Corrigendum

Reese, S. A., Ultsch, G. R. and Jackson, D. C. (2004). Lactate accumulation, glycogen depletion, and shell composition of hatchling turtles during simulated aquatic hibernation. *J. Exp. Biol.* **207**, 2889-2895.

In Fig. 3, the ratio of shell CO_2 to whole-body lactate for adult *Chrysemys picta bellii* was calculated incorrectly. Recalculating the ratio using the correct numbers yielded a smaller ratio, although it does not change any of the conclusions presented in the text. The figure is reprinted below; no changes have been made to the text.

The authors apologise for any inconvenience this may have caused.

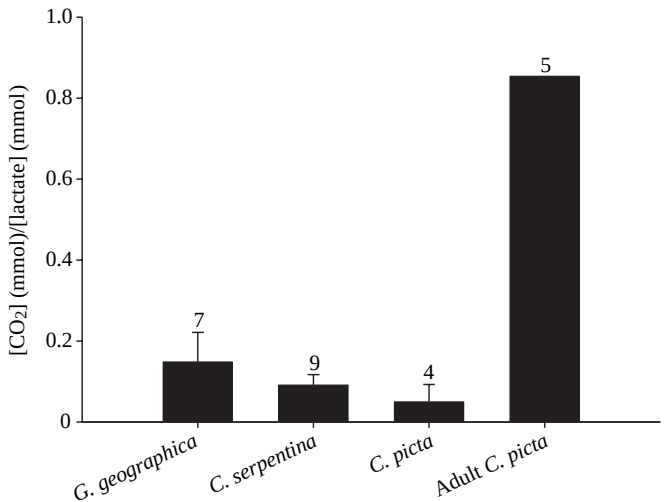

Fig. 3. Shell CO_2 mobilization (mean ± S.E.M.) as a function of whole-body lactate for hatchling *Chelydra serpentina*, *Chrysemys picta bellii* and *Graptemys geographica* and adult *Chrysemys picta bellii* submerged in anoxic water at 3°C. Sample sizes are listed above the bars. Data for adult *Chrysemys picta bellii* are from Warburton and Jackson (1995).

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Lactate accumulation, glycogen depletion, and shell composition of hatchling turtles during simulated aquatic hibernation

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Summary

We submerged hatchling western painted turtles *Chrysemys picta* Schneider, snapping turtles *Chelydra serpentina* L. and map turtles *Graptemys geographica* Le Sueur in normoxic and anoxic water at 3°C. Periodically, turtles were removed and whole-body [lactate] and [glycogen] were measured along with relative shell mass, shell water, and shell ash. We analyzed the shell for [Na^+], [K^+], total calcium, total magnesium, P_i and total CO_2. All three species were able to tolerate long-term submergence in normoxic water without accumulating any lactate, indicating sufficient extrapulmonary O_2 extraction to remain aerobic even after 150 days. Survival in anoxic water was 15 days in map turtles, 30 days in snapping turtles, and 40 days in painted turtles. Survival of hatchlings was only about one third the life of their adult conspecifics in anoxic water. Much of the decrease in survival was attributable to a dramatically lower shell-bone content (44% ash in adult painted turtles *vs.* 3% ash in hatchlings of all three species) and a smaller buffer content of bone (1.3 mmol g^{-1} CO_2 in adult painted turtles *vs.* 0.13–0.23 mmol g^{-1} CO_2 in hatchlings of the three species). The reduced survivability of turtle hatchlings in anoxic water requires that hatchlings either avoid aquatic hibernacula that may become severely hypoxic or anoxic (snapping turtles), or overwinter terrestrially (painted turtles and map turtles).

Key words: hatchling turtle, lactate, buffering, hibernation physiology, anoxia, shell composition.

Introduction

North American amphibians and reptiles encounter extended periods of freezing temperatures in the northern areas of their habitat ranges. Adult freshwater turtles escape these freezing temperatures by hibernating aquatically. Aquatic hibernation, however, has its own suite of physiological challenges and, depending on the hibernaculum, the challenges can be significant. Western painted turtles *Chrysemys picta bellii* Schneider and snapping turtles *Chelydra serpentina* L. are found in most permanent bodies of water within their range and can be exposed to hypoxia (low O_2) or anoxia (no O_2) during winter submergence (Crawford, 1991). Map turtles *Graptemys geographica* Le Sueur, on the other hand, have rather restricted habitats within the northern portions of their range, being found mostly in large lakes and rivers. Recent studies suggest that their restricted habitat may be a function of their inability to tolerate anoxic environments (Crocker et al., 2000; Reese et al., 2001).

