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Geographic Variation of the Physiological Response to Overwintering in the Painted Turtle (*Chrysemys picta*)

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ABSTRACT

We compared the physiological responses of latitudinal pairings of painted turtles submerged in normoxic and anoxic water at 3°C: western painted turtles (*Chrysemys picta bellii*) from Wisconsin (WI) versus southern painted turtles (*Chrysemys picta dorsalis*) from Louisiana (LA), Arkansas (AR), and Alabama (AL), and eastern painted turtles (*Chrysemys picta picta*) from Connecticut (CT) versus *C. p. picta* from Georgia (GA). Turtles in normoxic water accumulated lactate, with *C. p. bellii* accumulating less than (20 mmol/L) the other groups (44–47 mmol/L), but with relatively minor acid-base and ionic disturbances. *Chrysemys picta bellii* had the lowest rate of lactate accumulation over the first 50 d in anoxic water (1.8 mmol/d vs. 2.1 for AR *C. p. dorsalis*, 2.4 mmol/d for GA *C. p. picta*, and 2.5 mmol/d for CT *C. p. picta* after 50 d and 2.6 mmol/d for AL *C. p. dorsalis* after 46 d). Northern turtles in both groups survive longer in anoxia than their southern counterparts. The diminished viability in *C. p. dorsalis* versus *C. p. bellii* can be partially explained by an increased rate of lactate accumulation and a decreased buffering capacity, but for the CT and GA *C. p. picta* comparison, only buffering capacity differences are seen to influence survivability.

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Introduction

Freshwater turtles in north-temperate regions may spend more than half of each year hibernating, and ice cover can prevent breathing for a large portion of that time. During this apneic period, they may or may not have access to dissolved oxygen, depending on the characteristics of their hibernacula (reviewed by Ultsch 1989). Some species live in aquatic habitats that may become hypoxic or anoxic during winter (Crawford 1990), and any species that hibernates buried in mud will be anoxic. Freshwater turtles, in particular painted turtles (*Chrysemys picta*), have been shown to be the most anoxia tolerant of vertebrates (reviewed by Jackson 2000; Jackson et al. 2001) and are well adapted to the physiological challenges associated with overwintering under water.

Chrysemys picta bellii, the western painted turtle, has been the subject of numerous studies since the original description (Jackson and Ultsch 1982; Ultsch and Jackson 1982) of its still unmatched ability to tolerate long-term submergence in anoxic water at 3°C (reviews cited above). Ultsch et al. (1985) showed that a southern subspecies, *Chrysemys picta dorsalis*, had a considerably reduced tolerance of anoxic submergence relative to *C. p. bellii*, thus demonstrating a physiological cline within the species in adaptations to hibernation, although there were two distinct subspecies involved. In contrast, Ultsch and Cochran (1994) found no such cline in common musk turtle populations separated by similar latitudes.

One purpose, therefore, of this study was to determine whether the physiological cline found in painted turtles was the result of using two subspecies that might differ physiologically or whether the cline was truly due to a difference in the severity of the winters in the turtles' home ranges. Therefore, we have conducted studies of simulated hibernation in normoxic and anoxic water at 3°C of eastern painted turtles (*Chrysemys picta picta*) from Georgia (GA) and compared them with a previous study (Ultsch et al. 1999) of the same subspecies from Connecticut (CT) that used the same protocol. We have also conducted simultaneous and similar studies with *C. p. bellii* from Wisconsin (WI) and *C. p. dorsalis* from Louisiana (LA) and Arkansas (AR; the study of Ultsch et al. 1985 did not include *C. p. dorsalis* in normoxic water and did not include some of the variables reported here).

The original studies on *C. p. bellii* (Jackson and Ultsch 1982; Ultsch and Jackson 1982) were done on catheterized turtles, which raises two concerns. First, the sample size decreased with time from 10 to 11 to as low as 2, since animals died during

the course of the experiments. Second, the effects of the operation were unknown and may have been responsible for the wide scatter in the results for turtles submerged in normoxic water; these turtles were also subject to a fungus that responded poorly to treatment. Although numerous subsequent studies have verified that the trends shown in the 1982 studies are all qualitatively valid (Jackson and Heisler 1982, 1983; Herbert and Jackson 1985; Jackson et al. 2000), one study (Jackson et al. 2000) suggested that the use of noncatheterized turtles produced quantitatively different results, especially for turtles submerged in normoxic water. Therefore, all studies reported here used turtles (including that of Ultsch et al. 1999 for *C. p. picta* from Connecticut and Ultsch et al. 1985 for *C. p. dorsalis* from Alabama) that had not been subjected to any invasive procedures, and all methods and treatments were identical for all four groups: *C. p. bellii* from Wisconsin; *C. p. dorsalis* from Louisiana, Arkansas, and Alabama; and *C. p. picta* from Connecticut and Georgia.

Material and Methods

Animals

Adult *Chrysemys picta picta* (body mass from 69 to 459 g) were collected from Montgomery County, Georgia, in August 2000 and 2002. Adult *Chrysemys picta dorsalis* (body mass from 83 to 479 g) were collected from Monroe County, Arkansas, in July and August 1999 and from Concordia Parish, Louisiana, in July and August of 2000; because the anoxic experiments were done first with the initial intention of not doing normoxic experiments, only Arkansas turtles were used in experiments involving anoxic water. Data from Ultsch et al. (1985) for AL *C. p. dorsalis* are included in some of the analyses. Methods for this previous study are similar to current methods; only sampling periods vary significantly. Adult *Chrysemys picta bellii* (body mass from 160 to 934 g) were collected in LaCrosse County, Wisconsin, in July and August 2001. To minimize the number of animals used, we did not repeat previous studies of northern *C. p. picta*. Therefore, data for CT *C. p. picta* in normoxic and anoxic water are from the study of Ultsch et al. (1999), which used the same methods we use here. The animals were housed in an aquatic facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International at the University of Alabama, and all experiments were approved by the internal Animal Care and Use Committee (IN 185). Water temperature was 20° ± 2°C, and a 12L : 12D photoperiod was provided with full-spectrum bulbs. Animals were allowed to bask and were fed a diet of commercial catfish food pellets supplemented with fresh fish and worms. All animals were used in the winter following their capture.

Sampling and Analysis

The methodologies used for blood sampling and for simulating hibernation were as in Reese et al. (2001). In brief, in mid-October, animals that were unfed for 3 d were placed in approximately 15 cm of water at 15°C with access to air. The temperature was lowered 1°C/d to 3°C, where it was maintained for 3 d. Following this acclimation, turtles ($n = 5$–10) were killed and blood was sampled through a cardiac puncture for control data. A 0.4-mL sample of blood was taken anaerobically for determination of Po_2, Pco_2, and pH (Radiometer BMS 3 MK2 Blood Micro System and PHM 73 pH/blood gas monitor thermostatted at 3°C). Pco_2 was read as pH and converted using the linear relationship between log Pco_2 and pH. Blood gas electrodes were calibrated with gas mixtures supplied by a Wösthoff M301/a-F gas-mixing pump; the pH electrode was calibrated using precision buffers (Radiometer). Plasma $[HCO_3^-]$ was calculated from the Henderson-Hasselbalch equation using $\alpha CO_2 = 0.0812$ (Reeves 1976) and a pK that depended on the blood pH (Jackson and Heisler 1983): 6.293 for control turtles and submerged turtles with a pH greater than or equal to the lowest pH for control animals and 6.350 for turtles with a pH less than the lowest control turtle pH.

The turtle was then elevated, and a cannula inserted into the heart (PE-90 tubing with a 20-gauge needle) was used to fill duplicate microhematocrit tubes and several microcentrifuge tubes with blood. Hematocrit was determined by centrifugation for 4 min at 13,000 *g*. The microcentrifuge tubes of blood were centrifuged (3 min at 10,000 *g*), the plasma decanted, and the plasma frozen for latter analysis of $[Na^+]$ and $[K^+]$ (Radiometer FLM3 flame photometer), $[Cl^-]$ (Radiometer CMT10 chloride titrator), total Ca and Mg (Buck Scientific 210 atomic Absorption spectrophotometer), lactate and glucose (YSI 2300 Stat-Plus Analyzer), and osmolality (Precision Systems μOsmette 5004).

