e. a turtle no longer attempted to escape the grasp). Because exercise usually lasted 5–8 min and was performed in a large volume of water, aquatic gas exchange was not measured during this period. Immediately following exercise, the turtle was returned to the chamber in a closed, water-filled container to prevent it from breathing during the transfer. The metabolic chamber was then sealed, and samples were taken during a 2.0 h recovery period in the same manner as during the recovery period in the forced submergence protocol.

Oxygen partial pressures were measured using a Radiometer PHM72 blood/gas monitor equipped with an E5046 P_{O_2} electrode. Aquatic oxygen partial pressures were converted into molar concentrations using solubility coefficients from Dejours (1975). Carbon dioxide concentrations were measured using a Capni-con 5 total CO_2 analyzer (Cameron Instrument). Mean aerial, aquatic and total $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated for each individual, and percentage aerial $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were calculated from these means. Individual means were used to determine the mean ± S.E.M. of each variable for each species. Data analyses were performed on the means of individual animals, not on individual observations.

Ventilation and diving behaviour

The ventilation and diving behaviour of each turtle was monitored during all experiments (4 h duration) using a Dash IV oscillographic chart recorder (Astro-Med). Fine-gauge wires were fitted at opposite sides of the air–water interface and connected to a Colborne 2991 impedance converter (Morrow Bay, California, USA). Emersion, expiration, inspiration and immersion were monitored as a function of time by following the characteristic changes in impedance at the air–water interface. During preliminary experiments, a video camera was used to confirm behavioural measurements observed on the chart recorder. 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 (Stone et al., 1992b). Incomplete immersion or emersion bouts in progress at the beginning or end of an experiment were excluded from the data.

Blood acid–base status

Surgery

Trachemys scripta were anaesthetized using a pre-anaesthetic of NO_2 gas (10 % in air) followed by Halothane (approximately 2 %). Surgical anaesthesia was characterized by the loss of a withdrawal reflex in response to pedal stimulation. A 2.5 cm diameter section of plastron was removed from the ventral side of *T. scripta*, exposing the right subclavian artery (Jackson et al., 1974). PE 90 tubing was used to catheterize this artery and was secured using silk ligatures. Heparinized reptilian Ringer's solution (Lippe et al., 1966) was frequently flushed through the cannula to prevent clotting. The cannula was then routed posterior to the right limb and secured to the shell using rubber strips and superglue. The circular piece of plastron was replaced and sealed using plastic epoxy glue and covered with a thin acrylic sheet to prevent exchange of fluids in either direction.

In *A. ferox*, surgical anaesthesia was obtained using 3-aminobenzoic acid ethyl ester (MS-222, Sigma Chemical Co., St Louis, USA). A dosage of 500 mg kg^{-1} was injected intracoelomically, after which the turtle was immersed in an aerated solution of 1 g l^{-1} MS-222 buffered to a pH of 7.0 (Bagatto et al., 1997a). After surgical anaesthesia had been achieved, a semicircular flap of skin was cut and folded back, exposing the left subclavian artery (Ultsch et al., 1984; Bagatto et al., 1997b). Cannulation proceeded in the same manner as for *T. scripta*, after which the flap of skin was sutured and sealed with superglue. Animals were allowed at least 24 h to recover from surgery.

Blood analyses

After a resting blood sample had been withdrawn, a turtle was given one of two treatments. Forced submergence of 1 h involved placing a turtle in aerated water and fitting the container with a plastic grate so that the turtle could not emerge. The exercise treatment was as described above. After a resting blood sample had been withdrawn, additional samples (800 µl each) were withdrawn at 0, 30, 60, 120, 240 and 480 min following the forced submergence or exercise; these were replaced by equal volumes of heparinized reptile Ringer's solution.

Each blood sample was withdrawn into a gas-tight Hamilton syringe and transferred into 0.5 ml polyethylene tubes, which were quickly sealed and were always full when sealed. Care was taken to minimize the exposure of the blood sample to environmental air. Plasma was then separated from the corpuscular component using a Fisher model 235B micro-centrifuge (3 min at 10 000 revs min^{-1}), and the true plasma pH (pHe) and plasma P_{CO_2} were immediately measured using a Radiometer PHM 72 blood/gas monitor with associated G297 micro-pH unit/K497 reference electrode (Radiometer, Copenhagen) and associated E201 CO_2 electrode (Cameron Instrument), respectively. Simultaneously, a Corning model 965 CO_2 analyzer was then used to measure the total carbon dioxide content (T_{CO_2}) of the blood plasma. The red cell pellet was subsequently frozen in liquid nitrogen and thawed, destroying the cellular structure and allowing determination of intracellular erythrocyte pH (pHi) (Zeidler and Kim, 1977). Plasma lactate concentrations were measured on perchloric acid extracts following the method of Lowry and Passonneau

(1972). Measured values of true plasma T_{CO_2} and P_{CO_2} were used to calculate true plasma bicarbonate concentration ($[HCO_3^-]_{tpl}$) using the following rearrangement of the Henderson–Hasselbach equation:

$$[HCO_3^-]_{tpl} = T_{CO_2} - (\alpha_{CO_2} \times P_{CO_2}),$$

using values for α_{CO_2} determined according to Boutilier et al. (1984).

Statistical analyses

The means of the respiration and behavioural data for both species at rest were compared using an analysis of variance (ANOVA) when assumptions of equal variance and normality were met. A Kruskal–Wallis ANOVA on ranks was used when either assumption failed. For percentage data, arcsine-square-root transformations were performed (Zar, 1984). The factors of mass and sex did not significantly affect the variables measured and were not therefore included as covariates. Comparisons among rest and recovery, species and treatment were calculated using a three-way ANOVA with repeated measures. For the blood characteristics, a two-way ANOVA with repeated measures was used to determine the differences among species, treatments and time intervals. All data were analyzed for significance at the $P<0.05$ level using SigmaStat for all procedures, except the three-way repeated-measures ANOVA, which was analyzed using SPSS. All values presented are means ±1 S.E.M.

