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Survival and Physiological Responses of Hatchling Blanding's Turtles (*Emydoidea blandingii*) to Submergence in Normoxic and Hypoxic Water under Simulated Winter Conditions

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ABSTRACT

Overwintering habits of hatchling Blanding's turtles (*Emydoidea blandingii*) are unknown. To determine whether these turtles are able to survive winter in aquatic habitats, we submerged hatchlings in normoxic (155 mmHg Po_2) and hypoxic (6 mmHg Po_2) water at 4°C, recording survival times and measuring changes in key physiological variables. For comparison, we simultaneously studied hatchling softshell (*Apalone spinifera*) and snapping (*Chelydra serpentina*) turtles, which are known to overwinter in aquatic habitats. In normoxic water, *C. serpentina* and *A. spinifera* survived to the termination of the experiment (76 and 77 d, respectively). Approximately one-third of the *E. blandingii* died during 75 d of normoxic submergence, but the cause of mortality was unclear. In hypoxic water, average survival times were 6 d for *A. spinifera*, 13 d for *E. blandingii*, and 19 d for *C. serpentina*. Mortality during hypoxic submergence was probably caused by metabolic acidosis, which resulted from accumulated lactate. Unlike the case with adult turtles, our hatchlings did not increase plasma calcium and magnesium, nor did they sequester lactate within the shell. Our results suggest that hatchling *E. blandingii* are not particularly well suited to hibernation in hypoxic aquatic habitats.

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Introduction

In northern latitudes, hatchling turtles face the challenge of surviving winters in which environmental temperatures can approach or drop below 0°C. Hatchlings of several species avoid extreme cold by overwintering in aquatic habitats (Ultsch 1989; Ernst et al. 1994; Sims et al. 2001). However, these turtles may be threatened by reduced levels of ambient oxygen (Ultsch and Jackson 1982*a*; St. Clair and Gregory 1990) and osmotic perturbations (Sims et al. 2001). Little research has been devoted to the ability of hatchling turtles to survive under these conditions.

Compared with adults, hatchling turtles survive only briefly when submerged in anoxic water (Reese et al. 2002*a*). Under these conditions, turtles depend upon anaerobic metabolism to meet their energetic requirements, and as a result they accumulate high lactate concentrations. The ability to buffer a developing lactic acidosis may explain differences in anoxia tolerance between hatchling and adult turtles (Ultsch and Reese, forthcoming). Adult turtles use reserves of calcium and magnesium carbonates in the shell and long bones to buffer the accumulating lactate in the plasma (Jackson and Heisler 1982; Jackson et al. 2000). In addition, adult turtles accumulate, buffer, and store large amounts of lactate within the shell (Jackson 1997; Jackson et al. 2000). In fact, the shell sequesters 44% of the total body lactate in an adult turtle submerged at 3°C (Jackson 1997). Hatchlings may be unable to buffer lactate as well because they lack a completely ossified skeleton and, therefore, the large reserves of calcium and magnesium carbonates (Ultsch and Reese, forthcoming). Indeed, the shell of a hatchling turtle is mostly cartilage, and bone accounts for only 2% of the total body weight (Iverson 1982), compared with the 5%–40% of adult turtles (Iverson 1984).

Turtle species may vary in the size and composition of the shell, which in turn may influence anoxia tolerance (Jackson et al. 2000; Reese et al. 2002*b*). For instance, adult softshell (*Apalone spinifera*) and painted turtles (*Chrysemys picta*) have nearly the same proportion of shell mass to total body mass; however, the shell is less mineralized in *A. spinifera* compared with *C. picta*, and these differences are manifested in their relative tolerances to anoxia (Jackson et al. 2000; Reese et al. 2002*b*).

Blanding's turtle (*Emydoidea blandingii*) is a northerly distributed species that reportedly inhabits marshes and shallow

lakes and frequently encounters severe winters throughout its range (Sexton 1995; Sajwaj and Lang 2000). Ice cover may force turtles to remain submerged, possibly buried in anoxic mud, for extended periods. Adult *E. blandingii* reportedly overwinter this way (Graham and Butler 1993; Sexton 1995; Piepgras and Lang 2000), but the overwintering habits of hatchlings are unknown. Terrestrial overwintering of hatchling *E. blandingii* has been suggested or implied (Congdon et al. 1983, 1993, 2000; Butler and Graham 1995; Standing et al. 1997; McNeil et al. 2000; Pappas et al. 2000), and recent study has shown that these turtles are adapted to survive frost exposure (Dinkelacker et al. 2004). In contrast, some researchers have surmised that hatchling *E. blandingii* overwinter underwater (Congdon et al. 1983; Butler and Graham 1995; Packard et al. 1999, 2000). However, it is unknown whether these turtles can tolerate the stresses associated with prolonged submergence, especially in hypoxic water.

The purpose of this study was to determine whether hatchling *E. blandingii* can tolerate long-term submergence, which may be necessary for successfully overwintering in aquatic habitats. Given the lack of information regarding submergence tolerance in hatchling turtles, we also studied snapping turtles (*Chelydra serpentina*) and *A. spinifera* because these species are considered to be among the most anoxia tolerant and intolerant, respectively (Reese et al. 2002*b*).