The remaining turtles were divided into two groups, each submerged under a plastic grating to prevent surfacing. In one group, air from outside the cold room was used to aerate the water above the grating to maintain the water Po_2 throughout at or near air saturation (approximately 158 mmHg). In the second group, the top was sealed, except for small gas vents. Nitrogen was bubbled through the water above the grating and kept the water Po_2 <5 mmHg. The water in both tanks was flushed periodically using preequilibrated water. We removed turtles for sampling on days 10, 25, 50, 75, 100, 125, and 150 and prevented them from breathing by clamping the neck before they were removed from the water. In some groups, survival was <150 d, and the last day of sampling occurred when mortality was considered high (Fig. 1). The anoxic AL *C. p. dorsalis* (Ultsch et al. 1985) were sampled on days 0, 7, 20, 28, 38, and 46. A 12L : 12D photoperiod was maintained throughout the experiment.

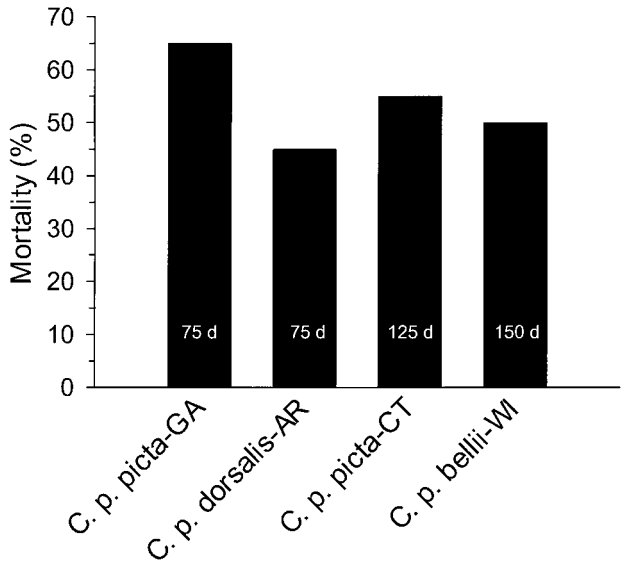

Figure 1. Mortality of painted turtles (*Chrysemys picta)* submerged in anoxic water at 3°C, as a percentage of turtles remaining, when limits of anoxia exposure were determined and reached and the experiment terminated. Day of final sample is labeled in white and considered the survival limit for each group.

Statistics

Statistical analysis was done using the 1999 edition of STATISTICA (Statsoft), using an α of 0.05 to determine significance. Comparisons among groups were done using a factorial ANOVA with subspecies/locality (N, S, NE, SE) and sampling day as independent variables. Analyses of variables within each group used a one-way ANOVA when appropriate ($[Cl^-]$, [glucose], $[Na^+]$, and body mass) but used a Kruskal-Wallis median ranks test when data did not meet parametric assumptions (pH, $[HCO_3^-]$, CO_2, Po_2, $[K^+]$, total calcium, total magnesium, [lactate], and osmolality). For certain calculated variables (e.g., rate of lactate accumulation and total body buffering), mass was used as a covariate and an ANCOVA was performed. Tukey's HSD was used for post hoc comparisons. Since data over short intervals respond linearly, we averaged the 5- and 15-d data for CT *C. p. picta* (Ultsch et al. 1999) to produce a day 10 for use in the factorial ANOVA only. For the analysis of buffering ions, a least squares two-line regression was performed using the method of Yeager and Ultsch (1989). All variables are reported as mean ± 95% confidence interval except in the figures where standard errors were used for clarity.

Results

Survival

All turtles that were submerged were intended as samples; however, some individuals died along the way. A large proportion of turtles dead on a given sampling day indicates the absolute survival ability in a particular treatment for a group. In submerged *Chrysemys picta bellii*, there were no deaths among 35 turtles in normoxic water during the 150 d of sampling; among 41 turtles submerged in anoxic water, one was dead on day 125 and five out of 10 remaining turtles were dead on day 150. There were no deaths among 39 *Chrysemys picta dorsalis* from this study submerged in normoxic water for up to 150 d; among 18 in anoxic water, two out of seven remaining turtles were dead on day 75. The *C. p. dorsalis* from Ultsch et al. (1985) had three out of four turtles dead on day 46 of anoxic submergence; however, the remaining turtle survived until day 86. Among CT *Chrysemys picta picta*, none of the 49 turtles submerged in normoxic water for up to 150 d died; among 51 submerged in anoxic water, four turtles were dead on day 75, two were dead on day 100, and five of the nine remaining turtles were dead on day 125. Among GA *C. p. picta*, one out of 31 submerged in normoxic water was dead on day 150; among 66 turtles submerged in anoxic water, 24 out of 37 remaining turtles were dead on day 75, all from the 2002 data set (Fig. 1).

Acid-Base Statuses

In turtles submerged in normoxic water, blood Po_2 dropped to 0.0–1.5 mmHg and remained low. Pco_2 fell slightly in all four groups but returned to control values in *C. p. dorsalis*, GA *C. p. picta*, and CT *C. p. picta* (Table 1). Although the Po_2 was low, aerobic metabolism was evident, as indicated by a much slower lactate accumulation (lowest among *C. p. bellii*) than in anoxic turtles (Fig. 2*A*, 2*C*; Table 1). After 150 d of submergence, *C. p. dorsalis*, CT *C. p. picta*, and GA *C. p. picta* had lactate concentrations of 45.0 ± 13.6, 46.7 ± 15.9, and 44.4 ± 17.2 mmol/L, respectively, more than twice that of *C. p. bellii* (20.4 ± 11.3 mmol/L; Table 1). Blood pH fell slightly in *C. p. dorsalis* (7.929 ± 0.031 to 7.607 ± 0.102 after 150 d) but was maintained at control values in the others (Fig. 2*B*). Blood $[HCO_3^-]$ for all groups of turtles fell 22–26 mmol/L, regardless of initial concentration (Table 1).

In turtles submerged in anoxic water, pH fell to 7.215 ± 0.122 in *C. p. bellii* after 150 d, to 7.119 ± 0.254 in *C. p. dorsalis* after 75 d in this study and to 6.961 ± 0.538 after 46 d in the Ultsch et al. (1985) study, to 7.032 ± 0.225 in CT *C. p. picta* after 125 d, and to 7.021 ± 0.207 in GA *C. p. picta* after 75 d (Fig. 2*D*; Table 1). *Chrysemys picta bellii* accumulated lactate slower (1.8 mmol/d after 50 d) than the other groups (2.4 mM/d, 2.1 mM/d, and 2.5 mM/d for GA *C. p. picta*, AR *C. p. dorsalis*, and CT *C. p. picta* after 50 d, respectively, and 2.6 mM/d for AL *C. p. dorsalis* after 46 d; Fig. 2*C*). In addition, the northern group of *C. picta* had accumulated an average of 41% more lactate by the end of the experiment, as evidenced by near death or 150 d, than had the southern group. The increase in [lactate] quickly titrated $[HCO_3^-]$ to low levels, which remained low throughout the experiment but never fell below 2 mmol/L. A

Table 1: Acid-bases statuses of *Chrysemys picta* subspecies from WI, CT, GA, AR, and LA submerged in anoxic and normoxic water at 3°C