Results

Respiratory gas exchange

All three species relied primarily on air for O_2 uptake; however, both *A. spinifera* and *A. ferox* were able to acquire aquatic O_2 at a significantly greater rate than *T. scripta* (Fig. 1A). The total $\dot{V}_{O_2}$ of *T. scripta* was 62.7±7.8 ml kg^{-1} h^{-1}; twice the value reported by Belkin (1968). The total $\dot{V}_{O_2}$ of *A. ferox* was similar to that of *T. scripta* at 52.0±3.4 ml kg^{-1} h^{-1}, and *A. spinifera* had a significantly lower total $\dot{V}_{O_2}$ than the other two species of 21.6±2.0 ml kg^{-1} h^{-1} (H=23.6; P<0.0001). The total $\dot{V}_{O_2}$ of *A. spinifera* was similar to but slightly lower than the value of 28.6 ml kg^{-1} h^{-1} measured by Stone et al. (1992a). The ratio of aquatic $\dot{V}_{O_2}$ to total $\dot{V}_{O_2}$, a standard measure of an animal's degree of reliance on water for gas exchange, was significantly different among the three species (H=35.1; P<0.0001). *T. scripta* relied the least on aquatic $\dot{V}_{O_2}$, exchanging 5.1 % of the total O_2 *via* water, while *A. ferox* was intermediate (11.7 %) and *A. spinifera* wasthe most reliant on aquatic gas exchange (21.7 %).

Because of the greater solubility of CO_2 than O_2 in water, rates of aquatic CO_2 excretion ($\dot{V}_{CO_2}$) were approximately five times greater than those for aquatic $\dot{V}_{O_2}$. *A. spinifera* and *A. ferox* had a significantly higher aquatic $\dot{V}_{CO_2}$ than *T. scripta* (H=30.6; P<0.0001), whereas *T. scripta* and *A. ferox* had a significantly higher aerial $\dot{V}_{CO_2}$ than *A. spinifera* (H=23.3; P<0.0001) (Fig. 1B). Therefore, *A. ferox* maintained a high CO_2 excretion rate both aquatically and aerially, which resulted in the highest total $\dot{V}_{CO_2}$ of 64.1±6.0 ml kg^{-1} h^{-1} compared with 45.0±4.6 ml kg^{-1} h^{-1} for *T. scripta* (half the value measured by Jackson et al., 1976) and 29.0±3.0 ml kg^{-1} h^{-1} for *A. spinifera* (almost identical to the value measured by Stone et al., 1992a). With aquatic and aerial CO_2 excretion partitioned almost equally in *A. ferox*, the ratio of aquatic to total $\dot{V}_{CO_2}$ was 55.0 %. *T. scripta* relied the least on water for CO_2 excretion (27.7 %), while *A. spinifera* was the most reliant on water (81.8 %) (F=245.1; P<0.0001).

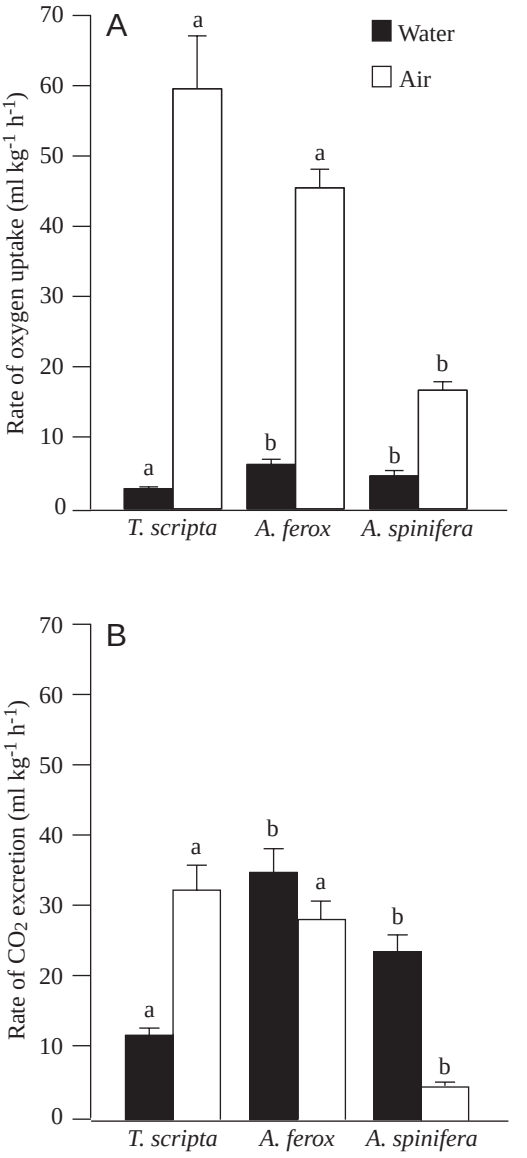

Fig. 1. Resting mean aquatic and aerial $\dot{V}_{O_2}$ (A) and $\dot{V}_{CO_2}$ (B) in *Trachemys scripta* (N=23), *Apalone ferox* (N=15) and *Apalone spinifera* (N=7). Letter groups indicate those species that are not significantly different from each other within each variable measured. Values are means + S.E.M.

Aquatic $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ in *T. scripta* did not change significantly in response to either exercise or forced submergence. Exercise did not have a significant effect on aquatic gas exchange in *A. ferox*; however, this was not true during forced submergence. *A.*

ferox demonstrated the ability to increase aquatic $\dot{V}_{O_2}$ (F=10.4; P<0.0036) and $\dot{V}_{CO_2}$ significantly (F=19.8; P<0.0003), each by twofold, during the submergence period.

Exercise and forced submergence both produced an increased aerial demand for O_2 in *T. scripta* and *A. ferox* (Fig. 2A). In *T. scripta*, aerial $\dot{V}_{O_2}$ increased by fourfold in response to exercise and forced submergence, and these values remained significantly higher than resting values until 30 min after each treatment (exercise F=76.82; P<0.0001; submergence F=188.9; P<0.0001). In *A. ferox*, aerial $\dot{V}_{O_2}$ increased by fourfold following exercise and by fivefold following forced submergence. The increase following forced submergence in *A. ferox* was significantly greater than that exhibited by *T. scripta* following the same treatment (F=4.86; P<0.042). Aerial $\dot{V}_{O_2}$ in *A. ferox* returned to resting values by 60 min post-submergence, but aerial $\dot{V}_{O_2}$ remained significantly higher than resting values even at 120 min post-exercise.

In addition to increased aerial O_2 demand, the rate of aerial CO_2 excretion increased in both species in response to exercise and forced submergence (exercise F=47.09; P<0.0001; submergence F=111.3; P<0.0001) (Fig. 2B). In *T. scripta*, aerial $\dot{V}_{CO_2}$ increased by fivefold following both exercise and forced submersion and returned to resting values by 60 min after each treatment. Aerial $\dot{V}_{CO_2}$ in *A. ferox* increased by sevenfold immediately after both exercise and forced submergence and returned to values for resting turtles by 60 min following each treatment. The increase following forced submergence in *A. ferox* was significantly greater than that exhibited by *T. scripta* following the same treatment (F=4.53; P<0.0036).