Differences in anoxia tolerance can affect habitat selection in adult turtles and may influence life history traits of hatchling conspecifics. Turtles lay eggs in shallow nests on land, which hatch in late summer or early fall. Snapping turtle hatchlings emerge immediately after hatching and move to water (Congdon et al., 1987; Costanzo et al., 1995, 1999), where they spend their first winter submerged. In contrast, map and painted turtle hatchlings remain in the nest, surviving the freezing temperatures of winter *via* supercooling or freeze-tolerance, and emerge from the nest the following spring (map turtles: Costanzo et al., 2001b; Pappas et al., 2000; Baker et al., 2003; painted turtles: Packard, 1997; Weisrock and Janzen 1999). Delayed emergence by hatchlings has been viewed as an advantage, allowing young animals to minimize exposure to predators during late summer and early autumn, when resources are in decline. The hatchlings then enter the water in spring, when resources are increasing in quality and abundance, allowing the turtles to grow rapidly (Wilbur, 1975; Gibbons and Nelson, 1978).

We hypothesized that hatchling turtles spending their first winter aquatically possess similar abilities for dealing with potential hypoxia/anoxia as previously described for adults. We also hypothesized that overwintering in the nest may be an evolutionary response to the relatively low anoxia tolerance of hatchlings, and that those species that do not overwinter terrestrially are either at risk of death during the first winter, or must hibernate in a different microenvironment than adults.

Materials and methods

Animals

We obtained *Chrysemys picta* Schneider, *Chelydra serpentina* L. and *Graptemys geographica* Le Sueur within 2 weeks of hatching from Michigan, Indiana and Minnesota. Hatchlings were housed in an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved aquatic facility at 20°C, with access to basking platforms illuminated by full spectrum lighting on a 12 h:12 h D:L cycle, and fed commercial turtle pellets (Reptomin) daily. Following reabsorption of the yolk, animals were placed in 5 cm of water in a temperature-controlled chamber at 20°C and cooled by 1°C per day to 3°C, where they were maintained for 1 week. Following acclimation to 3°C hatchlings were randomly assigned to an experimental treatment. Turtles assigned as control animals were immediately sampled as described below.

There were three experimental treatments, all at 3°C, for hatchling turtles. Containers of similar dimensions were used for each treatment and all treatments were run concurrently. The first group was submerged, without access to air, in air-equilibrated water (P_{O_2}=158 mmHg). The second group was forcibly submerged in nitrogen-equilibrated water, also without access to air (P_{O_2}<5 mmHg). The final group was placed in sealed containers (except for inlet and outlet ports) and humidified nitrogen was passed through the containers to maintain a gaseous anoxia. Turtles were checked daily to determine survival. Animals were removed on days 5, 10, 15, 20, 25, 30 and 40 (N=5–15) of aquatic or gaseous anoxic exposure for sampling, as described below. Animals submerged in normoxic water were sampled on days 10, 25, 50, 75, 100, 125 and 150 (N=5–15).

Lactate and glycogen

Animals used for the determinations of whole-body concentrations of lactate and glycogen were removed from the experimental treatment, immediately frozen in liquid nitrogen, and stored at –80°C until analysis. To measure whole body lactate concentrations, animals were homogenized (Virtis, Gardiner, NY, USA) in 0.6 mol l^{-1} perchloric acid (5× volume to mass) and a 0.2 ml sample of the homogenate was saved for glycogen analysis (see below). The remainder of the homogenate was centrifuged at 4°C (3000 ***g*** for 10 min, Beckman GS-15R, Fullerton, CA, USA), the supernatant decanted and filtered, and the filtrate centrifuged at 4°C (12 000 ***g*** for 10 min, Beckman GS-15R). The supernatant from the second centrifugation was decanted and used for the determination of lactate and free glucose concentrations (YSI 2300 Stat Plus-D lactate/glucose analyzer, Yellow Springs, OH, USA).

Glycogen was determined with amyloglucosidase as described by Keppler and Decker (1974). The sample of homogenate for glycogen analysis was incubated (2 h at 40°C with continuous shaking in stoppered test tubes) with 0.1 ml of a 1 mol l^{-1} $KHCO_3$ and 2.0 ml of a 10 mg ml^{-1} amyloglucosidase (Sigma #A-3514, St Louis, MO, USA) solution. The amyloglucosidase was suspended in a 0.2 mol l^{-1} acetate buffer, pH 4.8. The incubation was stopped by adding 1.0 ml of cold 0.6 mol l^{-1} perchloric acid. The incubated solution was centrifuged (10 000 ***g*** for 15 min) and the supernatant analyzed for glucose (YSI 2300 Stat Plus-D lactate/glucose analyzer). Glycogen content was calculated as the glucose concentration measured by this method minus the free glucose concentration measured as above.