	C. p. bellii (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (AR)	*C. p. bellii* (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (LA)
n	10 (0)	10 (0)	5 (0)	5 (0)				
pH	7.993 ± .061	8.004 ± .028	7.962 ± .025	7.929 ± .031				
Pco_2 (mmHg)	12.30 ± 2.12	10.43 ± 1.03	11.65 ± 1.74	11.91 ± 4.38				
$[HCO_3^-]$ (mmol/L)	48.8 ± 3.5	43.16 ± 2.32	44.20 ± 7.74	40.49 ± 13.23				
[Lactate] (mmol/L)	1.7 ± .6	2.0 ± .3	1.4 ± .5	1.4 ± .3				
	Anoxic Water (<5 mmHg)				Normoxic Water (150–160 mmHg)			
n	5 (10)	6 (5)	5 (10)	5 (10)	5 (10)	5 (5)	5 (10)	5 (10)
pH	7.668 ± .106	7.699	7.644 ± .069	7.434 ± .060	8.013 ± .127^{A}	7.934 ± .111	7.806 ± .160	7.604 ± .090^{A,*}
Pco_2	17.52 ± 3.98	14.62 ± 2.12	13.71 ± 2.31	13.72 ± 2.6	9.85 ± 2.95	10.57 ± 1.77	11.76 ± 2.32	14.21 ± 2.35
$[HCO_3^-]$ (mmol/L)	29.26 ± 2.22^{A}	26.46 ± 3.39	21.86 ± 3.41	13.63 ± 3.57^{A}	40.22 ± 4.04^{A}	33.76 ± 5.46	30.18 ± 5.44^{A,*}	20.78 ± 3.92^{A,*}
[Lactate] (mmol/L)	33.4 ± 5.1^{A}	30.2 ± 11.9	47.1 ± 5.0	64.8 ± 16.5^{A}	5.7 ± 5.8^{AB}	11.5 ± 7.7	27.2 ± 9.8^{A}	39.8 ± 7.7^{B,*}
n	5 (25)	5 (15)	5 (25)	5 (25)	5 (25)	5 (15)	5 (25)	5 (25)
pH	7.605 ± .124	7.577	7.395 ± .078	7.447 ± .080	8.120 ± .083^{CDE}	8.031 ± .090	7.904 ± .120^{AD}	7.738 ± .208^{BE}
Pco_2 (mmHg)	16.3 ± 3.4	13.23 ± 2.21	14.96 ± 3.25	15.00 ± 2.19	6.87 ± 1.02^{AB,*}	8.31 ± 1.34	8.95 ± 1.53^{A}	9.13 ± 1.48^{B}
$[HCO_3^-]$ (mmol/L)	28.3 ± 12.2^{ABC}	18.05 ± 2.34	13.65 ± 1.75^{B}	15.39 ± 3.64^{C}	37.23 ± 2.07^{CDE,*}	36.98 ± 7.02	27.28 ± 4.18^{AD,*}	19.24 ± 7.52^{BE,*}
[Lactate] (mmol/L)	68.8 ± 10.1	78.0 ± 27.3	73.8 ± 6.2	68.0 ± 11.1	4.9 ± 2.8^{BCD}	14.2 ± 11.9	35.0 ± 14.6^{AC,*}	42.1 ± 24.1^{D,*}
n	5 (50)	6 (25)	4–5 (50)	5 (50)	5 (50)	5 (25)	4 (50)	5 (50)
pH	7.499 ± .045^{A}	7.441 ± .095	7.235 ± .069^{A}	7.278 ± .096	8.061 ± .105^{CDE}	7.909 ± .196^{ABC}	7.761 ± .094^{AD}	7.844 ± .189^{BE}
Pco_2	11.8 ± 3.2	11.32 ± 1.80	11.34 ± 3.27	16.09 ± 2.48	7.53 ± 1.42^{AB,*}	8.21 ± 1.62	9.84 ± .78^{A}	7.51 ± 1.05^{B,*}
$[HCO_3^-]$ (mmol/L)	13.5 ± 3.3	11.40 ± 2.20^{A}	7.58 ± 1.43	11.90 ± 3.66	35.63 ± 3.23^{CDE,*}	26.76 ± 9.40^{ABC,*}	20.74 ± 3.37^{AD,*}	20.72 ± 7.82^{BE,*}
[Lactate] (mmol/L)	89.7 ± 10.3^{AB}	87.0 ± 11.9	122.3 ± 10.1^{B}	104.3 ± 19.4	15.9 ± 14.4^{BCD}	25.0 ± 12.2^{AB}	48.6 ± 14.4^{AC,*}	25.4 ± 21.1^{D}
n	5 (75)	5 (50)	5 (75)	4 (75)	5 (75)	5 (50)	5 (75)	5 (75)
pH	7.389 ± .195^{AB}	7.330 ± .080	7.021 ± .207^{BC}	7.119 ± .254^{A}	8.035 ± .067^{CDE}	8.052 ± .124^{ABC}	7.724 ± .098^{AD}	7.755 ± .123^{BE}
Pco_2 (mmHg)	11.3 ± 3.7	8.27 ± .79	8.25 ± 1.27^{A}	12.94 ± 3.64^{A}	6.96 ± 1.07^{AB,*}	6.24 ± .92*	7.75 ± .77^{A}	9.13 ± .60^{B}
$[HCO_3^-]$ (mmol/L)	10.1 ± 3.6	6.42 ± 1.79	3.64 ± 1.40	6.34 ± 3.02	30.98 ± .79^{CDE,*}	29.15 ± 5.24^{ABC,*}	14.92 ± 1.92^{AD,*}	19.17 ± 5.07^{BE,*}

[Lactate] (mmol/L)	102.8 ± 21.2^{ABC}	125.0 ± 21.3^{A}	146.0 ± 12.2^{B}	144.8 ± 32.3^{C}	18.0 ± 11.4^{BCD}	19.9 ± 15.9^{AB}	53.3 ± 12.7^{AC,*}	37.7 ± 26.1^{D,*}
n	4 (100)	5 (75)			5 (100)	5 (75)	5 (100)	5–7 (100)
pH	7.301 ± .223^{A}	7.239 ± .110^{C}			8.056 ± .128^{CDE}	7.878 ± .200^{ABC}	7.800 ± .116^{AD}	7.616 ± .115^{BE,*}
Pco_2 (mmHg)	8.78 ± 2.56	9.78 ± 2.00			7.13 ± .38^{AB,*}	8.81 ± 1.63	6.91 ± 1.98^{A,*}	10.02 ± 1.68^{B}
[HCO_3^-] (mmol/L)	6.41 ± 2.31^{A}	6.10 ± .56			34.10 ± 8.79^{CDE,*}	26.08 ± 8.90^{ABC,*}	16.28 ± 4.92^{AD,*}	15.14 ± 4.51^{BE,*}
[Lactate] (mmol/L)	149.8 ± 17.4^{A}	150.0 ± 24.0^{A}			17.8 ± 14.6^{BCD}	38.9 ± 17.7^{AB,*}	48.2 ± 14.3^{AC,*}	39.6 ± 16.4^{D,*}
n	5 (125)	5 (100)			5 (125)	5 (100)	5 (125)	5–6 (125)
pH	7.223 ± .174^{A}	7.075 ± .250^{A}			7.914 ± .218^{CDE}	7.888 ± .292^{ABC}	7.800 ± .116^{AD,*}	7.698 ± .196^{BE}
Pco_2 (mmHg)	8.40 ± 1.55	9.80 ± 3.36			9.15 ± 2.80^{AB}	8.28 ± 1.38	6.91 ± 1.98^{A}	9.76 ± 2.57^{B}
[HCO_3^-] (mmol/L)	5.14 ± 1.38^{A}	4.20 ± 1.35^{A}			28.84 ± 8.63^{CDE,*}	24.24 ± 11.47^{ABC,*}	16.28 ± 4.92^{AD,*}	19.53 ± 10.89^{BE,*}
[Lactate] (mmol/L)	152.8 ± 22.7^{A}	179.0 ± 39.0^{A}			27.0 ± 27.5^{BCD,*}	43.5 ± 30.4^{AB,*}	48.2 ± 14.3^{AC,*}	36.3 ± 13.8^{D,*}
n	5 (150)	4 (125)			5 (150)	5 (125)	5 (150)	6 (150)
pH	7.215 ± .122	7.032 ± .225^{A}			7.923 ± .180^{CDE}	7.813 ± .178^{ABC}	7.748 ± .095^{AD,*}	7.607 ± .102^{BE,*}
Pco_2 (mmHg)	9.98 ± 1.90	10.97 ± 3.01			7.34 ± 1.25^{AB,*}	8.26 ± .96	9.04 ± 1.66^{A}	9.78 ± 1.10^{B}
[HCO_3^-] (mmol/L)	6.19 ± 2.90	4.30 ± 1.46^{A}			23.97 ± 7.76^{CDE,*}	19.95 ± 6.55^{ABC,*}	18.24 ± 2.54^{AD,*}	14.48 ± 2.57^{BE,*}
[Lactate] (mmol/L)	193.4 ± 12.4	201.9 ± 8.7^{A}			20.4 ± 11.3^{BCD}	36.9 ± 18.5^{AB,*}	44.4 ± 17.2^{AC,*}	45.0 ± 13.6^{D,*}
n						5 (150)		
pH						7.825 ± .122^{ABC}		
Pco_2 (mmHg)						8.23 ± 1.04		
[HCO_3^-] (mmol/L)						20.14 ± 4.33^{ABC,*}		
[Lactate] (mmol/L)						46.7 ± 15.9^{AB,*}		

Note. WI = Wisconsin, CT = Connecticut, GA = Georgia, AR = Arkansas, LA = Lousiana. Values are mean ± 95% confidence interval. Days submerged are in parentheses after the sample size (*n*). Data for *C. p. picta* from CT are from Ultsch et al. (1999). Letters indicate significant differences for a given sampling day within a particular treatment.