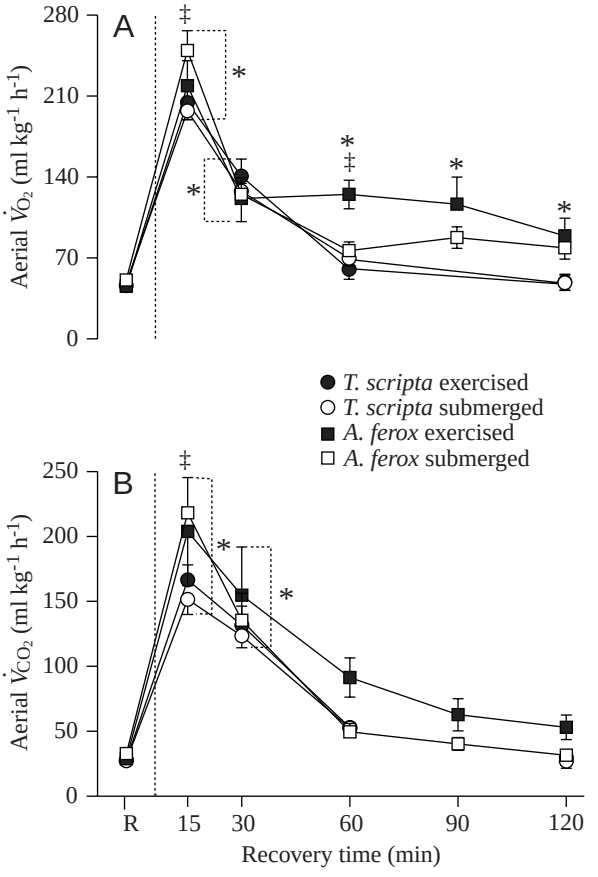

Fig. 2. Mean aerial $\dot{V}_{O_2}$ (A) and $\dot{V}_{CO_2}$ (B) in *Trachemys scripta* (N=8 exercise; N=8 forced submergence) and *Apalone ferox* (N=6 exercise; N=6 forced submergence) at rest (R) and after exercise and forced submergence. The dotted vertical line represents the time of application of the treatment. Asterisks indicate significant differences from corresponding resting values. The dotted brackets represent a grouping of data for the purpose of expressing significance. A double dagger (‡) denotes a significant difference between species at that corresponding time interval and treatment. Values are means ± S.E.M.

Ventilation and diving behaviour

At rest, each of the three species had a distinct ventilatory and diving pattern. Individual dives in *T. scripta* were short, with a mean duration of 5.3±0.6 min (Fig. 3A). In *A. ferox*,

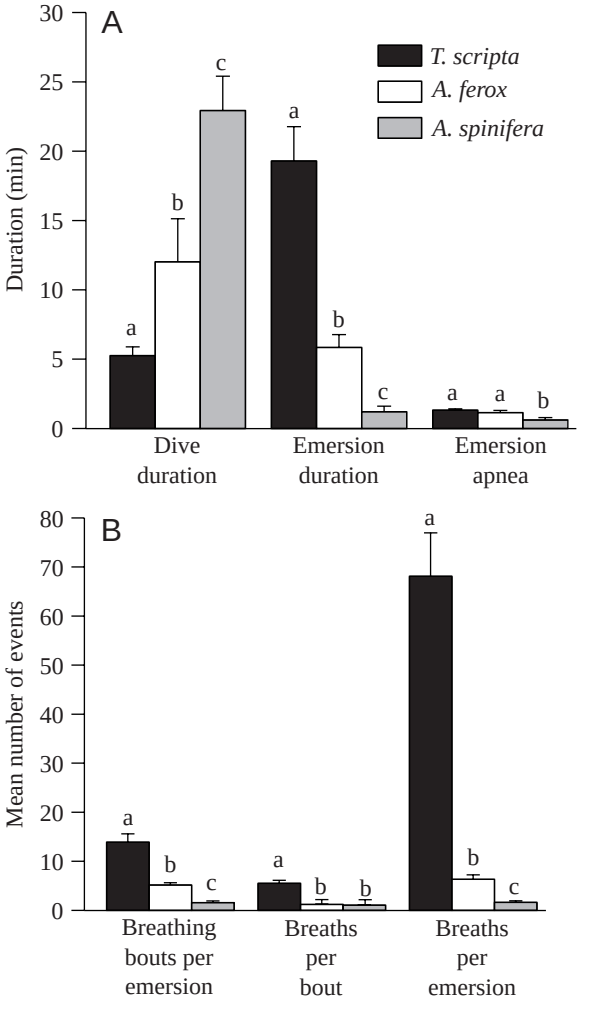

Fig. 3. (A) Mean durations of immersion and emersion behaviour and (B) mean number of ventilation behaviours in *Trachemys scripta* (N=23), *Apalone ferox* (N=15) and *Apalone spinifera* (N=7) at rest. Letter groups indicate those species that are not significantly different from each other within each variable measured. Values are means + S.E.M.

mean dive duration was significantly longer (12.0±3.1 min) (H=26.7; P<0.0001), and mean dive duration was longer still in *A. spinifera* (22.9±2.5 min, a value twice that measured by Stone et al., 1992b). Dive duration was not significantly altered in *T. scripta* or in *A. ferox* by either exercise or submersion (Table 1).

While dive duration increased significantly from the least aquatically dependent species (*T. scripta*) to the most aquatically dependent species (*A. spinifera*), the reverse trend was true for emersion duration. The mean emersion duration of *T. scripta* was significantly longer than that for *A. ferox*, and both these species had significantly longer emersion times than *A. spinifera* (H=32.3; P<0.0001). Emersion duration in *T. scripta* was unaffected by exercise and, although an 83 % increase was noted after forced submergence, it was not significant (Table 1). Emersion duration in *A. ferox* increased fourfold after forced submergence (H=11.5; P<0.009), but did not increase significantly after exercise.

Emersion in all species was characterized by intermittent bouts of breathing separated by periods of apnoea. *A. spinifera* displayed significantly shorter apnoeic bouts than both *T. scripta* and *A. ferox* (F=5.76; P<0.006) (Fig. 3A). Emersion apnoea was not significantly altered by the treatments except in *A. ferox* after exercise, in which the mean apnoeic bout length decreased by 58 % compared with the corresponding resting value (F=10.8; P<0.02) (Table 1).

Another diving behaviour measured was the ratio of total apnoeic time during a given period of emersion to total emersion time. This conveyed the proportion of breathing time *versus* apnoeic time when the turtle was at the surface. *A. spinifera* spent a greater proportion of emersion time breathing compared with the other two species, with 73.0±7.8 % of the emersion time allotted to apnoea (F=4.01; P<0.025). *T. scripta* and *A. ferox* both spent less time at the surface breathing, with 86.4±1.6 % and 88.3±2.6 % of the emersion time spent in apnoea, respectively. However, when *T. scripta* and *A. ferox* were subjected to exercise and forced submergence, the resultant increase in breathing frequency significantly reduced the ratio of apnoeic time to total emersion time.