Shell analyses

Turtles used for shell analyses were removed from the treatment, patted dry and weighed. Animals were then killed by decapitation and pithing, and the plastron and carapace were dissected out and weighed. The shells were placed in a drying oven and dried to constant mass. Dried shells were randomly assigned to carbonate analysis or ion analysis. Dried shell was powdered at liquid nitrogen temperatures (SPEX 6700 freezer mill, Metuchen, NJ, USA) and carbonate content was measured using an incubation system previously described (Jackson et al., 1999). Pre-weighed shell powder was placed in a custom sample chamber that had two compartments, the contents of which could be combined without breaking a seal. Powder was added to 15 ml of 2 mol l^{-1} HCl without opening the chamber, liberating any carbonate as CO_2 gas. Nitrogen gas was humidified, passed through the chamber, dried, and then analyzed for CO_2 content (Applied Electrochemistry CD-3A CO_2 analyzer, Naperville, IL, USA). The CO_2 analyzer was calibrated with a precision gas (verified by analysis with a Scholander 0.5 cc analyzer), the signal digitized (Biopac MP100, Goleta, CA, USA), and the resultant trace integrated (Acknowledge 3.7) to determine total CO_2 content of the gas passing through the system. Flow rate through the system was measured using a Brooks Vol-U-Meter (Hatfield, PA, USA).

For ion analyses, dried shell was ashed (24 h at 450°C, Thermolyne 1300 furnace, Dubuque, IA, USA) and weighed. The ash was dissolved in 1 mol l^{-1} HCl (20× volume to mass). The resultant solution was used to measure $[Na^+]$ and $[K^+]$ (Instrumentation Laboratory 943 flame photometer, Lexington, MA, USA). Total magnesium was measured following a further dilution to 60× (Perkin-Elmer 280 atomic absorption spectrophotometer, Boston, MA, USA). Total calcium (Perkin-Elmer 280 atomic absorption spectrophotometer) and inorganic phosphates (Sigma kit 670-A with a Milton Roy Spectronic 601 spectrophotometer) were measured following a dilution to 1800×.

Statistics

Data were analyzed with STATISTICA ’99 edition (Statsoft Inc., Tulsa, OK, USA). Analysis of covariance (ANCOVA) was used to compare lactate accumulation and glycogen depletion among species with time and mass as covariates. Kruskal–Wallis analysis of variance (ANOVA) or Mann–Whitney U tests (where appropriate) were used to test end-point determinations in [lactate], [glycogen], energy utilization, shell characteristics and shell composition. *Post-*

hoc comparisons used Tukey's HSD for unequal *N* values. Significance was accepted at the $P<0.05$ level and all values are mean ± S.E.M. The University of Alabama and the Brown University Internal Animal Care and Use Committees (IACUC #26-03) approved this study.

Results

Metabolites

None of the hatchlings in normoxic water accumulated lactate during 150 days of submergence (Fig. 1A). *Chelydra serpentina* had higher [glycogen] than *C. picta* and *G. geographica*. The rate of glycogen depletion was lower in C. *picta* than *C. serpentina* or *G. geographica* (Fig. 1B). After 150 days of submergence *C. serpentina* had utilized 45% of their glycogen and *C. picta* utilized 28%. *Graptemys geographica* utilized 53% of their glycogen stores after only 125 days submerged.

Hatchling *Chrysemys picta* survived 40 days submerged in anoxic water, *Chelydra serpentina* survived 30 days and *Graptemys geographica* survived 15 days. All of the animals submerged in anoxic water accumulated lactate, and at similar rates (Fig. 1A), but to different levels by the survival limit (3.5±0.1 mg g^{-1} in *G. geographica*, 5.5±0.1 mg g^{-1} in *C. serpentina*, and 6.7±0.2 in *C. picta*) (15, 30 and 40 days, respectively; Fig. 1A). When held in gaseous nitrogen, lactate accumulated more rapidly than during anoxic submergence (0.36±0.03 *vs.* 0.24±0.01 mg g^{-1} day^{-1} in *C. picta*, 0.39±0.01 *vs.* 0.28±0.02 mg g^{-1} day^{-1} in *G. geographica*, and 0.43±0.02 *vs.* 0.33±0.01 mg g^{-1} day^{-1} in *C. serpentina* after 10 days of anoxic exposure) and survivability decreased (maximum survival was 10 days for *G. geographica*, 20 days for *C. serpentina*, and 30 days for *C. picta* in gaseous anoxia). Anoxic glycogen utilization rates were 10× faster than normoxic rates (Fig. 1B). *Graptemys geographica* had used 48%, *Chelydra serpentina* used 67%, and *Chrysemys picta* used 66% of their glycogen by the limits of survival. Utilization rates were slower in *C. picta* than in *C. serpentina* or *G. geographica*.