* Significantly different from controls for those submerged in normoxic water; all values in anoxic water are significantly different from controls except Pco_2, which is only significantly different on day 10 for *C. p. bellii* and *C. p. picta*.

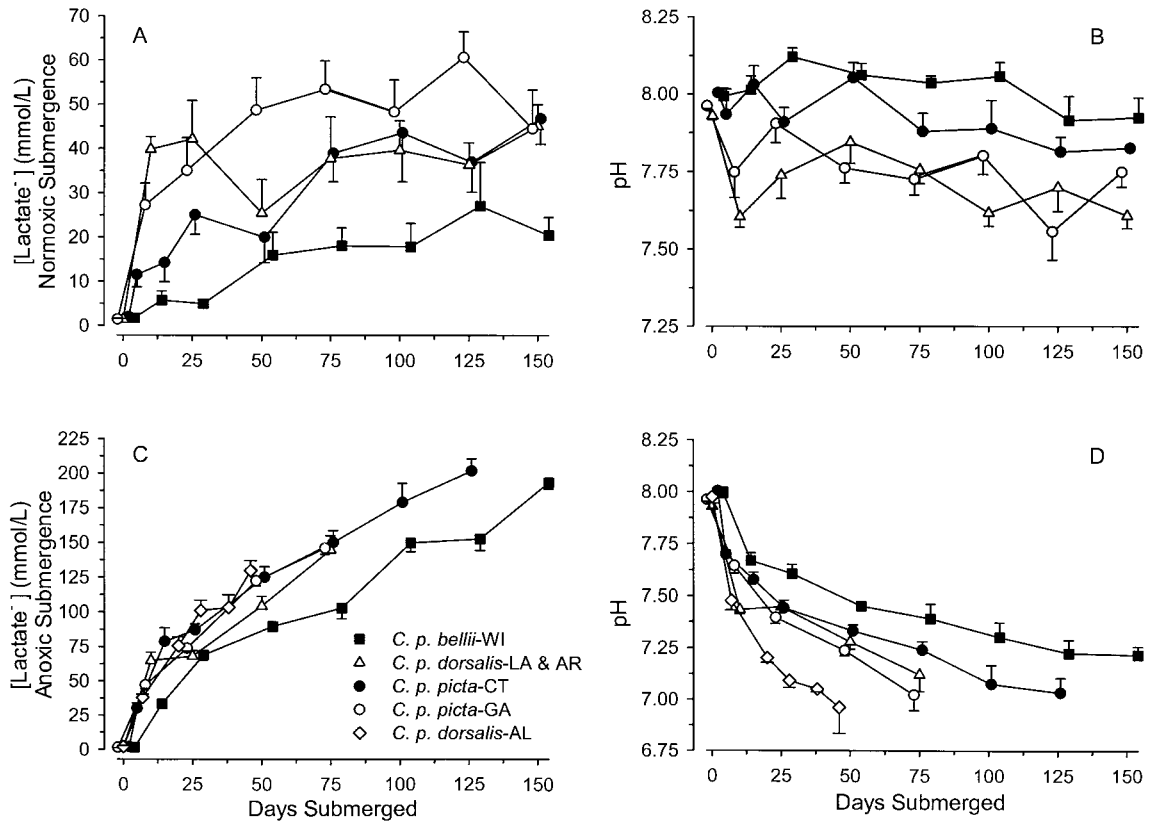

Figure 2. *A*, Plasma lactate concentrations for turtles submerged in normoxic water at 3°C. Solid squares are *Chrysemys picta bellii* from Wisconsin (*WI*), open triangles are *Chrysemys picta dorsalis* from Louisiana (*LA*), solid circles are *Chrysemys picta picta* from Connecticut (*CT*), and open circles are *C. p. picta* from Georgia (*GA*). Values are mean ± SE. *B*, As in *A* for blood pH. *C*, As in *A* for turtles submerged in anoxic water with *C. p. dorsalis* from Alabama (*AL*). D, As in *C* for blood pH.

pH-[HCO_3^-] graph (Davenport diagram; Fig. 3) suggests the acid-base response to anoxia was similar in all groups, with an initial combined respiratory-metabolic acidosis that progressed to a strictly metabolic acidosis, though AL *C. p. dorsalis* had a combined acidosis for all but the final 2 sampling days. All turtles reached similar pHs before death.

Ionic Statuses

In turtles submerged for 150 d in normoxic water, plasma [Na^+] fell significantly in *C. p. bellii*, GA *C. p. picta*, and *C. p. dorsalis* and remained unchanged in CT *C. p. picta* (Table 2). Plasma [Cl^-] dropped in GA *C. p. picta*, CT *C. p. picta*, and *C. p. dorsalis* but remained unchanged in *C. p. bellii*. Total Ca and Mg increased in all turtles except *C. p. bellii*.

In all turtles submerged in anoxic water, plasma [Cl^-] fell, and plasma [K^+], total Ca, and total Mg increased throughout the experiment (Table 2). Plasma [Na^+] fell in *C. p. bellii*, GA *C. p. picta*, and *C. p. dorsalis* but not in CT *C. p. picta*. However, the final [Na^+] concentrations were similar in all four groups (Table 2).

Hematocrit, Glucose, and Osmolality

In all turtles submerged in normoxic water, hematocrit varied considerably; glucose and osmolality remained unchanged (Table 3). In anoxic water, hematocrit and glucose remained unchanged, while osmolality increased in all groups; osmolality was 17% higher for turtles from northern latitudes by the experimental end point.

Discussion

*Western Painted Turtles (*Chrysemys picta bellii*) versus Southern Painted Turtles (*Chrysemys picta dorsalis*)*

One purpose of this study was to reinvestigate the response of *Chrysemys picta bellii* to submergence in normoxic water. Although we found the responses to be qualitatively similar to those reported in Jackson and Ultsch (1982) and Ultsch and Jackson (1982), there were some significant quantitative differences. In particular, the lactate concentration after 149 d (at which time only two turtles remained) reported by Ultsch and Jackson (1982) was 70.0 mmol/L at a pH of 7.170, while in

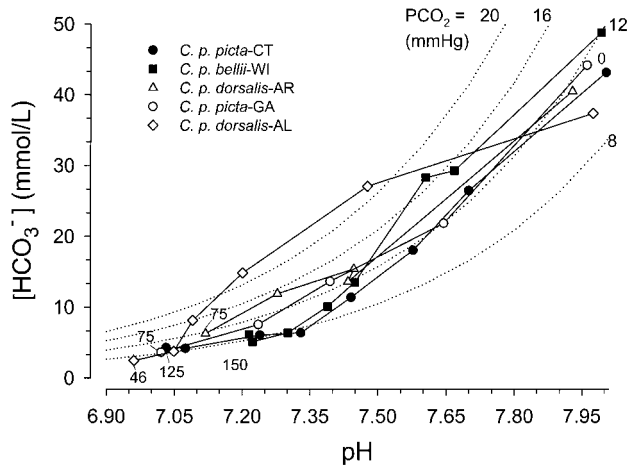

Figure 3. Changes in acid-base status of turtles submerged in anoxic water at 3°C as depicted by a pH-$[HCO_3^-]$ (Davenport) plot. Initial pH was ≈8 for all groups and declined over time. Symbols as in Figure 2, with day 0 values and termination of experiment values labeled. Values are means for a given sampling day.