The longer emersion durations in *T. scripta* allowed them to undergo a significantly greater number of breathing bouts per emersion period (13.9±1.7 *versus* 5.1±0.5 for *A. ferox*), and both performed significantly more breathing bouts per emersion period than *A. spinifera* (1.6±0.3) (H=32.0; P<0.0001) (Fig. 3B). The number of breathing bouts per emersion period did not change in *T. scripta* following either exercise or forced submersion (Table 1). However, *A. ferox* significantly increased the number of breathing bouts per emersion in response to exercise and forced submergence by factors of 5.5 and 5.8, respectively (H=17.4; P<0.0006).

At rest, *A. spinifera* and *A. ferox* were more similar in ventilatory characteristics compared to *T. scripta* (Fig. 3B). Both species retained a one-breath-per-bout behaviour that was unaltered by either submergence or exercise. *T. scripta* utilized a multi-breath bout, demonstrating a significantly greater number of breaths per bout than both *A. spinifera* and *A. ferox* (H=36.1; P<0.0001). In response to exercise, *T. scripta* significantly increased the number of breaths per bout by twofold (F=4.0; P<0.0172) (Table 1).

In *T. scripta* at rest, the number of breaths taken per emersion period was significantly greater than in *A. ferox*, and values for both these species were significantly greater than for *A. spinifera* (H=38.5; P<0.0001) (Fig. 3B). Following exercise and forced submersion, *T. scripta* increased the total number of breaths per emersion each by twofold (F=2.58; P<0.042) (Table 1). *A. ferox*, however, responded to exercise and forced submergence by increasing the number of breaths per emersion by factors of 5.5 and 5.8, respectively (F=7.58; P<0.0014). *T. scripta* exhibited significantly more breaths per emersion period than *A. ferox* for any given treatment.

The mean breath length (measured from the beginning of exhalation to the end of inhalation) of *T. scripta* (2.1±0.1 s) was

Table 1. *Mean durations and numbers of ventilation behaviours in* Trachemys scripta *and* Apalone ferox *at rest, during and after forced submergence and after exercise*

	T. scripta				*A. ferox*			
	Exercise		Submergence		Exercise		Submergence	
	Before	After	Before	After	Before	After	Before	After
Dive duration (min)	4.61±0.97	4.55±2.86	4.73±1.17	4.56±1.39	8.25±1.00	6.03±1.90	9.31±1.80	6.76±1.36
Emersion duration (min)	19.0±3.3	19.5±3.9	24.4±5.4	43.8±16.8	6.8±1.8‡	20.0±8.4	5.2±1.0‡	19.4±3.0*
Emersion apnea (min)	1.10±0.13	0.87±0.15	1.50±0.18	1.13±0.19	1.29±0.22	0.54±0.09*	1.17±0.33	0.65±0.09
Number of bouts per emersion	15.1±2.1	16.2±3.3	16.8±3.9	29.4±10.3	5.0±0.8‡	27.6±6.9*	5.4±0.8‡	31.6±7.7*
Number of breaths per bout	5.2±0.7	10.7±1.8*	5.8±1.4	6.3±0.8	1.1±0.1‡	1.1±0.1‡	1.2±0.2‡	1.3±0.3‡
Number of breaths per emersion	75.3±12.3	167.3±36.4*	76.4±16.3	163.3±40.7*	5.8±1.5‡	32.0±9.3‡,*	6.4±1.2‡	37.3±7.4‡,*

Values are means ± S.E.M., N=8 for *T. scripta*; N=6 for *A. ferox*.

Asterisks indicate significant differences from corresponding resting values; double daggers (‡) denote significant differences between species at corresponding treatment values.

significantly shorter than that for *A. ferox* and *A. spinifera* (4.7±0.6 s and 5.1±0.3 s, respectively) (H=29.1; P<0.0001). Mean breath length was not significantly altered by either of the treatments in *A. ferox* or *T. scripta*.

Blood acid–base status

In *A. ferox* and *T. scripta*, plasma pH (pHe) and intracellular pH (pHi) decreased significantly in response to exercise and forced submergence (Figs 4, 5). The acidosis produced by exercise was primarily metabolic in origin for both species, whereas the acidosis produced by forced submergence was almost completely of respiratory origin in *T. scripta* and mixed in *A. ferox* (see below).

Plasma pH in *T. scripta* decreased by 0.18 pH units in response to exercise, returning to resting values by 30 min (F=45.33; P<0.0001) (Fig. 4A). In *A. ferox*, pHe decreased by the same amount; however, resting values were not restored until 60 min post-exercise (Fig. 5A). Plasma pH in *T. scripta* and *A. ferox* decreased by 0.20 and 0.31 pH units, respectively,

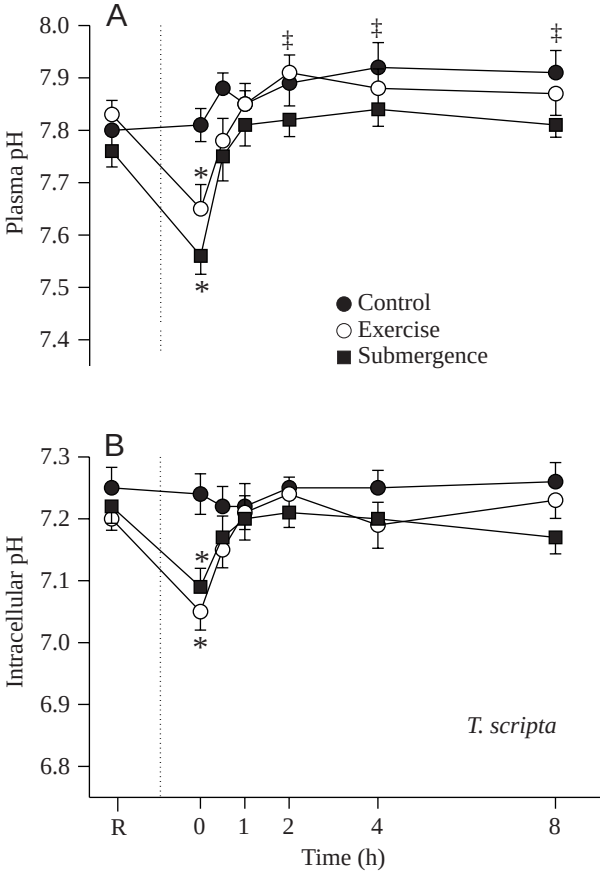

Fig. 4. Mean blood plasma pH (A) and intra-erythrocyte pH (B) in *Trachemys scripta* (N=7 control; N=9 exercise; N=10 forced submergence) at rest (R) and after exercise and forced submergence. The dotted vertical line represents the time of application of either treatment. Asterisks denote a significant difference from both the resting value and the corresponding control value. Double daggers (‡) indicate a significant difference from the resting value only. Values are means ± S.E.M.