Material and Methods

Animal Source and Acclimation

In June and July 2002, female *Emydoidea blandingii* collected at Beem Lake in Hyannis, Nebraska, and *Apalone spinifera* from Rattlesnake Pond, near Oshkosh, Nebraska, were injected with synthetic oxytocin to induce oviposition (Ewert and Legler 1978). *Chelydra serpentina* eggs were collected from nests at Crescent Lake National Wildlife Refuge, near Oshkosh. All eggs were incubated at 28°C on a substratum of vermiculite (1.0 g water g^{-1} dry vermiculite) in an environmental chamber (model I-35X; Percival, Boone, IA). Animal care and experimental procedures were approved by the Animal Care and Use Committee of Miami University (protocol 518).

On September 1, 2002, hatchlings were transferred to six 45 × 35-cm tubs (two tubs of 70 *E. blandingii*, two tubs of 25 *C. serpentina*, two tubs of 15 *A. spinifera*) filled halfway (approximately 6 cm) with tap water at 20°C. Ambient temperature was adjusted to 15°C on October 1, 10°C on October 15, and 4°C on November 1. Corresponding photoperiod was 14L : 10D during September, 12L : 12D during October, and 10L : 14D thereafter. The tubs were covered with cloth sheets on January 15, 2003 in order to simulate the reduced light penetration caused by ice cover. Hatchlings were denied food throughout the acclimation period because hatchlings presumably do not feed until the following spring (Sims et al. 2001). Air was bubbled in the water, which was changed weekly.

Experimental Treatment

Winter-acclimated hatchlings were transferred to two tubs (75 × 35 cm) containing water approximately 12 cm deep. Seventy *E. blandingii*, 25 *C. serpentina*, and either 10 or 17 *A. spinifera* were segregated by species and confined to mesh-walled compartments within each tub. In order to maintain normoxic or hypoxic conditions, we bubbled air or nitrogen, respectively, into the water (Ultsch and Jackson 1982*a*). Pulmonary breathing was impeded by a Plexiglas cover placed just beneath the water surface. Dissolved oxygen concentrations, measured daily with a probe (model 55; YSI, Yellow Springs, OH) showed that the tub receiving nitrogen remained hypoxic (average, 6 mmHg Po_2; range = 2–12 mmHg), whereas the tub receiving air remained normoxic (average, 155 mmHg Po_2; range = 141–159 mmHg). Ammonia, nitrite, and pH were measured periodically throughout the experiment. The water was not filtered; however, approximately half of the water in the normoxic tub was replaced with chilled, oxygenated water on day 29. Temperature was held at 3°–4°C throughout the experiment.

Sampling Regimen

Before installing the Plexiglas covers, we sampled turtles (*E. blandingii*, $n = 10$; *C. serpentina*, $n = 8$; *A. spinifera*, $n = 7$) in order to establish baseline values for hematocrit, plasma osmolality, and the plasma concentrations of various ions and metabolites. At the same time, we used additional turtles (*E. blandingii*, $n = 10$; *C. serpentina*, $n = 8$) to determine presubmergence values of body water content. Remaining hatchlings were then submerged in either normoxic (*E. blandingii*, $n = 70$; *C. serpentina*, $n = 25$; *A. spinifera*, $n = 10$) or hypoxic (*E. blandingii*, $n = 70$; *C. serpentina*, $n = 25$; *A. spinifera*, $n = 17$) water. Viability of these animals was assessed daily by prodding them with a blunt probe. Live turtles characteristically withdrew their appendages into their shell or swam away, whereas dead turtles were unresponsive and typically swollen. We promptly removed dead animals and recorded the time of death to the nearest day.

We sampled 10 *E. blandingii* in hypoxic water on days 3 and 7 in order to track changes in physiology. In addition, hypoxic turtles were used to measure physiological variables (*E. blandingii*, $n = 10$; *C. serpentina*, $n = 8$; *A. spinifera*, $n = 8$) and body water contents (*E. blandingii*, $n = 10$; *C. serpentina*, $n = 8$) after about 50% of each group had died. From the pool of live animals kept in normoxic water, we sampled *E. blandingii* on days 3, 7, 14, 30, and 75 ($n = 10$, each day), measuring the physiological variables listed above. We sampled *C. serpentina* ($n = 10$) and *A. spinifera* ($n = 9$) after approximately 75 d of