this study we found it to be 20.4 mmol/L at a pH of 7.923 (not significantly different from the control pH) after 150 d, a value similar to the 25.2 mmol/L at a pH of 7.81 after 125 d found by Jackson et al. (2000). Therefore, we conclude that *C. p. bellii* do, in fact, have a mixed anaerobiosis/aerobiosis during long-term submergence in normoxic water. Although lactate does accumulate in the blood, concentrations do not reach those found in completely anoxic animals (193 mmol/L); thus, animals in normoxic water must be using avenues of extrapulmonary oxygen uptake that may include the skin, the buccopharyngeal cavity, and the cloacal bursae, although the skin is probably the more important site (D. Jackson, E. Rauer, R. Feldman, and S. Reese, unpublished data). The oxygen extraction abilities of these methods are inadequate to maintain total metabolism; however, the anaerobic component is much smaller than previously reported and does not result in major acid-base disturbances.

Chrysemys picta dorsalis had higher plasma [lactate] than *C. p. bellii* after 150 d submerged in normoxic water. In both subspecies, extrapulmonary uptake of O_2 enabled a largely but not completely aerobic metabolism. Increases in lactate were associated with qualitatively similar but proportionally reduced compensatory responses as seen in anoxic turtles. However, a decrease in P_{CO_2} allowed *C. p. bellii* to maintain pH throughout the 150 d of submergence, while *C. p. dorsalis* had a slight drop in pH because of a higher lactate load and no compensatory P_{CO_2} drop. Despite the slight differences, both subspecies were capable of surviving extended periods of submergence in normoxic water.

Chrysemys picta bellii responded to anoxic submergence as reported in previous studies (Jackson and Ultsch 1982; Ultsch and Jackson 1982; Jackson et al. 2000). Lactate increased to as high as 200 mmol/L in one animal and was buffered by mechanisms that have been reviewed elsewhere (Jackson 2000; Jackson et al. 2001). In brief, the acidosis effects a mobilization of calcium and magnesium carbonates from bone, the dry weight of which accounts for approximately 25% of wet mass (Iverson 1982). The carbonate ions react with protons, producing CO_2 that is lost to the water, and calcium and magnesium ions electrically balance the lactate either by complexing (≈two-thirds of the divalent cation, calcium, has been shown to be complexed at these concentrations) with it or as free ions in solution (Jackson and Heisler 1982). Besides releasing carbonate buffers into the plasma, the shell also sequesters about 47% of the total body lactate and buffers lactate in situ (Jackson 2000; Jackson et al. 2000). The increase in $[K^+]$ and the decrease in $[Cl^-]$ are compensatory in that they increase the strong ion difference (SID) minimizing the fall in pH (Stewart 1981).

Chrysemys picta dorsalis responded to anoxic submergence in a qualitatively similar fashion as *C. p. bellii*, as found by Ultsch et al. (1985). AR *C. p. dorsalis* in this study had a slower pH fall than Alabama turtles from Ultsch et al. (1985; Fig. 2*D*). The faster rate of pH fall in the earlier work is mostly attributable to the retention of CO_2 (Fig. 3). The differences between subspecies submerged in anoxic water can be traced partially to differences in the rates of lactate accumulation, which is lower in *C. p. bellii*, and partially to differences in lactate-buffering ability, which is better in *C. p. bellii*. Comparing the two subspecies at the last sampling date that we have data for both subspecies (day 50), AR *C. p. dorsalis* accumulated lactate at 1.9 mmol/d, while *C. p. bellii* accumulated lactate at 1.4 mmol/d. AL *C. p. dorsalis* accumulated lactate at 2.6 mmol/d using day 46 data. Using $\Delta pH/\Delta[\text{lactate}]$ as a measure of whole-body buffering capacity, the two subspecies differ in end buffering capacity (Fig. 4*A*). Thus, the disparity in survival between *C. p. dorsalis* (≈75 d) and WI *C. p. bellii* (≈150 d) can be partially explained by a difference in acid-base status, resulting from differential lactate accumulation and differential buffering of the accumulating lactate. The accumulation of lactate is probably due to a latitudinal cline in body size. *Chrysemys picta dorsalis* averaged 172.0 g, while *C. p. bellii* averaged 451.7 g, and when weight was used as a covariate comparing lactate accumulation rates, there was no difference between the northern and southern groups. It is interesting that the difference in buffering capacity is evident only after day 75 (*C. p. bellii* was −0.0061/mmol/L and *C. p. dorsalis* was −0.0058/mmol/L from 0 to 75 d, but *C. p. bellii* was 0.0018/mmol/L from 75 to 150 d). This difference in buffering capacity does not appear to be associated with the clinal variation in body size (as evidenced by an ANCOVA) but may stem from dissimilarities in SID compensation that are difficult to detect with our data.

Table 2: Ion statuses of *Chrysemys picta* subspecies from WI, CT, GA, AR, and LA submerged in anoxic and normoxic water at 3°C