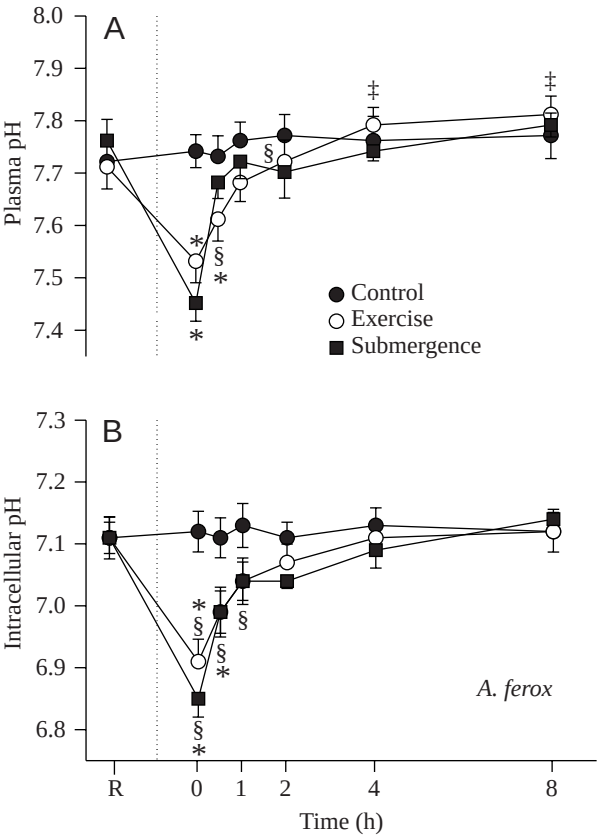

Fig. 5. Mean blood plasma pH (A) and intra-erythrocyte pH (B) in *Apalone ferox* (N=5 control; N=7 exercise; N=6 forced submergence) at rest (R) and after exercise and forced submergence. The dotted vertical line represents the time of application of either treatment. Asterisks denote a significant difference from both the resting value and the corresponding control value. Double daggers (‡) indicate a significant difference from the resting value only. § signs indicate a significant difference from the value for *Trachemys scripta* (see Fig. 4) at the corresponding treatment and time. Values are means ± S.E.M.

in response to forced submergence, and resting values returned by 30 min in each species (F=45.52; P<0.0001).

Intracellular erythrocyte pH also exhibited a significant decline after exercise in each species (F=24.08; P<0.0001) (Figs 4B, 5B). In *T. scripta*, pHi decreased by 0.15 pH units in response to exercise, with resting values returning by 30 min. In *A. ferox*, a similar decrease was noted which recovered by 60 min. *A. ferox* pHi values remained significantly lower than the corresponding *T. scripta* values at each sampling interval until 120 min post-exercise (F=13.3; P<0.0026). Intracellular pH in *T. scripta* decreased by 0.13 pH units in response to forced submergence, returning to resting pHi values by 30 min (F=24.26; P<0.0001). In *A. ferox*, pHi decreased by 0.26 units immediately after forced submergence and had recovered by 60 min. *A. ferox* pHi values remained significantly lower than the corresponding *T. scripta* values at each sample time until 120 min post-submergence.

As pH declined following exercise, a concurrent decrease in plasma bicarbonate concentration was also noted in each species (F=16.63; P<0.0001) (Figs 6A, 7A). Plasma

bicarbonate concentrations decreased by 25 % immediately following exercise in *T. scripta*, with resting values being achieved by 60 min post-exercise. In *A. ferox*, plasma bicarbonate concentration did not reach its maximal decrease of 25 % until 30 min post-exercise, but this also recovered by 60 min. Forced submergence had no effect on HCO_3^- concentrations in *T. scripta*. Thirty minutes post-submergence, a maximal decrease of 30 % in [HCO_3^-] was exhibited in *A. ferox*, and [HCO_3^-] did not return to resting levels until 240 min post-submergence (F=5.08; P<0.0002).

The partial pressure of CO_2 (P_{CO_2}) in the plasma did not change in either species after exercise (Figs 6B, 7B). Plasma P_{CO_2} did not change in *T. scripta* after forced submergence; however, there was a transient increase of 30 % in P_{CO_2} in *A. ferox* immediately after forced submergence.