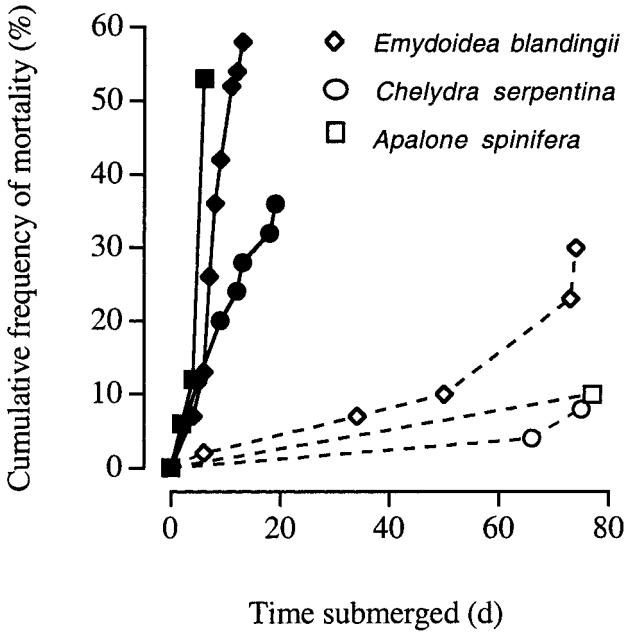

Figure 1. Cumulative frequency of mortality in hatchling turtles submerged in normoxic (*dashed lines, open symbols*) and hypoxic (*solid lines, solid symbols*) water at 4°C. Diamonds represent *Emydoidea blandingii*, circles represent *Chelydra serpentina*, and squares represent *Apalone spinifera*. Initially, 70 *E. blandingii* hatchlings were placed in each group. However, we sampled 20 in hypoxic submergence and 40 in normoxic submergence before the end of the experiment. Therefore, the cumulative frequency of mortality was based on groups of 50 animals for the hypoxic submergence and 30 animals for the normoxic submergence. For *C. serpentina*, groups of 25 animals were used in both conditions. For *A. spinifera*, 17 hatchlings were used in hypoxic submergence, and 10 hatchlings were used in the normoxic submergence.

normoxic submergence, and at this time we determined body water content in *E. blandingii* ($n = 10$) and *C. serpentina* ($n = 8$).

Specimen Collection and Analysis

Hatchlings used in the analyses were removed from the tub, blotted dry with a paper towel, weighed to the nearest 0.1 g, and immediately killed by severing the spinal cord. Breathing was possible during this brief (<1 min) procedure. Blood was drawn from the severed neck vessels into heparinized microhematocrit tubes, which were centrifuged for 5 min at 2,000 *g*. Hematocrit was recorded and aliquots of plasma placed in plastic vials or clay-sealed microcapillary tubes, frozen in liquid N_2, and stored at −80°C until analyzed for metabolite and ion concentrations.

Turtles were measured to the nearest 0.1 mm using dial calipers to determine the lengths and widths of the carapace and plastron. We then removed the carapace from the carcass, separately weighing each to the nearest 0.1 mg, and then we wrapped them in aluminum foil. The samples were flash frozen in liquid N_2 and subsequently stored at −80°C.

Plasma osmolality was determined using a vapor pressure osmometer (Vapro 5520; Wescor, Logan, UT). Plasma lactate and glucose were measured using Sigma Diagnostic kits 735 and 510, respectively (Sigma, St. Louis, MO). Total calcium concentrations were determined colorimetrically (no. 140–20; Diagnostic Chemicals, Prince Edward Island, Canada). Portions of the plasma were diluted with known portions of 0.1% nitric acid and analyzed for $[Na^+]$, $[K^+]$, and total Mg using flame photometry (Spectra AA 20, Varian Analytical, Palo Alto, CA).

Lactate concentrations in turtle shell were measured using methods similar to those reported by Jackson (1997). Five samples (≈200 mg) of the carapace from each turtle were collected using a 7-mm hole punch, weighed, dried for 48 h in a 70°C oven, and reweighed. Water content of the samples was determined from the mass lost during drying. The dried samples were then ground to a fine powder using a mortar and pestle under liquid N_2 and then mixed with 12 parts of 8% perchloric acid and incubated at room temperature for 24 h. The mixture

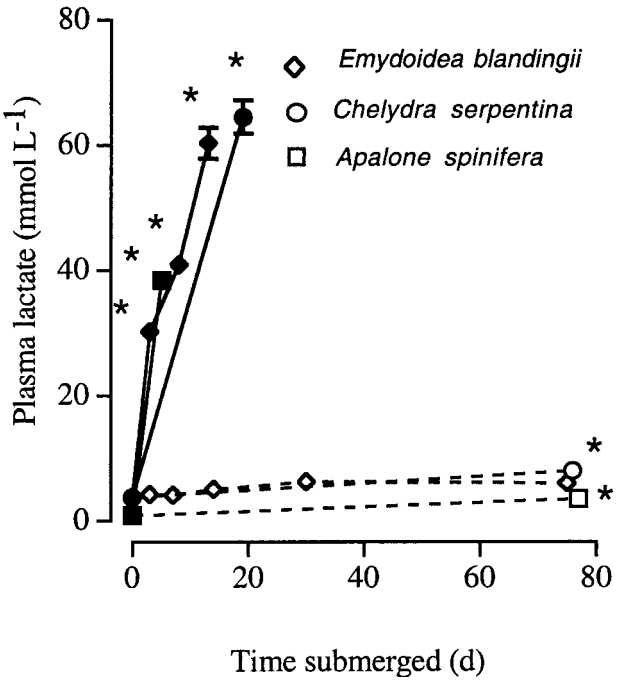

Figure 2. Changes in plasma lactate concentrations in hatchling turtles submerged in normoxic (*dashed lines, open symbols*) and hypoxic (*solid lines, solid symbols*) water at 4°C. Diamonds represent *Emydoidea blandingii*, circles represent *Chelydra serpentina*, and squares represent *Apalone spinifera*. All values are means ± SE. Data points lacking error bars have SEs too small to be depicted. Sample groups contained 10–12 *E. blandingii*, 8–10 *C. serpentina*, or 7–9 *A. spinifera*. Values for turtles in hypoxic and normoxic submergence that differed significantly ($P < 0.05$) from control values are denoted by an asterisk.