	C. p. bellii (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (AR)	*C. p. bellii* (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (LA)
n	10 (0)	14 (0)	5 (0)	5 (0)				
$[Na^+]$ (mmol/L)	130.2 ± 2.1^{AD}	114.8 ± 3.4^{ABC}	122.7 ± 4.1^{CDE}	126.4 ± 5.0^{BE}				
$[K^+]$ (mmol/L)	$2.43 \pm .28$	$2.58 \pm .27$	$2.66 \pm .32$	$2.90 \pm .30$				
$[Cl^-]$ (mmol/L)	81.4 ± 3.4^{A}	74.5 ± 4.4	$73.0 \pm .8^{A}$	80.6 ± 7.9				
Total Ca	$2.5 \pm .6$	$2.8 \pm .56$	2.2 ± 1.0	1.6 ± 1.5				
Total Mg	$2.6 \pm .3$	$2.2 \pm .5$	$2.6 \pm .2$	2.3 ± 1.6				
	Anoxic Water (<5 mmHg)				Normoxic Water (150–160 mmHg)			
n	5 (10)	4 (5)	5 (10)	5 (10)	5 (10)	5 (5)	5 (10)	5 (10)
$[Na^+]$ (mmol/L)	130.8 ± 2.7^{A}	115.5 ± 18.4	118.0 ± 5.8^{AB}	127.2 ± 4.7^{B}	130.4 ± 6.8^{A}	113.1 ± 10.2	113.4 ± 4.6^{AB}	125.3 ± 2.4^{B}
$[K^+]$ (mmol/L)	$3.17 \pm .46$	$2.58 \pm .03$	$3.08 \pm .64$	$4.42 \pm .39$	$2.95 \pm .56$	$2.58 \pm .46$	$2.87 \pm .50$	$3.60 \pm .31$
$[Cl^-]$ (mmol/L)	72.9 ± 10.4	74.5 ± 18.7	67.1 ± 4.1	71.9 ± 4.5	81.7 ± 4.5^{A}	68.6 ± 5.9	64.5 ± 5.4^{A}	75.2 ± 1.8^{A}
Total Ca	3.8 ± 1.3	5.3 ± 1.4	$6.9 \pm .6$	8.7 ± 1.4	$1.6 \pm .4^{A}$	3.3 ± 1.0	4.2 ± 1.2^{A}	6.6 ± 1.8^{A}
Total Mg	$3.9 \pm .7$	3.6 ± 1.4	$4.8 \pm .4$	$4.8 \pm .7$	$2.7 \pm .6^{A}$	2.8 ± 1.6	$3.3 \pm .5^{B}$	$4.7 \pm .4^{AB,\star}$
n	5 (25)	5 (15)	5 (25)	5 (25)	5 (25)	5 (15)	5 (25)	5 (25)
$[Na^+]$ (mmol/L)	127.5 ± 8.7^{BC}	118.1 ± 12.7	115.1 ± 9.2^{AC}	118.2 ± 10.6^{AD}	126.8 ± 2.6^{BC}	106.3 ± 12.0	112.1 ± 6.5^{AB}	114.8 ± 4.0^{AC}
$[K^+]$ (mmol/L)	$3.81 \pm .46$	$3.81 \pm .67$	$4.58 \pm .79$	4.96 ± 1.02	$2.60 \pm .46$	$2.58 \pm .57$	$2.64 \pm .50^{A}$	$3.23 \pm .36$
$[Cl^-]$ (mmol/L)	$65.8 \pm 9.8^{ABC,\star}$	67.8 ± 10.8	$59.3 \pm 7.7^{B,\star}$	$60.0 \pm 7.0^{C,\star}$	78.1 ± 3.0^{CDE}	61.0 ± 11.0	62.0 ± 4.1^{ABD}	66.0 ± 6.8^{BE}
Total Ca	10.0 ± 3.0	10.2 ± 2.0	10.8 ± 3.9	$11.7 \pm 3.2^{\star}$	$1.2 \pm .4^{CDE}$	4.1 ± 1.8	3.3 ± 2.1^{AD}	$8.4 \pm 4.3^{BE,\star}$
Total Mg	$6.9 \pm .7^{\star}$	$4.4 \pm .8$	$6.7 \pm .7^{\star}$	5.3 ± 1.4^{A}	$2.2 \pm .6^{CDE}$	2.7 ± 1.4	2.9 ± 1.5^{AD}	$5.3 \pm 1.8^{BE,\star}$
n	5 (50)	5 (25)	4–5 (50)	5 (50)	4–5 (50)	5 (25)	4 (50)	5 (50)
$[Na^+]$ (mmol/L)	122.9 ± 8.0^{BC}	110.4 ± 7.5^{BD}	113.9 ± 5.2^{AC}	$112.1 \pm 7.3^{AD\star}$	$116.1 \pm 8.5^{BC,\star}$	110.2 ± 10.1	$105.4 \pm 4.4^{AB,\star}$	105.7 ± 7.5^{AC}
$[K^+]$ (mmol/L)	$4.7 \pm .8$	$4.29 \pm .70$	$6.67 \pm 1.01^{\star}$	$6.22 \pm 2.18^{\star}$	$2.4 \pm .4^{D}$	$2.70 \pm .09^{A}$	$2.18 \pm .39^{AC}$	$3.09 \pm .54^{BCD,\star}$
$[Cl^-]$ (mmol/L)	$63.7 \pm 9.3^{ABC,\star}$	$59.6 \pm 6.7^{A,\star}$	$56.9 \pm 3.1^{B,\star}$	$53.1 \pm 9.2^{C,\star}$	70.4 ± 4.4^{CDE}	67.6 ± 10.0^{AC}	$56.9 \pm 4.1^{ABD,\star}$	$59.9 \pm 2.6^{BE,\star}$
Total Ca	$15.2 \pm 2.1^{A,\star}$	11.4 ± 3.8	$23.8 \pm 4.6^{\star}$	$22.6 \pm 6.8^{\star}$	3.3 ± 1.9^{CDE}	4.0 ± 1.6^{ABC}	8.2 ± 1.8^{AD}	6.9 ± 4.9^{BE}
Total Mg	$9.8 \pm 1.1^{\star}$	$9.1 \pm 1.8^{A,\star}$	$10.1 \pm 1.1^{\star}$	$8.3 \pm 2.2^{\star}$	$2.4 \pm .6^{CDE}$	3.7 ± 1.7^{ABC}	$4.5 \pm .8^{AD}$	4.5 ± 1.6^{BE}
n	5 (75)	5 (50)	5 (75)	3–4 (75)	4–5 (75)	5 (50)	5 (75)	5 (75)
$[Na^+]$ (mmol/L)	$116.5 \pm 7.5^{BC,\star}$	114.4 ± 11.6^{BD}	$102.8 \pm 6.8^{AC,\star}$	$110.1 \pm 13.5^{AD,\star}$	118.0 ± 4.3^{BC}	111.9 ± 12.1	$101.1 \pm 3.7^{AB,\star}$	103.8 ± 4.6^{AC}
$[K^+]$ (mmol/L)	$5.30 \pm .90^{ABC,\star}$	$6.32 \pm .93^{\star}$	$8.84 \pm 1.98^{B,\star}$	$7.64 \pm 1.23^{C,\star}$	$2.73 \pm .4^{D}$	$2.41 \pm .45^{AB}$	$2.72 \pm .77^{AC}$	$3.65 \pm 1.14^{BCD,\star}$
$[Cl^-]$ (mmol/L)	57.1 ± 5.6^{ABC}	$58.9 \pm 8.9^{A,\star}$	$46.1 \pm 4.2^{B,\star}$	$55.9 \pm 10.9^{C,\star}$	68.6 ± 10.2^{CDE}	68.2 ± 8.5^{AC}	$53.2 \pm 4.4^{ABD,\star}$	$57.7 \pm 5.8^{BE,\star}$
Total Ca	$24.3 \pm 6.3^{AB,\star}$	$26.8 \pm 11.5^{A,\star}$	$34.1 \pm 5.7^{B,\star}$	$32.0 \pm 10.9^{\star}$	2.7 ± 1.7^{CDE}	3.3 ± 1.0^{ABC}	7.9 ± 2.4^{AD}	7.4 ± 4.2^{BE}
Total Mg	$12.2 \pm 2.5^{\star}$	$10.8 \pm 3.2^{\star}$	$12.2 \pm 1.1^{\star}$	$11.3 \pm 1.2^{\star}$	$2.8 \pm .9^{CDE}$	3.1 ± 1.0^{ABC}	5.0 ± 1.1^{AD}	$4.8 \pm 1.1^{BE,\star}$

n	5 (100)	5 (75)	5 (100)	5–7 (100)	5 (100)	5 (75)	5 (100)	5–7 (100)
$[Na^+]$ (mmol/L)	120.2 ± 5.9^{A}	109.2 ± 18.5^{BD}	113.1 ± 11.7^{BC,*}	111.4 ± 5.8	106.2 ± 3.5^{AB,*}	100.2 ± 16.1^{AC,*}	108.5 ± 6.3	
$[K^+]$ (mmol/L)	7.33 ± 1.02^{A,*}	7.95 ± 1.23^{A,*}	2.88 ± .57	3.27 ± .39^{AB}	3.17 ± .74^{A}	3.66 ± .70	3.58 ± .55^{A,*}	
$[Cl^-]$ (mmol/L)	52.0 ± 2.7*	53.4 ± 11.6^{A,*}	62.4 ± 8.6^{CDE,*}	58.7 ± 10.8^{AC}	49.8 ± 3.6^{ABD,*}	56.4 ± 13.8^{BE,*}	56.4 ± 9.9^{AC,*}	
Total Ca	35.5 ± 7.7^{A,*}	34.6 ± 7.2^{A,*}	3.0 ± 1.3^{CDE}	5.1 ± 4.6^{ABC}	8.3 ± 2.8^{AD}	7.6 ± 5.2^{BE,*}	7.9 ± 3.4^{ABC,*}	
Total Mg	14.3 ± 1.9*	11.8 ± 1.6*	3.0 ± .4^{CDE}	4.3 ± 1.8^{ABC}	5.4 ± .9^{A,*}	4.3 ± 1.4^{BE}	4.7 ± 1.0^{ABCD,*}	
n	4–5 (125)	5 (100)	5 (125)	5 (100)	5 (125)	5 (125)		
$[Na^+]$ (mmol/L)	110.6 ± 10.6*	109.6 ± 7.0^{A}	108.7 ± 11.6^{BC,*}	115.3 ± 11.3	91.6 ± 4.6^{AB,*}	97.2 ± 11.3^{AC,*}		
$[K^+]$ (mmol/L)	7.57 ± .95^{A,*}	10.86 ± 3.36^{A,*}	2.67 ± .64^{B}	3.28 ± .83^{A}	3.49 ± .63^{A}	3.64 ± 1.66		
$[Cl^-]$ (mmol/L)	50.2 ± 8.0*	49.7 ± 5.8*	58.6 ± 15.6^{CDE,*}	60.3 ± 14.5^{AC}	40.2 ± 4.1^{ABD,*}	55.1 ± 11.6^{BE,*}		
Total Ca	42.0 ± 9.7^{A,*}	48.6 ± 10.2^{A,*}	4.4 ± 3.4^{CDE}	7.6 ± 4.4^{ABC,*}	12.5 ± 3.4^{AD,*}	6.6 ± 2.9^{BE}		
Total Mg	15.3 ± 2.8*	15.8 ± 4.8*	3.9 ± 2.0^{CDE}	4.4 ± 1.5^{ABCD,*}	6.3 ± 1.2^{AD,*}	4.2 ± 1.7^{BE}		
n	5 (150)	4 (125)	5 (150)	4 (125)	5 (150)	6 (150)		
$[Na^+]$ (mmol/L)	107.0 ± 8.9*	111.0 ± 12.4	108.5 ± 7.9^{BC,*}	111.0 ± 12.4	91.8 ± 10.4^{AB,*}	97.7 ± 13.8^{AC,*}		
$[K^+]$ (mmol/L)	8.46 ± 1.43*	12.00 ± 3.00^{A,*}	2.75 ± .82	12.00 ± 3.00^{AB,*}	2.20 ± .97^{A}	3.39 ± .95		
$[Cl^-]$ (mmol/L)	50.9 ± 5.1*	50.5 ± 7.2*	67.8 ± 8.5^{CDE}	50.5 ± 7.2^{AC,*}	47.1 ± 9.5^{ABD,*}	54.7 ± 6.2^{BE,*}		
Total Ca	53.1 ± 4.4*	56.5 ± 12.0^{A,*}	3.5 ± .8^{CDE}	56.5 ± 12.0^{ABC}	8.7 ± 3.9^{AD,*}	8.0 ± 1.7^{BE,*}		
Total Mg	17.8 ± 2.2*	16.5 ± 3.8*	3.2 ± 1.1^{CDE}	16.5 ± 3.8^{ABC}	4.8 ± 1.8^{A}	4.6 ± .9^{BE,*}		