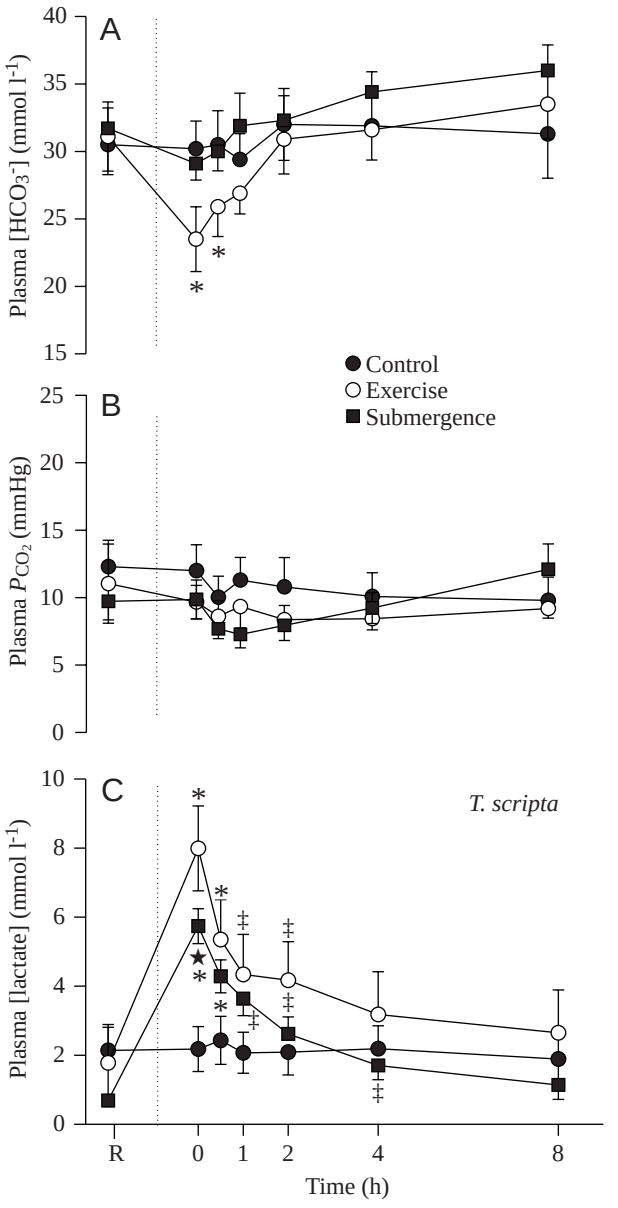

Fig. 6. Mean plasma bicarbonate concentration (A), plasma CO_2 partial pressure (B) and plasma lactate concentration (C) in *Trachemys scripta* (N=7 control; N=9 exercise; N=10 forced submergence) at rest (R) and after exercise and forced submergence. The dotted vertical line represents the time of application of either treatment. Asterisks denote a significant difference from both the resting value and the corresponding control value. Double daggers (‡) indicate a significant difference from the resting value only. A star indicates a significant difference between treatments at a given time interval. Values are means ± S.E.M. 1 mmHg=0.133 kPa.

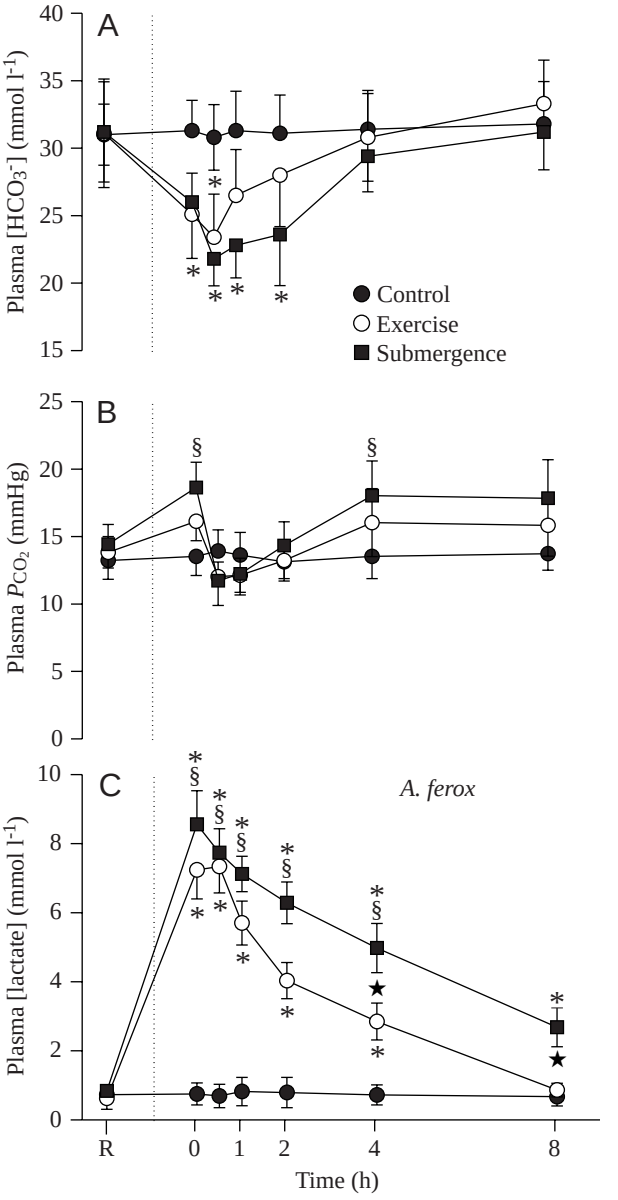

Fig. 7. Mean plasma bicarbonate concentration (A), plasma CO_2 partial pressure (B) and plasma lactate concentration (C) in *Apalone ferox* (N=5 control; N=7 exercise; N=6 forced submergence) at rest (R) and after exercise and forced submergence. The dotted vertical line represents the time of application of either treatment. Asterisks denote a significant difference from both the resting value and the corresponding control value. § signs indicate a significant difference from the value for *Trachemys scripta* (see Fig. 6) at the corresponding treatment and time value. A star indicates a significant difference between treatments at a given time interval. Values are means ± S.E.M. 1 mmHg=0.133 kPa.

With the significant decrease in blood pH, there was also a concurrent increase in plasma lactate concentration immediately following exercise (F=53.36; P<0.0001) (Figs 6C, 7C). In *T. scripta*, plasma lactate concentration increased by 6.21 mmol l^{-1}. Recovery in this species was variable after exercise; however, resting lactate concentrations returned by 240 min. An increase in lactate concentration of 6.61 mmol l^{-1} was observed in *A. ferox* following exercise, with recovery apparent by 480 min. In *T. scripta*, forced submergence increased plasma lactate concentration by 5.05 mmol l^{-1}. Significantly raised lactate levels were maintained following the forced submergence treatment in *T. scripta* until 240 min post-treatment. *A. ferox* exhibited a remarkable trend in that forced submergence produced an increase in lactate concentration of 7.72 mmol l^{-1}, the largest increase of the study (F=90.83; P<0.0001). During forced submergence, lactate levels in *A. ferox* were significantly higher than the corresponding values for *T. scripta* at each sampling time until 480 min post-treatment (F=17.73; P<0.001). Lactate levels in *A. ferox* remained six times higher than at rest at 480 min post-submergence.

Discussion

Respiratory gas exchange

Rest

The partitioning of oxygen uptake between air and water was unique for each species. These data, which were consistent with previous studies of *T. scripta* and *A. spinifera*, support the idea that among bimodal gas exchangers there is a spectrum of ability with regard to aquatic gas exchange that is related to overall reliance on water (Stone et al., 1992a). Absolute values for total and aquatic $\dot{V}_{O_2}$ in the present study were different from those reported previously for the same species (Belkin, 1968; Stone et al., 1992a), but these differences did not change the general pattern of how gas exchange was partitioned between air and water. Although *A. spinifera* and *A. ferox* had a similar aquatic $\dot{V}_{O_2}$, it accounted for a significantly larger proportion of aquatic gas exchange in *A. spinifera*. *A. ferox* is generally found in sluggish streams, lakes and ponds and may not, therefore, be able to rely on aquatic O_2 as much as *A. spinifera* (Mount, 1975). Slow-moving or stagnant water has a variable partial pressure of O_2 and is often hypoxic. It would not, therefore, be advantageous for *A. ferox* to have a high aquatic $\dot{V}_{O_2}$ if this source of O_2 were not constantly available. Furthermore, animals that have high aquatic $\dot{V}_{O_2}$ values could potentially lose O_2 from the blood if the animal were exposed to a hypoxic environment (Belkin, 1968). Randall et al. (1981) documented such a transfer of oxygen from blood to water *via* the gills when *Amia calva*, a bimodally breathing fish, was exposed to hypoxic water.