Table 1: Changes in plasma ion concentrations during forced submergence in normoxic and hypoxic water at 4°C

	Days Submerged	Total Ca (mmol L^{-1})	Total Mg (mmol L^{-1})	Na^+ (mmol L^{-1})	K^+ (mmol L^{-1})
Emydoidea blandingii:					
Control	0	1.8 ± .1 (12)	2.8 ± .2 (12)	114.6 ± 3.5 (12)	4.6 ± .5 (12)
Normoxia	3	1.9 ± .1 (10)	1.9 ± .1*** (10)	118.0 ± 1.9 (10)	3.2 ± .2 (10)
	7	1.8 ± .1 (9)	1.9 ± .1*** (10)	119.0 ± 1.8 (9)	4.5 ± .3 (10)
	14	1.8 ± .1 (9)	1.8 ± .1*** (10)	109.0 ± 4.7 (10)	4.1 ± .4 (10)
	30	1.9 ± .1 (5)	1.8 ± .1*** (8)	112.8 ± 4.3 (8)	4.0 ± .3 (8)
	75	2.0 ± .1 (5)	2.1 ± .2*** (9)	106.7 ± 2.7 (8)	4.4 ± .4 (9)
Hypoxia	3	3.2 ± .1*** (9)	2.4 ± .1 (10)	106.5 ± 4.8 (10)	4.5 ± .5 (10)
	7	4.7 ± .2*** (9)	2.7 ± .1 (10)	111.8 ± 3.1 (10)	4.7 ± .5 (10)
	13	7.1 ± .3*** (10)	3.6 ± .2** (10)	110.2 ± 1.5 (10)	4.6 ± .4 (10)
Chelydra serpentina:					
Control	0	1.7 ± .04 (8)	2.2 ± .2 (8)	108.5 ± 4.4 (8)	3.7 ± .5 (8)
Normoxia	76	1.6 ± .1 (9)	1.3 ± .04*** (10)	82.9 ± 3.9** (10)	4.3 ± .4 (10)
Hypoxia	19	8.2 ± .4*** (8)	2.6 ± .1* (8)	91.2 ± 2.2* (8)	6.3 ± .4* (8)
Apalone spinifera:					
Control	0	1.0 ± .03 (7)	1.4 ± .1 (7)	103.1 ± 1.6 (7)	4.8 ± .6 (6)
Normoxia	77	1.4 ± .1*** (9)	1.4 ± .04 (9)	103.3 ± 1.4 (9)	4.3 ± .3 (9)
Hypoxia	6	4.5 ± .2*** (8)	2.0 ± .1** (8)	95.9 ± .9** (8)	5.2 ± .4 (8)

Note. Values are means ± SE; sample sizes in parentheses. Significant differences from control values indicated with asterisks.
* $P < 0.05$.
** $P < 0.001$.
*** $P < 0.0001$.

was centrifuged and the supernatant removed and analyzed for lactate (Sigma Diagnostic Kit 735).

Additional samples of the carapace were used to determine the mineral content of hatchling shell. Dried samples were weighed, placed in crucibles, and incinerated for 24 h at 550°C in a muffle furnace. The organic-free residue was then weighed to the nearest 0.1 mg.

Body water content of euthanized turtles was determined from the change in carcass mass after drying for 7 d in a 70°C oven. We did not collect data for *A. spinifera* because too few animals were available for study.

Statistical Treatment

Variation in body mass, water content, hematocrit, osmolality, and concentrations of ions and metabolites was tested for each species in hypoxic and normoxic water using separate ANOVAs (SAS, ver. 8, SAS Institute, Cary, NC). Multiple contrasts were made using the Bonferroni procedure. Significance was reported at $P \leq 0.05$.

Results

Submergence in Normoxic Water

Nine of 30 (30%) *Emydoidea blandingii* died during 75 d of normoxic submergence (Fig. 1), although most deaths occurred near the end of the experiment. In comparison, two of 25 (8%) *Chelydra serpentina* died during a 76-d experiment and only one of 10 (10%) *Apalone spinifera* died during a 77-d experiment.

Normoxic *E. blandingii* did not accumulate lactate; however, both *C. serpentina* and *A. spinifera* did (Fig. 2). Of the ions we examined, only total magnesium changed significantly during submergence of *E. blandingii* (Table 1). However, in *C. serpentina*, we found decreases in plasma osmolality, total magnesium, and sodium (Tables 1, 2). In *A. spinifera*, total calcium, hematocrit, and osmolality increased, but glucose decreased.

Submergence in Hypoxic Water

Twenty-nine of 50 (58%) *E. blandingii* died within 13 d of hypoxic submergence (Fig. 1). Deaths occurred sporadically during the experiment. Nine of 25 (36%) *C. serpentina* died (also sporadically) within 19 d of hypoxic submergence, and nine of 17 (53%) *A. spinifera* died in only 6 d.