Shell

The amount of shell (as % of wet body mass) differed among species with *G. geographica* (23.3±0.4% shell) higher than *C. serpentina* (18.3±0.3%) or *C. picta* (19.4±0.4%; Fig. 2). There were some differences in the composition of shell among species of hatchling turtles. *Chelydra serpentina* had higher water content (80.0±0.3%) and lower organic content (16.9±0.4%) than *C. picta* (74.3±0.7% and 22.9±0.9%, respectively), which was higher and lower, respectively, than in *G. geographica* (69.4±0.4% and 27.2±0.6%, respectively; Fig. 2). However, ash content was the same among hatchlings (3.0%; Fig. 2).

Shell $[Na^+]$, $[K^+]$ and total Mg were at similar levels in control animals, but *G. geographica* had higher total Ca and $[P_i]$ (Tables 1 and 2). Total CO_2 was highest in control *C. serpentina*. Submergence in normoxic water for 75–81 days had little impact on shell composition. $[P_i]$ tended to increase in all three species, *C. picta* had elevated $[Na^+]$ and $[K^+]$, and *C. serpentina* had elevated total Ca. Shell CO_2 was unaffected by normoxic submergence.

Nitrogen exposure did not affect shell composition any differently than submergence in anoxic water, except for $[K^+]$ in *C. serpentina*, which was elevated after anoxic submergence (Table 1). Anoxic treatment had no dramatic effects on shell

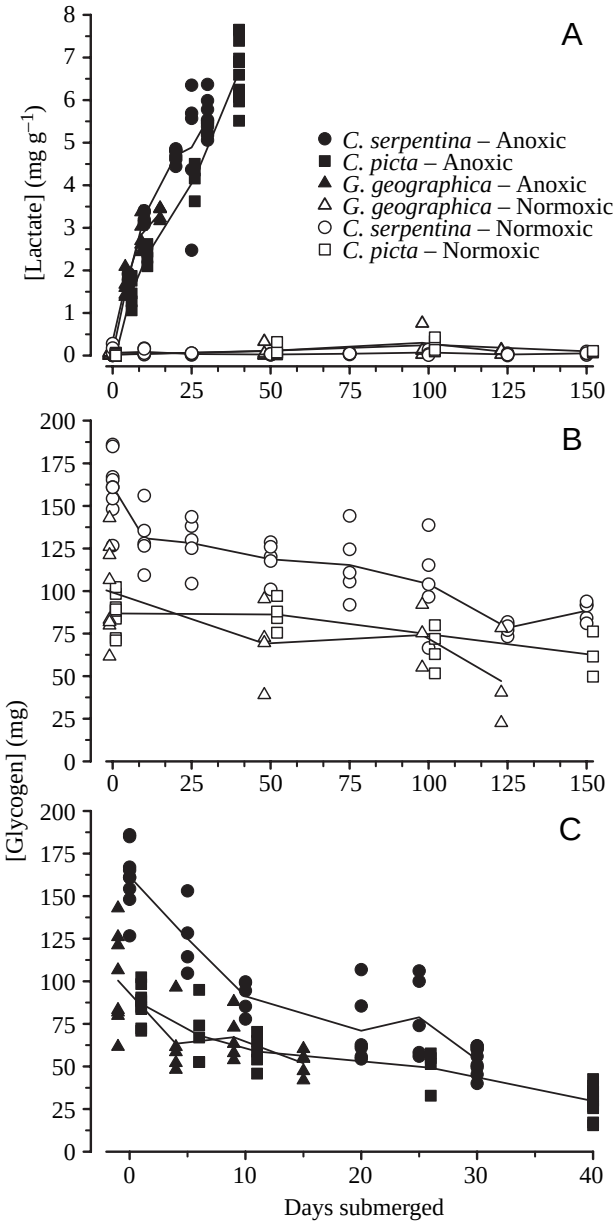

Fig. 1. (A) Whole-body [lactate] of hatchling *Chelydra serpentina* (circles), *Chrysemys picta* (squares) and *Graptemys geographica* (triangles) submerged in normoxic (open symbols) and anoxic (closed symbols) water at 3°C. (B) Whole-body glycogen of hatchling *Chelydra serpentina* (circles), *Chrysemys picta* (squares) and *Graptemys geographica* (triangles) submerged in normoxic water at 3°C. Glycogen levels were corrected *via* ANCOVA to a common mass (7.5 g). (C) As B for animals in anoxic water.

ionic composition. *Chelydra serpentina* had elevated total Ca and [P_i] after 31 days of anoxia and *Chrysemys picta* had elevated [P_i]. Total CO_2 decreased to similar levels in all three species after anoxia treatment.