Aquatic $\dot{V}_{CO_2}$ has always been found to exceed $\dot{V}_{O_2}$ in bimodal gas exchangers, primarily because of the greater solubility of CO_2 in water (Wood and Lenfant, 1976). Again, even though the absolute values of total and aquatic $\dot{V}_{CO_2}$ in the present study differ from those reported previously, the partitioning pattern remains the same. Furthermore, the value of 27.7 % aquatic $\dot{V}_{CO_2}$ for *T. scripta*, a poor aquatic gas exchanger, may be more indicative of the minimal ability of aquatic turtles in general because this is consistent with aquatic $\dot{V}_{CO_2}$ values from another poor aquatic gas exchanger, *Chelydra serpentina* (30 %; B. Bagatto and R. P. Henry, unpublished observations). Jackson et al. (1976) reported an aquatic $\dot{V}_{CO_2}$ of 10.5 % for *T. scripta*; however, their total $\dot{V}_{CO_2}$ was more than twice that of the present study and was probably not a true resting value, which may have led to an underestimation of the importance of aquatic $\dot{V}_{CO_2}$.

Effects of submergence and exercise

In response to forced submersion, the first sign of respiratory plasticity was noted in *A. ferox* because this species increased both aquatic $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ by twofold. Increasing aquatic gas exchange may involve more frequent buccal pumping (Belkin, 1968) and/or increased capillary perfusion to the skin and buccal cavity, especially during exercise *via* increased cardiac output. The present study confirms that highly aquatic turtle species can alter their aquatic gas exchange and that this can be achieved more rapidly that previously reported (Belkin, 1968). Aquatic gas exchange remained minimal in *T. scripta* and did not change in response to either of the treatments. This confirms the aerial dependence of *T. scripta* and suggests that aquatic gas exchange is not plastic for every species; certainly not in species that are highly dependent on air.

Although *A. ferox* has the ability to increase cutaneous respiration during continued forced submergence, this probably did not occur during intense anaerobic exercise. Additionally, as *A. ferox* recovered from each of the treatments, direct access to air reduced the degree of utilization of aquatic gas exchange. This was especially apparent in *A. ferox* during recovery from forced submergence. During submergence, *A. ferox* increased both aquatic O_2 uptake and aquatic CO_2 excretion by twofold; however, these rates decreased to resting levels once access was given to air during recovery.

Forced submergence had a greater impact on *A. ferox* than on *T. scripta*, an unexpected result (Fig. 2). Because *A. ferox* ventilate their lungs far less frequently than *T. scripta*, we hypothesized that *A. ferox* could easily withstand forced immersion for 1 h. Apparently the 22 breaths h^{-1} are vital to resting metabolism; denying aerial respiration to *A. ferox*, even for 1 h, resulted in a large shift towards anaerobiosis as shown by the blood acidosis and increase in circulating lactic acid levels. Preliminary data using *A. spinifera* show the same trend even though this species only breathes approximately twice per hour. *Apalone* species appears to operate at the aerobic/anaerobic threshold, taking in enough O_2 to remain aerobic under resting conditions. Thus, a disturbance in the natural rhythm of ventilatory and diving behaviour in these highly aquatic species seems to force them into anaerobic respiration.

Ventilation and diving behaviour

Rest

The more dependent a turtle species was on aerial respiration, the longer it stayed at the surface and the shorter were its dives (Fig. 3). *A. spinifera* support a large percentage of their resting

metabolism *via* non-pulmonary avenues and have adopted a diving pattern similar to that of lungfish (Graham and Baird, 1982; Kramer, 1988), which come to the surface only for a single breath, then submerge again. *A. spinifera* have also evolved a one-breath bout, characteristic of highly aquatic species such as sea turtles *Chelonia mydas* (Butler et al., 1984) and sea snakes (Graham, 1974). This one-breath breathing pattern was also present in *A. ferox*; however, their increased reliance on aerial respiration was associated with increased numbers of breathing bouts per emersion and, thus, more breaths per emersion than *A. spinifera*. *T. scripta* was highly dependent on aerial respiration and, thus, displayed characteristics typical of terrestrial breathing patterns (a multi-breath bout).

Most reptiles undergo variable periods of apnoea (Wood and Lenfant, 1976). Mean lengths of apnoeic periods in *T. scripta* and *A. ferox* were 90 and 60 s, respectively. The mean emersion apnoea duration for *A. spinifera* was 36 s and was very similar to the value of 29 s measured by Stone et al. (1992b). As the capacity of a species for aquatic gas exchange increased, emersion times decreased (Stone et al., 1992b) and so did the duration of apnoea. It appears that the more efficient a species becomes at aquatic gas exchange, the more it approaches the condition observed in primitive lungfish; a one-breath bout and virtually no apnoeic period at the surface (Kramer et al., 1983; Kramer, 1988).

Agassiz (1857) noted that the more aquatic turtle species had significantly reduced lung volumes. In contrast, both *A. spinifera* and *A. ferox* had significantly longer breath durations than *T. scripta*, even after correction for differences in mass. Lung surface area and diffusion distance aspects aside, it seems that *A. spinifera* and *A. ferox* are able to ventilate more deeply as a result of having a less rigid outer shell structure and a reduced plastron. This would allow the visceral volume to vary to a greater extent, creating large intrapulmonary subatmospheric pressures, as found in *Chelydra serpentina* by Gaunt and Gans (1969). Although *T. scripta* have a larger lung volume per kilogram, their shell morphology allows them to produce only shallow breaths, requiring them to ventilate more quickly, as is the case for other strictly terrestrial species (Gans and Hughes, 1967). Thus, a one-breath bout is probably related to more than just a high level of aquatic gas exchange. Anatomical as well as other physiological variables may also relate to the existence of this seemingly unalterable phenomenon.

Another explanation of the utilization of the one-breath bout may involve the higher blood levels of CO_2 in terrestrial reptiles. Because *A. spinifera* and *A. ferox* are able to transfer a significant proportion of CO_2 into water, they may not need to increase ventilation frequency in order to void CO_2 aerially. *T. scripta*, as well as other terrestrial reptiles, may use many short breaths to reduce blood CO_2 levels, while concurrently obtaining the required amount of O_2 (Rahn and Howell, 1976).

Effects of submergence and exercise

Because *A. ferox* did not alter their apnoeic period durations following forced submergence, compared with at rest, the emersion durations had to increase to allow for the increase in the number of ventilation bouts per emersion (Table 1). However, *A. ferox* displayed a significant decrease in emersion apnoea duration after exercise, which may be the reason that the increase in emersion duration after exercise was not significant. Although there were no significant differences in diving behaviour after either treatment, *T. scripta* spent almost twice as much time at the surface after forced submergence, most likely to accommodate the increased number of bouts per emersion.