Plasma lactate increased universally during hypoxic submergence (Fig. 2). Ultimate concentrations of lactate in shell (mmol kg^{-1} dry mass) were lowest in *E. blandingii* and highest in *C. serpentina* (Table 3). Shell lactate levels in *A. spinifera* after 6 d of hypoxic submergence were higher than those in *E. blandingii* after 13 d.

In hypoxic *E. blandingii*, plasma concentrations of total cal-

Table 2: Changes in hematocrit, plasma osmolality, plasma glucose, and body water content during forced submergence in normoxic and hypoxic water at 4°C

	Days Submerged	Hematocrit (%)	Osmolality (mosmol kg^{-1})	Glucose (mmol L^{-1})	Water Content (% wet mass)
Emydoidea blandingii:					
Control	0	20 ± 1 (12)	231 ± 6 (12)	1.0 ± .2 (12)	83.6 ± .2 (10)
Normoxia	3	20 ± 1 (10)	237 ± 5 (10)	1.1 ± .1 (10)	…
	7	20 ± 1 (10)	228 ± 3 (10)	1.0 ± .1 (10)	…
	14	23 ± 1 (10)	214 ± 11 (10)	1.2 ± .1 (10)	…
	30	20 ± 1 (10)	219 ± 7 (10)	.9 ± .1 (10)	…
	75	21 ± 1 (10)	213 ± 5 (10)	1.2 ± .2 (8)	84.1 ± .4 (10)
Hypoxia	3	18 ± 2 (9)	216 ± 11 (9)	.9 ± .3 (9)	…
	7	17 ± 1 (10)	247 ± 8 (10)	1.5 ± .4 (10)	…
	13	17 ± 2 (10)	247 ± 4 (10)	.9 ± .2 (10)	83.8 ± .4 (10)
Chelydra serpentina:					
Control	0	20 ± 1 (8)	202 ± 7 (8)	1.3 ± .1 (8)	86.0 ± .4 (8)
Normoxia	76	20 ± 2 (10)	166 ± 8* (10)	1.7 ± .3 (10)	86.8 ± .5 (8)
Hypoxia	19	16 ± 1* (8)	212 ± 6 (8)	2.4 ± .4* (8)	87.8 ± .4* (8)
Apalone spinifera:					
Control	0	18 ± 2 (7)	196 ± 3 (7)	3.3 ± .5 (7)	…
Normoxia	77	26 ± 2* (9)	208 ± 2* (9)	2.0 ± .3* (9)	…
Hypoxia	6	21 ± 1 (8)	218 ± 3** (8)	13.0 ± 3.2* (8)	…

Note. Values are means ± SE; sample sizes are in parentheses. Missing data for water content are denoted by ellipses. Significant differences from control values indicated with asterisks.

* $P < 0.05$.

** $P < 0.001$.

cium and magnesium increased (Table 1). In *C. serpentina*, sodium concentrations decreased, but glucose, total calcium, magnesium, and potassium concentrations increased. Also, body water content increased with a concomitant decrease in hematocrit (Table 2). In *A. spinifera* we found increases in glucose, osmolality, total calcium, and total magnesium; however, sodium concentrations decreased.

Discussion

Normoxic Submergence

Several *Emydoidea blandingii* died during submergence in normoxic water, although the reasons for the deaths remain unclear. The turtles did not appear to have been under physiological stress because total magnesium was the only measured variable that changed during 75 d of submergence. The mortality we observed may ultimately prove to be an artifact, given that the hatchlings (Reese et al. 2002*a*) and adults (Ultsch 1985, 1988; Saunders et al. 2000; Reese et al. 2001) of various species readily survive submergence in cold, normoxic water for at least 150 d.

Submergence in normoxic water appeared to be relatively benign for *Chelydra serpentina*. Hatchlings accumulated lactate, although the increases were relatively minor and probably do not represent a strong reliance on anaerobic metabolism (Ultsch 1988). Hatchlings did not gain or lose water, but osmolality decreased, probably because of decreases in total magnesium and sodium. Body water content of hatchling *C. serpentina* was remarkably high (86% of wet mass). This finding, coupled with low values for hematocrit (20%) and plasma osmolality (202 mosmol kg^{-1}), suggests that these turtles were hyperhydrated. Water retention could be a response to cold acclimation in water because hatchling *C. serpentina* acclimated to cold in a terrestrial setting have a lower body water content (77.3%) and higher hematocrit (30%) and plasma osmolality (349 mosmol kg^{-1}; Costanzo et al. 2000). Furthermore, Sims et al. (2001) reported that osmolality was reduced in hatchling *C. serpentina* kept at low temperature in water relative to those kept in soil. Nevertheless, our findings, like those of Reese et al. (2002*a*), indicate that hatchling *C. serpentina* can tolerate long-term submergence in normoxic water.

Like *C. serpentina*, hatchling *Apalone spinifera* accumulated a small quantity of lactate, although it is doubtful that this change represents a reliance on anaerobic metabolism. Hematocrit increased in these turtles, perhaps functioning to improve oxygen carrying capacity (Saunders et al. 2000).