Discussion

Physiological considerations

Hatchling turtles submerged in normoxic water maintained near normal homeostasis even over prolonged periods (125–150 days). The turtles were able to extract enough O_2 from the water *via* extrapulmonary mechanisms to remain aerobic throughout their cold submergence, as indicated by their low [lactate]. In contrast, adult *C. picta* and *C. serpentina* submerged in normoxic water at 3°C could not extract sufficient O_2 to remain entirely aerobic (Ultsch and Jackson, 1982; Jackson et al., 2000; Reese et al., 2002; S. A. Reese, C. E. Crocker, D. C. Jackson and G. R. Ultsch, unpublished data). The ability of hatchlings to remain aerobic during submergence, while their adult conspecifics cannot, may stem from a more favorable surface area to volume ratio, allowing the turtles to extract more O_2 per gram of animal, rather than a more favorable metabolic state, since smaller animals typically have a higher mass-specific metabolism (Sievert et al., 1988; D. E. Warren and D. C. Jackson, manuscript submitted for publication), although ontogenetic changes to the diffusion capacity of the gas exchange surfaces (e.g. skin) may also influence this difference. The [glycogen] did not appear to be limiting during submergence in normoxic water, as the animals had glycogen remaining even after 125–150 days. The glycogen utilization rate was lower in *C. picta* than in the other species, which may help explain the difference in survival ability of anoxic submergence, at least between *C. picta* and *C. serpentina* (40 days *vs.* 30 days, respectively). The difference seen in glycogen utilization may represent a difference in metabolic rate due to a difference in size (snapping turtle hatchlings are 2–3 times larger) or to inherent

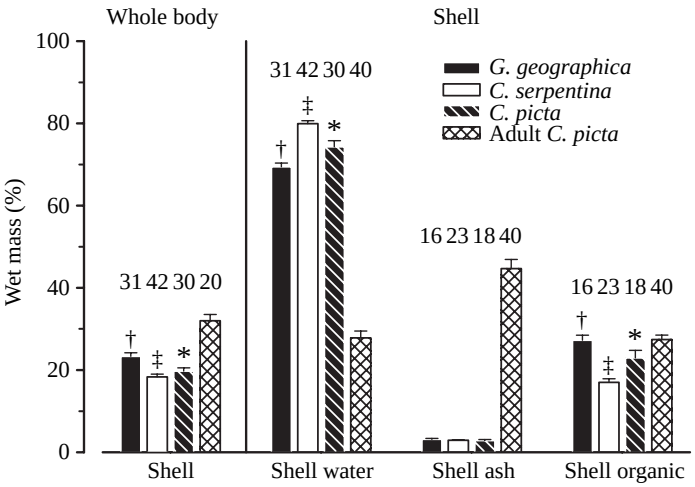

Fig. 2. Relative values (mean ± S.E.M.) of shell, shell water, shell ash and shell organic content of hatchling *Chelydra serpentina*, *Chrysemys picta* and *Graptemys geographica*, and adult *Chrysemys pict*. Different symbols indicate a significant difference among species. Sample sizes are listed above the bars. Data for adult *Chrysemys picta* (% shell) are from Jackson et al. (1996) and shell composition from Jackson et al. (2000).

Table 1. *Shell [Na^+], [K^+] and total Mg of hatchling* Graptemys geographica, Chelydra serpentina *and* Chrysemys picta, *and adult* C. picta *with air access (controls), submerged in normoxic or anoxic water, and exposed to nitrogen at 3°C*

		Hatchling			Adult
		G. geographica	*C. serpentina*	*C. picta*	*C. picta*
[Na^+] (μmol g^{-1} wet mass shell)					
	Air-access	46.2±13.3^{a} (5)	42.8±14.9 (8)	38.4±3.5^{a} (5)	
	Normoxic water	36.9±9.0^{a} (5)	41.5±16.7 (5)	66.2±32.0^{b} (2)	120.8±1.4 (5)
	Anoxic water		59.2±2.9 (5)	54.5±14.1^{b,†} (5)	96.9±2.7 (5)
	Nitrogen	20.0±7.6^{b,*} (5)	51.1±5.0 (5)	52.6±11.1^{b,†} (6)	
[K^+] (μmol g^{-1} wet mass shell)					
	Air-access	3.3±1.0^{a} (5)	6.1±4.7^{a} (5)	2.6±0.8^{a} (5)	
	Normoxic water	4.1±2.5^{a} (5)	6.1±3.7^{a} (5)	9.3±28.9^{b} (2)	4.3±0.2 (5)
	Anoxic water		10.3±1.7^{b,} (5)	5.4±4.6^{a,b,†} (5)	4.6±0.5 (5)
	Nitrogen	0.8±0.3^{b,*} (5)	4.3±0.8^{a,} (5)	5.4±2.3^{a,b,†} (6)	
Total Mg (μmol g^{-1} wet mass shell)					
	Air-access	11.4±2.2 (5)	9.2±0.7 (5)	11.0±2.2 (5)	
	Normoxic water	9.8±1.0 (5)	10.0±1.1 (5)	9.1±10.5 (2)	71.3±2.0 (5)
	Anoxic water		9.5±0.7 (5)	9.1±2.4 (5)	69.3±1.1 (5)
	Nitrogen	8.9±0.6 (5)	9.0±0.6 (5)	9.2±1.4 (6)	

Values are means ± S.E.M., *N* in parentheses.