A. ferox retained a one-breath-per-bout pattern at all times; thus, the response of increasing breath frequency was simply a reflection of increased bout frequency (Table 1). This indicates that the characteristic of a one-breath bout is not plastic or reserved only for resting metabolism. These aquatic turtles have evolved a trait that is seemingly unalterable, even under extreme physiological conditions. This species-specific number of breaths per breathing bout was also documented for *Kinosternon leucostomum* and *Staurotypus triporcatus* following forced submergence (B. Bagatto, B. Hange and R. P. Henry, unpublished results). It was therefore interesting to note that exercise resulted in a significant increase in the number of breaths per bout in *T. scripta*. It is not known whether exercise would have a similar effect on breathing bouts in other species highly dependent on air. Perhaps exercise is so stressful that the increase in breathing frequency required for recovery causes breathing bouts to fuse, creating one long bout. This was certainly the trend in *T. scripta* immediately following exercise.

Blood acid–base status

Exercise

The magnitudes of the decreases in pH and $[HCO_3^-]$ were similar in both *T. scripta* and *A. ferox*, indicating that brief intense anaerobic exercise had a similar physiological effect. It was apparent that the high aquatic to aerial gas exchange ratio in *A. ferox* neither delayed the onset of anaerobiosis nor aided the recovery from exhaustive exercise. In response to exercise, *T. scripta* was better able to cope with the metabolic acid load. Because of the compartmentalization of lactate within the body and long lag periods associated with intercompartmental transfers (especially from muscle to blood), blood lactate concentrations cannot be used to quantify the total amount of anaerobic metabolism (Bennett, 1994). However, relative levels of anaerobiosis can be compared between species and treatments using blood lactate level as an indicator, assuming equal perfusion and equal exchange rates between compartments. As with pH and $[HCO_3^-]$, exercise produced similar lactate levels in *T. scripta* and *A. ferox*. *T. scripta* metabolized lactate to within resting levels by 4 h post-exercise, whereas *A. ferox* did not reduce plasma lactate to resting levels until 8 h post- exercise. There are no published data describing the total blood volume of species in the genus *Apalone*; however, anecdotal evidence suggests that it is not significantly different from that of *T. scripta*. Therefore, assuming that exercise produced a similar amount of lactate in both species (even though relative levels in the blood plasma were slightly higher in *T. scripta*), *A. ferox* did not have the ability to remove plasma lactate as effectively as *T. scripta*.

Forced submergence

Both *A. ferox* and *T. scripta* utilized anaerobic metabolism during the forced submergence period. It was surprising that *A. ferox* could not withstand the short duration of forced submergence, which produced the largest pH decrease of any treatment. Even though aquatic gas exchange was significantly increased during forced submergence, this was apparently not sufficient to sustain aerobic metabolism.

Plasma bicarbonate level decreased profoundly in *A. ferox* following forced submergence; however, *T. scripta* maintained resting levels of bicarbonate throughout recovery, further supporting the suggestion that this treatment did not create a long-lasting physiological disturbance. This indicates that *T. scripta* may have a larger reservoir of HCO_3^- with which to buffer the by-products of anaerobic metabolism. Smith (1929) noted that freshwater turtles, such as *T. scripta*, possess a large volume of coelomic fluid with a pH more alkaline than plasma and having a HCO_3^- concentration three times that of plasma. Because *T. scripta* have a higher volume to surface area ratio, this coelomic fluid may be present in greater quantity and may be utilized to a greater extent in buffering acidoses produced by anaerobic respiration. *A. ferox*, having a large surface to volume ratio, is equipped for increases in aquatic gas exchange, indicating that the role of coelomic fluid as a buffer may have been reduced or lost.

The hypothesized advantage that *A. ferox* had over *T. scripta* in avoiding anaerobic respiration via non-pulmonary respiration during forced submergence was not confirmed. Even though *A. ferox* doubled its rate of aquatic CO_2 excretion during forced submergence, an increase in plasma P_{CO_2} was nonetheless observed (Fig. 7). Furthermore, since the resting aerial CO_2 excretion rate in *A. ferox* was as high as that in *T. scripta*, perhaps the increase in aquatic CO_2 excretion was not adequate to compensate for internal CO_2 produced by metabolism during forced submergence.

Lactate concentrations in *A. ferox* were almost double those of *T. scripta* after forced submergence. This supports the observation that *T. scripta* is able to tolerate and recover from long bouts of anaerobiosis, even at warmer temperatures (Belkin, 1968). This also confirms the metabolic portion of the acidosis created in *A. ferox* during and after forced submergence. Perhaps resting lactate levels indicate the relative importance of anaerobiosis in each species. Because the resting levels of lactate in *T. scripta* are approximately four times those in *A. ferox*, shorter bouts of anaerobiosis may not affect *T. scripta* as they would *A. ferox*. Frequent periods of lactate production and catabolysis may allow *T. scripta* to tolerate and efficiently metabolize higher concentrations of lactate (Robin et al., 1964, 1981).

The marked differences in the effects of forced submergence between species may also be related to hibernation. It has been documented that *Chrysemys picta*, a close relative of *T. scripta*, undergoes bouts of severe anaerobiosis during hibernation that allow this species to remain submerged for periods of up to 6 months (Ultsch and Jackson, 1982). Although metabolic depression is critically involved in surviving hibernation (Ultsch et al., 1985), massive amounts of lactic acid are nonetheless produced (Ultsch and Jackson, 1982, 1995). *A. ferox*, in contrast, has been documented to increase its aquatic oxygen uptake in response to decreasing water temperature and concurrent increasing oxygen solubility (S. Prassack, B. Bagatto and R. Henry, unpublished results). It is not known whether *A. ferox* undergoes anaerobic metabolism during the winter, but it is possible that gas exchange to support resting metabolism may be almost exclusively *via* the water. Given this, the severe anaerobic bouts that *T. scripta* experience during hibernation are likely to give them a physiological advantage when dealing with anaerobic respiration at any other time.

The findings of this study aid in defining more clearly the extent to which turtles rely on the aquatic environment for gas exchange. Even though the ability to increase aquatic gas exchange in response to forced submergence may be universal in species highly dependent on aquatic respiration, the resultant metabolic demands created by exercise and submergence force these species to revert to aerial gas exchange. Members of the family Trionychidae have evolved a remarkable ability to extract O_2 from and void CO_2 into the aquatic medium; however, this appears to be an integral part of respiration only under resting conditions.

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