Hypoxic Submergence

Hatchling *E. blandingii* poorly tolerated submergence in hypoxic water because 58% of the group died within 13 d. Death

Table 3: Shell characteristics and lactate concentrations of shell and plasma from hatchlings submerged in hypoxic water at 4°C

	Shell Water Content (% fresh mass)	Shell Mineral Content (mg g^{-1} dry mass)	Shell Lactate (mmol kg^{-1} dry mass)	Shell Lactate (mmol kg^{-1} H_2O)	Plasma Lactate (mmol kg^{-1} H_2O)
Emydoidea blandingii:					
Control	79.1 ± .4 (12)	85.6 ± 4.0 (12)	16.5 ± 1.4 (12)	4.3 ± .3 (12)	3.9 ± .4 (12)
Final	77.2 ± $.9^{A,*}$ (10)	76.8 ± 3.7^{A} (10)	138.6 ± $10.0^{A,*}$ (10)	39.9 ± $1.5^{A,*}$ (10)	62.9 ± $2.6^{A,*}$ (10)
Chelydra serpentina:					
Control	82.7 ± .4 (8)	157.2 ± 22.4 (8)	29.4 ± 2.7 (8)	6.2 ± .6 (8)	3.8 ± .5 (8)
Final	82.4 ± $.4^{B}$ (8)	113.2 ± 6.7^{B} (8)	239.2 ± $4.9^{B,*}$ (8)	50.9 ± $1.0^{B,*}$ (8)	67.3 ± $2.8^{A,*}$ (8)
Apalone spinifera:					
Control	84.9 ± .6 (7)	95.9 ± 2.8 (7)	25.7 ± 3.4 (7)	4.6 ± .7 (7)	.8 ± .2 (7)
Final	82.9 ± $.7^{B,*}$ (8)	93.7 ± 3.1^{C} (8)	189.9 ± $14.6^{C,*}$ (8)	38.5 ± $1.9^{A,*}$ (8)	40.0 ± $1.5^{B,*}$ (8)

Note. Control and final sample data are shown. Shell lactate data are presented as mmol kg^{-1} dry mass and as mmol kg^{-1} H_2O. Plasma lactate data are presented as mmol kg^{-1} H_2O (assuming 96% water content; Jackson 1997) for comparison with shell lactate data. All values are means ± SE; sample sizes are in parentheses. Statistical differences in final values among species are indicated by different letters. Final sample times were 13 d for *E. blandingii*, 19 d for *C. serpentina*, and 6 d for *A. spinifera*. Significant difference from control values indicated with asterisks.

* $P < 0.05$.

probably resulted from metabolic acidosis associated with lactate accumulation. Although *E. blandingii* mobilized calcium and magnesium, the increases were only on the order of two- to threefold (Table 1). Jackson and Ultsch (1982) studied adult *Chrysemys picta*, reporting that total concentrations of calcium and magnesium were closely associated with lactate concentrations expressed as mEq L^{-1}. Given the minor increases we observed in hatchlings, it is unlikely that plasma calcium and magnesium buffer a substantial amount of lactate. Furthermore, contrary to the case with adult turtles (Jackson 1997), the shell of hatchlings apparently does not sequester lactate because, when expressing concentrations as mmol kg^{-1} H_2O, lactate levels in the shell were no higher than those in the blood (Table 3). Because the immature carapace has not fully ossified, the water content of hatchling *E. blandingii* shell is much higher than that (≈32%) found in adult turtles (Jackson 1997).

Chelydra serpentina survived longer in hypoxia than *E. blandingii*, accumulating as much lactate in 19 d as *E. blandingii* did in 13 d (Fig. 2). Hatchling *A. spinifera* were the least tolerant of hypoxia: more than half the group died within 6 d of submergence. Reese et al. (2002*b*) suggested that species with lower metabolic rates (as indicated by slower lactate accumulation) should survive longer in anoxic conditions. Accordingly, our lactate data might indicate that *C. serpentina* had lower metabolism, which could forestall development of metabolic acidosis. In addition, *C. serpentina* might have a superior buffering capacity because the mineral density of its shell (carapace) was higher than that in *E. blandingii* or *A. spinifera* (Table 3). Shell is an important source of mineral used in lactate buffering (Jackson 1997; Jackson et al. 2000), and notably, the quantity of mineral present in the carapace was lowest in *A. spinifera* (18.7 ± 1.1 mg; mean ± SE), intermediate in *E. blandingii* (33.9 ± 1.8 mg), and highest in *C. serpentina* (53.5 ± 7.5 mg); this hierarchy accords with survival times recorded for these species. Nevertheless, given the low mineral density in the shell (<15% of dry mass), the failure to accumulate much calcium and magnesium in the blood, and the rapid accumulation of lactate, we suspect that variation in metabolic rate accounts for most of the difference in hypoxia tolerance among the species we examined.