Note. WI = Wisconsin, CT = Connecticut, GA = Georgia, AR = Arkansas, LA = Lousiana. Values are mean ± 95% confidence interval. Days submerged are in parentheses after the sample size (*n*). Data for *C. p. picta* from CT are from Ultsch et al. (1999). Letters indicate significant differences for a given sampling day within a particular treatment.

* Significantly different from controls.

Table 3: Blood and plasma variables of *Chrysemys picta* subspecies from WI, CT, GA, AR, and LA submerged in anoxic and normoxic water at 3°C

	C. p. bellii (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (AR)	*C. p. bellii* (WI)	*C. p. picta* (CT)	*C. p. picta* (GA)	*C. p. dorsalis* (LA)
n	10 (0)	14 (0)	5 (0)	5 (0)				
Hematocrit (%)	27.5 ± 2.7^{AB}	24.7 ± 3.5^{C}	22.1 ± 4.0^{ABC}	24.4 ± 4.3^{B}				
[Glucose] (mmol/L)	5.3 ± 2.5	2.8 ± 1.0	2.5 ± .8	3.4 ± 1.1				
Osmolality (mOsm/L)	270.7 ± 7.0	241.0 ± 8.0	237.0 ± 9.1	251.8 ± 6.6				
	Anoxic Water (<5 mmHg)				Normoxic Water (150 ± 160 mmHg)			
n	5 (10)	4–6 (5)	5 (10)	5 (10)	5 (10)	5 (5)	5 (10)	5 (10)
Hematocrit (%)	37.2 ± 4.8^{AB}	32.7 ± 8.2	21.5 ± 5.8^{A}	31.6 ± 7.5^{B}	43.3 ± 4.7^{AB}	34.8 ± 7.7	27.0 ± 3.5^{A}	30.9 ± 1.6^{B}
[Glucose] (mmol/L)	2.9 ± .5	3.8 ± 1.6	3.4 ± .7	5.2 ± 3.4	3.4 ± 1.0^{A}	4.0 ± 1.8	1.7 ± .3^{AB}	4.1 ± .9^{B}
Osmolality (mOsm/L)	272.1 ± 7.9	251.2 ± 30.0	261.3 ± 10.6	288.7 ± 16.0	262.1 ± 16.5^{A}	247.2 ± 32.2	238.3 ± 11.7^{AB}	272.1 ± 11.8^{B}
n	5 (25)	5 (15)	5 (25)	5 (25)	5 (25)	5 (15)	5 (25)	5 (25)
Hematocrit (%)	39.0 ± 5.4^{AB,*}	36.3 ± 3.8*	24.7 ± 6.9^{AC}	29.1 ± 8.1^{B}	37.9 ± 3.2^{CD}	38.2 ± 8.4*	34.6 ± 2.6^{AC,*}	30.9 ± 1.6^{ABD}
[Glucose] (mmol/L)	3.2 ± 1.5	4.0 ± 1.4	2.4 ± .4	3.4 ± 1.5	2.5 ± .5	1.8 ± 1.7	1.8 ± .4^{B}	4.1 ± .9^{AB}
Osmolality (mOsm/L)	280.5 ± 7.7	283.0 ± 21.0	277.9 ± 20.4	268.2 ± 23.0	240.2 ± 4.7^{AB}	246.0 ± 17.0	237.8 ± 17.0^{B}	272.1 ± 11.8
n	5 (50)	5 (25)	5 (50)	5 (50)	5 (50)	5 (25)	4 (50)	5 (50)
Hematocrit (%)	40.2 ± 8.5^{AB,*}	32.2 ± 6.0^{C}	25.5 ± 3.5^{AC}	24.2 ± 9.4^{B}	48.3 ± 14.3^{CD,*}	41.8 ± 3.9^{B,*}	32.8 ± 8.8^{BC}	38.6 ± 4.0^{ABD}
[Glucose] (mmol/L)	3.5 ± 1.0	2.6 ± .8	5.9 ± 2.7	2.2 ± .4	2.8 ± 1.0	1.6 ± 1.2^{A}	1.0 ± .5^{B}	1.6 ± 1.0^{AB}
Osmolality (mOsm/L)	312.8 ± 24.5	289.6 ± 48.5	330.5 ± 13.5*	308.5 ± 29.0	267.8 ± 74.4^{CD}	252.0 ± 34.0^{A}	242.8 ± 18.1^{BD}	308.5 ± 29.0^{AB,*}
n	5 (75)	5 (50)	5 (75)	4 (75)	5 (75)	5 (50)	5 (75)	5 (75)
Hematocrit (%)	30.3 ± 6.8^{AB}	36.4 ± 9.1^{C}	24.7 ± 5.5^{AC}	30.0 ± 18.4^{B}	51.7 ± 11.9^{CD,*}	41.9 ± 4.7^{A,*}	36.4 ± 5.3^{AC,*}	43.2 ± 8.6^{ABD,*}
[Glucose] (mmol/L)	5.6 ± 1.8	2.5 ± 1.3	11.3 ± 10.6	12.8 ± 16.7	3.1 ± 1.4	1.9 ± .7^{A}	1.2 ± .3^{B}	1.2 ± 1.1^{AB}
Osmolality (mOsm/L)	329.5 ± 20.7*	328.4 ± 34.6*	345.5 ± 36.6*	362.8 ± 55.8*	246.2 ± 14.8^{CDE}	231.6 ± 22.2^{AC}	242.2 ± 13.4^{BD}	308.5 ± 29.0^{ABE,*}
n	5 (100)	5 (75)			5 (100)	5 (75)	5 (100)	7 (100)
Hematocrit (%)	35.1 ± 7.3	31.6 ± 6.3^{C}			54.9 ± 11.4^{CD,*}	42.0 ± 5.7^{B,*}	40.0 ± 6.1^{BC,*}	41.0 ± 10.5^{ABD,*}
[Glucose] (mmol/L)	4.8 ± 2.1^{A}	6.6 ± 7.0			4.0 ± 1.5	1.5 ± .7^{A}	1.6 ± .6^{B}	7.9 ± 8.9^{AB}
Osmolality (mOsm/L)	356.2 ± 17.8^{A,*}	331.4 ± 49.3*			234.2 ± 22.1^{AB}	233.0 ± 13.7^{AC}	235.3 ± 14.2^{B}	231.0 ± 33.2
n	5 (125)	5 (100)			5 (125)	5 (100)	5 (125)	5 (125)
Hematocrit (%)	34.5 ± 7.6	28.0 ± 11.1			40.4 ± 19.6^{DE}	46.4 ± 3.8^{A,*}	30.9 ± 5.3^{ACD}	34.4 ± 13.2^{BCE}
[Glucose] (mmol/L)	7.3 ± 5.9^{A}	32.8 ± 21.5^{A,*}			3.3 ± 1.3	1.4 ± .8^{A}	3.9 ± 3.4^{B}	4.6 ± 6.6^{AB}
Osmolality (mOsm/L)	362.7 ± 21.6^{A,*}	382.9 ± 71.9^{A,*}			230.6 ± 23.2^{AB}	226.0 ± 25.9^{A}	211.6 ± 14.4^{B}	219.5 ± 20.1
n	5 (150)	4 (125)			5 (150)	5 (125)	5 (150)	5 (150)
Hematocrit (%)	36.3 ± 11.0	30.5 ± 16.0			51.9 ± 8.1^{DE,*}	48.9 ± 3.3^{AB,*}	28.9 ± 4.6^{ACD}	44.2 ± 6.5^{BCE,*}
[Glucose] (mmol/L)	14.8 ± 12.4*	36.1 ± 8.4^{A,*}			5.8 ± 2.6^{CD}	4.3 ± 6.3^{A}	2.0 ± 1.4^{BD}	5.7 ± 6.7^{AB}
Osmolality (mOsm/L)	419.2 ± 20.5*	410.8 ± 67.6^{A,*}			244.6 ± 14.6^{AB}	206.1 ± 19.3^{A,*}	197.1 ± 38.0^{B}	235.9 ± 44.9
n						5 (150)		
Hematocrit (%)						54.4 ± 12.6^{AB,*}		
[Glucose] (mmol/L)						1.6 ± .5^{AC}		
Osmolality (mOsm/L)						223.7 ± 12.5^{A}		