Different superscript letters indicate significant differences within a species; *,†significant differences among hatchling species.

Graptemys geographica were maintained in anoxic water or nitrogen for 15 days and submerged in normoxic water for 75 days. *Chelydra serpentina* and *Chrysemys picta* hatchlings were kept anoxic for 31 days and submerged in normoxic water for 81 days.

Data for adult *Chrysemys picta* anoxic for 90 days are from Jackson et al. (2000).

Table 2. *Shell total Ca, [Pi] and CO_2 of hatchling* Graptemys geographica, Chelydra serpentina *and* Chrysemys picta, *and adult* C. picta *with air access (controls), submerged in normoxic water or anoxic water, and exposed to nitrogen at 3°C*

		Hatchling			Adult
		G. geographica	*C. serpentina*	*C. picta*	*C. picta*
Total Ca (μmol g^{-1} wet mass shell)					
	Air-access	240.2±47.3* (5)	171.7±24.3^{a,†} (5)	172.1±50.4† (5)	
	Normoxic water	265.4±35.5 (5)	254.6±35.5^{b} (5)	183.1±268.3 (2)	3267.0±122.1 (5)
	Anoxic water		249.3±21.2^{b} (5)	240.0±77.4 (5)	3487.1±260.0 (4)
	Nitrogen	246.5±28.6 (5)	245.8±17.9^{b} (5)	224.5±33.5 (6)	
[P_i] (μmol g^{-1} wet mass shell)					
	Air-access	165.4±29.0* (5)	120.4±24.9^{a,†} (5)	115.8±30.0^{a,†} (5)	
	Normoxic water	189.0±14.6 (5)*	153.5±8.8^{b,†} (5)	127.5±208.2^{a,b,†} (2)	1491.0±82.2 (5)
	Anoxic water		178.1±15.4^{b} (5)	174.0±50.9^{b} (5)	1375.4±185.9 (4)
	Nitrogen	183.6±15.9 (5)	178.6±12.5^{b} (5)	178.4±29.6^{b} (6)	
Total CO_2 (mmol g^{-1} dry mass shell)					
	Air-access	0.13±0.02^{a,*} (5)	0.23±0.03^{a,†} (5)	0.16±0.06^{a,*} (4)	1.22±0.01 (8)
	Normoxic water	0.10±0.04^{a,*} (5)	0.25±0.06^{a,†} (5)		1.16±0.15 (8)
	Anoxic water		0.06±0.04^{b} (4)	0.04±0.02^{b} (4)	0.97±0.15 (6)
	Nitrogen	0.04±0.01^{b} (5)	0.07±0.04^{b} (5)	0.04±0.04^{b} (3)	

Values are means ± S.E.M., *N* in parentheses.

Different superscript letters indicate significant differences within a species; *,†significant differences among hatchling species.

Graptemys geographica were maintained in anoxic water or nitrogen for 15 days and submerged in normoxic water for 75 days. *Chelydra serpentina* and *Chrysemys picta* hatchlings were kept anoxic for 31 days and submerged in normoxic water for 81 days.

Data for adult *Chrysemys picta* ions after 90 days of anoxia are from Jackson et al. (2000); total CO_2, D. C. Jackson, S. A. Reese, J. M. Gall, C. Ruckdeschel and C. R.. Shoop, unpublished data.

differences in the ability of the two species to depress their metabolism, since adult *C. picta* live longer submerged in anoxic water than do adult *C. serpentina* (Reese et al., 2002).

Hatchling turtles placed in nitrogen accumulated lactate and lost glycogen faster than their conspecifics submerged in anoxic water, indicating a reduction in metabolism due to submergence. A dramatic depression in metabolism (10 000× below that of a similarly sized, euthermic, resting mammal; Jackson, 2002) is a mechanism central for extended survival in adult painted turtles (Jackson, 2002) and has been attributed to the inherent lowering of metabolism in all ectotherms (Bennett and Ruben, 1979), the Q_{10} effect of temperature (Bennett and Dawson, 1976; Herbert and Jackson, 1985), and the reduction induced by anoxia (Jackson, 1968; Buck et al., 1993). The reduced metabolism of hatchling turtles due to submergence, independent of [O_2] or temperature, may also be applicable to adult turtle hibernation, and thus would be another factor influencing whole-animal metabolic reduction.