Submergence Tolerance in Hatchling and Adult Turtles

Given that adult *E. blandingii* overwinter in marshes and shallow ponds (Ernst et al. 1994; Sexton 1995; Sajwaj and Lang 2000), this species probably tolerates extended submergence in hypoxic water. In contrast, anoxia-intolerant species such as *Graptemys geographica* and *A. spinifera* overwinter in rivers and large lakes (Reese et al. 2001, 2003). Profound hypoxia tolerance in *E. blandingii* is lacking in hatchlings but probably is acquired coincident with the ontogenetic development of the skeleton and formation of large buffer reserves.

Hatchling *C. serpentina* apparently utilized different strategies from adults to survive winter submergence. In normoxic water, adults are unable to maintain aerobiosis and must invoke anaerobic pathways to energy production (Reese et al. 2002*b*). However, our hatchlings remained aerobic (as evidenced by a lack of lactate), perhaps because oxygen uptake is facilitated by their relatively high surface area per unit body mass. In contrast, whereas adult *C. serpentina* survive 100 d in hypoxic water (Reese et al. 2002*b*), our hatchlings survived only 18 d, probably because of their inability to sequester lactate within the shell and the limited buffer reserves of the shell.

Hatchling *A. spinifera* apparently utilize similar strategies as the adults to survive submergence in normoxic water. For example, hatchlings apparently extract enough oxygen from the water to sustain aerobic metabolism and essentially become aquatic organisms, as do the adults (Reese et al. 2003). Both

hatchlings and adults are highly intolerant of hypoxic submergence; our hatchlings survived only 6 d, and adults reportedly (Reese et al. 2003) survive only 14 d. In hatchlings as well as adults, calcium and magnesium reserves appear insufficient, and the shell, which is poorly ossified, cannot sequester lactate (Jackson et al. 2000).

Hibernation Habits of Hatchling Blanding's Turtles

Regarding the supposition that hatchling *E. blandingii* overwinter underwater (Congdon et al. 1983; Butler and Graham 1995; Packard et al. 1999, 2000), our results indicate that they cannot tolerate hypoxic submergence. Possibly these hatchlings shuttle between anoxic mud and oxygenated water (Graham and Butler 1993) or simply extend the head into the water column to permit buccopharyngeal gas exchange (Ultsch and Jackson 1982*a*; Ultsch 1989). However, Ultsch and Jackson (1982*b*) found that adult *C. picta* submerged in anoxic water at 3°C required access to air for perhaps several weeks to mitigate the attendant physiological stress. If hatchling *E. blandingii* do overwinter aquatically, they may be restricted to highly oxygenated microhabitats, preferably with access to air. One such microhabitat is the pond edge, where hatchlings could readily breathe air during intermittent thaws. On the other hand, hatchlings hibernating in shallow water may be exposed to predators and freezing (Ultsch 1989).

Overall, our findings rather lend support to the suggestion that hatchling *E. blandingii* hibernate terrestrially (Congdon et al. 1993, 2000; Standing et al. 1997; McNeil et al. 2000; Pappas et al. 2000). Hatchlings might survive winter on land by virtue of a tolerance to the freezing of their body fluids, and a recent study has demonstrated that they can recover after being frozen at −3.5°C for at least 72 h (Dinkelacker et al. 2004). Nevertheless, careful field study is needed to confirm the hibernation habits of these turtles.

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Literature Cited

Butler B.O. and T.E. Graham. 1995. Early post-emergent behavior and habitat selection in hatchling Blanding's turtles, *Emydoidea blandingii*, in Massachusetts. Chelonian Conserv Biol 1:183–196.

Congdon J.D., A.E. Dunham, and R.C. van Loben Sels. 1993. Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. Conserv Biol 7: 826–833.

Congdon J.D., R.D. Nagle, O.M. Kinney, M. Osentoski, H.W. Avery, R.C. van Loben Sels, and D.W. Tinkle. 2000. Nesting ecology and embryo mortality: implications for hatching success and demography of Blanding's turtles (*Emydoidea blandingii*). Chelonian Conserv Biol 3:569–579.

Congdon J.D., D.W. Tinkle, G.L. Breitenbach, and R.C. van Loben Sels. 1983. Nesting ecology and hatching success in the turtle *Emydoidea blandingii*. Herpetologica 39:417–429.

Costanzo J.P., J.D. Litzgus, J.B. Iverson, and R.E. Lee. 2000. Seasonal changes in physiology and development of cold hardiness in the hatchling painted turtle, *Chrysemys picta*. J Exp Biol 203:3459–3470.

Dinkelacker S.A., J.P. Costanzo, J.B. Iverson, and R.E. Lee. 2004. Cold-hardiness and dehydration resistance of hatchling Blanding's turtles (*Emydoidea blandingii*): implications for overwintering in a terrestrial habitat. Can J Zool 82:594–600.

Ernst C.H., R.W. Barbour, and J.E. Lovich. 1994. Turtles of the United States and Canada. Smithsonian Institution, Washington, DC.

Ewert M.A. and J.M. Legler. 1978. Hormonal induction of oviposition in turtles. Herpetologica 34:314–318.

Graham T.E. and B.O. Butler. 1993. Metabolic rates of wintering Blanding's turtles, *Emydoidea blandingii*. Comp Biochem Physiol 106A:663–665.