Compared to adults of the same species, hatchling turtles in this study had significantly shorter survival times when submerged in anoxic water. Adult *Chrysemys picta bellii* can survive submerged in anoxic water for 150 days (Ultsch and Jackson, 1982; S. A. Reese, C. E. Crocker, D. C. Jackson and G. R. Ultsch, unpublished data) while conspecific hatchlings survive only 40 days. Similar reductions of survival time were seen in hatchling turtles of other species (50 days *vs.* 15 days in *Graptemys geographica*, Reese et al., 2001; and 100 days *vs.* 30 days in *Chelydra serpentina*, Reese et al., 2002). This represents a little over a 3× shorter survival time of hatchling turtles whether they are anoxia-tolerant (e.g. *C. picta* and *C. serpentina*) or anoxia-intolerant (e.g. *G. geographica*) as adults and whether they spend their first winter aquatically (e.g. *C. serpentina*) or terrestrially (e.g. *C. picta* and *G. geographica*) as hatchlings. Two integrative physiological mechanisms are central to the anoxia tolerance of adult painted turtles. First, mentioned above, is a dramatic reduction in metabolic rate (Jackson, 1968; Jackson and Heisler, 1982; Jackson et al., 2000) and second, the effective buffering of lactic acid (Jackson et al., 1999, 2000; Jackson, 2000). The lower anoxia tolerance of hatchling turtles may be attributed to inferior effectiveness of these mechanisms.

Anoxia tolerance

Measurements of whole-body [lactate] in the present study permit us to calculate anaerobic metabolic rate directly. Anoxic metabolism, in the form of adult whole-body [lactate], has not been measured in hibernating turtles; however, Gatten (1981) measured whole-body [lactate] after 16 days of cold, anoxic submergence in painted turtles and estimates of whole-body [lactate] for cold, anoxic painted turtles have been made from plasma [lactate] using water content of various body compartments (Jackson, 1997). We assume that *C. serpentina* and *G. geographica* are similar to *C. picta* in the relative amount of lactate distributed in various body compartments. After 10 days of anoxic submergence, adult *C. picta* have whole-body [lactate] of 2.25 mg g^{-1} (Gatten, 1981) while hatchling [lactate] range from 2.36–3.27 mg g^{-1}, indicating very little difference in metabolic rate. Adult *C. picta* have

accumulated 4.7 mg g^{-1} of lactate after 40 days of anoxic submergence, while hatchlings have accumulated 6.7 mg g^{-1} (40 days is the limit of hatchling *C. picta* survival). A similar comparison for *C. serpentina* at 30 days yields 4.2 mg g^{-1} for adults and 5.5 mg g^{-1} for hatchlings (30 days is the limit of hatchling *C. serpentina* survival), while for *G. geographica* adults accumulate 3.7 mg g^{-1} after 15 days and hatchlings 3.4 mg g^{-1} (15 days is the limit of hatchling *G. geographica* survival). Although the coarse nature of the calculation for adult [lactate] prevents us from concluding a dramatic difference between adults and hatchlings, taking the greatest difference (e.g. *C. picta*) there is still only ≈1.4-fold increase in metabolic rate, which cannot account for the threefold reduction in survival time seen in hatchlings from all three species.

Lactic acid buffering

Adult painted turtles can maintain a viable pH by the release of calcium and magnesium carbonates from the bone into the extracellular fluid (ECF), and by sequestration of lactate within bone, buffering it *in situ* and removing it from the ECF (Jackson et al., 2000, 1999; Jackson, 2000). In adult animals, the first part of this mechanism is readily apparent as a loss of Mg^{2+} from the shell (Jackson et al., 2000) and accumulation of Ca^{2+} and Mg^{2+} *in vivo* to similar levels as seen in bone incubated at the same pH *in vitro* (Jackson et al., 1999). In addition, loss of CO_2 from the shell during incubation is indicative of mobilization of carbonates (Jackson et al., 1999) and is readily apparent during anoxic submergence in intact animals (Warburton and Jackson, 1995). Although there is no loss of calcium or magnesium from the shells of anoxic hatchlings, there is a decrease in CO_2 content (Table 2). However, when compared to the initial shell [CO_2] in adult painted turtles (1.325 mmol g^{-1}; Warburton and Jackson, 1995) and the magnitude of CO_2 loss in adult painted turtles for a given amount of lactate accumulated (Fig. 3), hatchling turtles do not appear to have adequate buffer stores.

Two aspects of hatchling turtles account for their inability to tolerate anoxic submergence as well as their conspecific adults. The first is the reduced content of bone, particularly in the shell. Bone ossification in turtles occurs over the first year of the animal's life after emerging from the nest (Ewert, 1985), thus hatchling turtles do not have substantial amounts of bone to utilize as an ionic buffer reserve. This is readily apparent when comparing adult and hatchling shell ash content even though the relative amount of shell on the hatchlings is similar to that of adults (Fig. 2). Secondly, the buffering content of the bone that is available in hatchling turtles is reduced when compared to adult turtles. The Ca:P ratio in adult turtles is 2:1 while in hatchling turtles it ranges from 1.4–1.5:1. This difference in Ca:P ratios is attributable to reduced calcium carbonates within the structure of the bone, apparent from the reduced CO_2 content of hatchling bone *vs.* adult bone. Thus the small amount of bone that is present in hatchling turtles is very low in the available buffer stores that adults normally utilize for dealing with lactate accumulation.

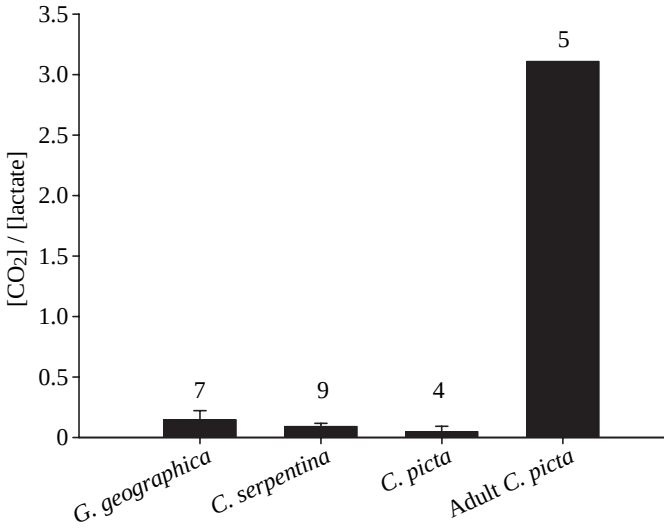

Fig. 3. Shell CO_2 mobilization (mmol) as a function of whole-body lactate (mmol) for hatchling *Chelydra serpentina*, *Chrysemys picta* and *Graptemys geographica*, and adult *Chrysemys picta* submerged in anoxic water at 3°C. Values are means ± S.E.M.; sample sizes are listed above the bars. Data for adult *Chrysemys picta* are from Warburton and Jackson (1995).

Ecological considerations

Delayed emergence has been viewed as a strategy that allows hatchling turtles to enter an environment expanding in resources (Wilbur, 1975; Gibbons and Nelson, 1978). However, since adult painted turtles routinely inhabit aquatic systems that are likely to become hypoxic or anoxic during winter months (Ultsch, 1989), evolution of delayed emergence may have been influenced by the hatchling turtle's inability to tolerate long-term anoxia.

Map turtles are anoxia-intolerant as adults, and thus do not inhabit aquatic systems that are likely to become hypoxic (Crocker et al., 2000; Reese et al., 2001). Hatchling map turtles display delayed emergence, but they are unlikely to experience long-term anoxia, so development of this overwintering strategy may be more influenced by spring resource conditions (Baker et al., 2003). The limited anoxia tolerance that is displayed in hatchling map turtles may be important for survival in a supercooled state. During supercooling, blood flow may be minimal or nil and the animals may experience a functional hypoxia that lasts for several days (Birchard and Packard, 1997; Hartley et al., 2000). Thus an ability to deal with increases in lactate may be important for surviving these subfreezing episodes in map and painted turtles (Costanzo et al., 2001a).

The shorter survival time of hatchling turtles submerged in anoxic water may have some consequences for the life history of a species. Populations of *C. serpentina* that inhabit aquatic ecosystems with the potential for hypoxic/anoxic winter conditions (e.g. swamps and eutrophic ponds) will probably have large hatchling mortality during winter-kill years unless

the hatchlings can find a hibernaculum that contains O_2 for most of the winter. Because snapping turtles are a long-lived species, a single year of winter-kill that results in low recruitment may not have a telling effect on the total population; however, several consecutive years of low O_2 may produce a population bottleneck. A traditional population bottleneck has been recognized in all species of turtles due to the high mortality associated with nesting and emergence. In this new bottleneck, only those hatchlings that can find microenvironments within the aquatic system that will remain oxygenated for the majority of the winter months will survive to the following spring. It is also possible that there is segregated hibernation among adults and juveniles, with juveniles actively seeking out microenvironments that are less likely to become oxygen deficient, such as streams. New studies are required to elucidate the hibernation habits of hatchlings that spend their first winter submerged, since their hibernacula are currently unknown.

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