Iverson J.B. 1982. Ontogenetic changes in relative skeletal mass in the painted turtle *Chrysemys picta*. J Herpetol 16:412–414.

———. 1984. Proportional skeletal mass in turtles. Fla Sci 47: 1–11.

Jackson D.C. 1997. Lactate accumulation in the shell of the turtle *Chrysemys picta bellii* during anoxia at 3°C and 10°C. J Exp Biol 200:2295–2300.

Jackson D.C., C.E. Crocker, and G.R. Ultsch. 2000. Bone and shell contribution to lactic acid buffering of submerged turtles *Chrysemys picta bellii* at 3°C. Am J Physiol 278:R1564–R1571.

Jackson D.C. and N. Heisler. 1982. Plasma ion balance of submerged anoxic turtles at 3°C: the role of calcium lactate formation. Respir Physiol 49:159–174.

Jackson D.C. and G.R. Ultsch. 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.

McNeil J.A., T.B. Herman, and K.L. Standing. 2000. Movement of hatchling Blanding's turtles (*Emydoidea blandingii*) in Nova Scotia in response to proximity to open water: a manipulative experiment. Chelonian Conserv Biol 3:611–617.

Packard G.C., M.J. Packard, and J.W. Lang. 2000. Why hatchling Blanding's turtles don't overwinter inside their nest. Herpetologica 56:367–374.

Packard G.C., M.J. Packard, J.W. Lang, and J.K. Tucker. 1999. Tolerance for freezing in hatchling turtles. J Herpetol 33: 536–543.

Pappas M.J., B.J. Brecke, and J.D. Congdon. 2000. The Blanding's turtles (*Emydoidea blandingii*) of Weaver Dunes, Minnesota. Chelonian Conserv Biol 3:557–568.

Piepgras S.A. and J.W. Lang. 2000. Spatial ecology of Blanding's turtle in central Minnesota. Chelonian Conserv Biol 3:589–601.

Reese S.A., C.E. Crocker, M.E. Carwile, D.C. Jackson, and G.R. Ultsch. 2001. The physiology of hibernation in common map turtles (*Graptemys geographica*). Comp Biochem Physiol A 130:331–340.

Reese S.A., D.C. Jackson, and G.R. Ultsch. 2002*a*. Lactate accumulation and glycogen depletion in hatchling turtles (*Chrysemys picta bellii*, *Chelydra serpentina*, and *Graptemys geographica*) submerged at 3°C. Integr Comp Biol 42:1299.

———. 2002*b*. The physiology of overwintering in a turtle that occupies multiple habitats, the common snapping turtle (*Chelydra serpentina*). Physiol Biochem Zool 75:432–438.

———. 2003. Hibernation in freshwater turtles: softshell turtles (*Apalone spinifera*) are the most intolerant of anoxia among North American species. J Comp Physiol B 173:263–268.

Sajwaj T.D. and J.W. Lang. 2000. Thermal ecology of Blanding's turtle in central Minnesota. Chelonian Conserv Biol 3:626–636.

Saunders D.K., A.C. Roberts, and G.R. Ultsch. 2000. Blood viscosity and hematological changes during prolonged submergence in normoxic water of northern and southern musk turtles (*Sternotherus odoratus*). J Exp Zool 287:459–466.

Sexton O.J. 1995. Miscellaneous comments on the natural history of Blanding's turtle (*Emydoidea blandingii*). Trans Mo Acad Sci 29:1–13.

Sims P.A., G.C. Packard, and P.L. Chapman. 2001. The adaptive strategy for overwintering by hatchling snapping turtles (*Chelydra serpentina*). J Herpetol 35:514–517.

Standing K.L., T.B. Herman, D.D. Hurlburt, and I.P. Morrison. 1997. Postemergence behavior of neonates in a northern peripheral population of Blanding's turtle, *Emydoidea blandingii*, in Nova Scotia. Can J Zool 75:1387–1395.

St. Clair R.C. and P.T. Gregory. 1990. Factors affecting the northern range limit of painted turtles (*Chrysemys picta*): winter acidosis or freezing? Copeia 1990:1083–1089.

Ultsch G.R. 1985. The viability of nearctic freshwater turtles submerged in anoxia and normoxia at 3 and 10°C. Comp Biochem Physiol A 81:607–611.

———. 1988. Blood gases, hematocrit, plasma ion concentrations, and acid-base status of musk turtles (*Sternotherus odoratus*) during simulated hibernation. Physiol Zool 61:78–94.

———. 1989. Ecology and physiology of hibernation and overwintering among freshwater fishes, turtles, and snakes. Biol Rev 64:435–516.

Ultsch G.R. and D.C. Jackson. 1982*a*. 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.

———. 1982*b*. Long-term submergence at 3°C of the turtle, *Chrysemys picta bellii*, in normoxic and severely hypoxic water. III. Effects of changes in ambient Po_2 and subsequent air breathing. J Exp Biol 97:87–99.

Ultsch G.R. and S.A. Reese. Forthcoming. Ecology and physiology of overwintering. In R.J. Brooks, A.C. Steyermark, and M.S. Finkler, eds. The Biology of the Snapping Turtle. Johns Hopkins University Press, Baltimore.