Note. WI = Wisconsin, CT = Connecticut, GA = Georgia, AR = Arkansas, LA = Lousiana. Values are mean ± 95% confidence interval. Days submerged are in parentheses after the sample size (*n*). Data for *C. p. picta* from CT are from Ultsch et al. (1999). Letters indicate significant differences for a given sampling day within a particular treatment.

* Significantly different from controls.

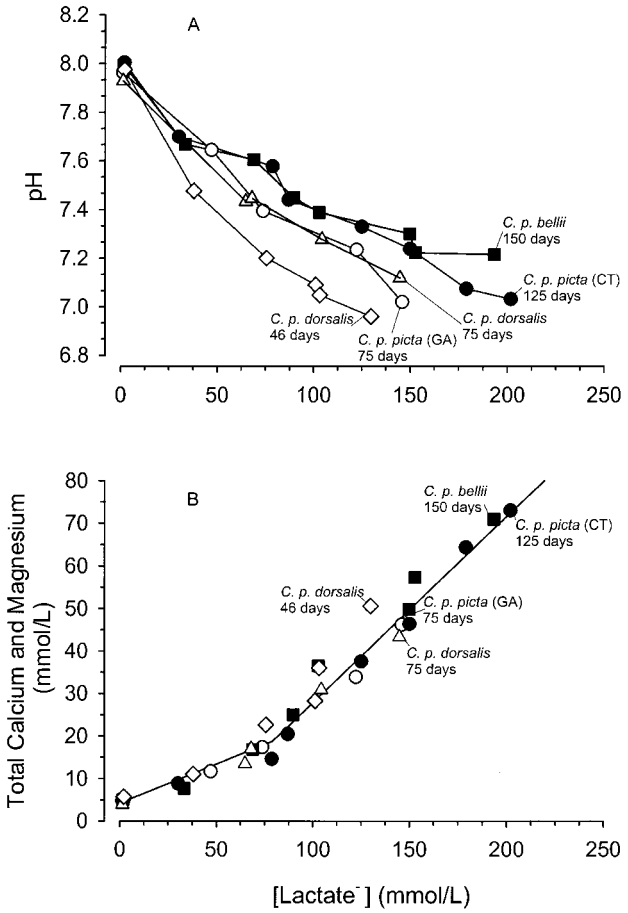

Figure 4. *A*, Whole-body buffering of plasma lactate for turtles submerged in anoxic water at 3°C. Initial pH was ≈8 for all groups and declined over time. Termination of experiment values for each experiment are labeled. Values are means for a given sampling day. *B*, Ratio of total calcium and magnesium released to plasma lactate evolved in three subspecies of painted turtle from four geographic locations. The line is fitted using the method of Yeager and Ultsch (1989). Values are means for a given sampling day.

*Georgia versus Connecticut Eastern Painted Turtles (*Chrysemys picta picta*)*

All subspecies, regardless of latitude of origin, have no marked differences among their physiological responses to submergence in normoxic water, which effect only minor acid-base and ionic perturbations (Tables 1–3). There is also no clinal variation in body size within this subspecies (CT *Chrysemys picta picta* averaged 257.7 g and GA *C. p. picta* averaged 261.3 g). In anoxic water, physiological responses found between GA *C. p. picta* and CT *C. p. picta* were significant, although not as dramatic as those found between *C. p. bellii* and *C. p. dorsalis*. The rate of pH fall in GA *C. p. picta* (0.016/d) was higher than that seen in CT *C. p. picta* (0.010/d) after 75 d of submergence, but the rate of lactate accumulation was similar (Fig. 2*C*, 2*D*; Table 1). Whole-body buffering was reduced in the southern population of *C. p. picta* (Fig. 4*A*).

Although this reduced buffering ability may account for some of the difference in survival between northern and southern groupings of *C. p. picta*, there are two points that suggest another unmeasured variable is also influencing survival in these turtles. First, the difference in survival between the northern and southern *C. p. picta* is similar to that between *C. p. bellii* and *C. p. dorsalis* over similar latitudinal ranges, but the physiological responses are not equally different. Second, GA *C. p. picta* were studied on two separate years, 2000 and 2002. The day 75 pH values for those 2 yr differ quite dramatically (7.226 ± 0.070 for 2000 and 6.816 ± 0.145 for 2002), and turtles were dead by day 75 in 2002, while turtles in 2000 were not dying until day 84. Thus, the physiological response to anoxia in *C. p. picta* is quite variable, and acid-base status is probably only part of the reason that GA *C. p. picta* do not survive as long as CT *C. p. picta* in anoxic submergence. One hypothesis that may help explain an earlier death is that the Georgia turtles started submergence with lower glycogen stores than the Connecticut turtles and, lacking a proportionately lowered metabolic rate, depleted those stores sooner.

Intraspecific and Interspecific Considerations

One variable that responded similarly to normoxic submergence in most groups was an increase in hematocrit (Table 3). This same response has been seen in several studies (Ultsch et al. 1985; Ultsch and Cochran 1994; Reese et al. 2000, 2001; Saunders et al. 2000) and may be a response to increase oxygen carrying capacity of the blood by increasing red blood cell count (Saunders et al. 2000). However, map turtles exposed to low temperatures sequestered plasma in parts of the peripheral vasculature, which may also account for the increase in hematocrit in these species (Semple et al. 1970; Stitt and Semple 1971; Stitt et al. 1971). In addition to the increased hematocrit, Maginniss et al. (1983) found that cold exposure causes an extreme left shift in the oxygen dissociation curve of blood from *Chrysemys picta* that would help increase the ability of the turtles to pick up oxygen from the water.

Another aspect that is similar in all groups is the calcium and magnesium buffering response to the lactacidosis during anoxic submergence. Similar amounts of lactate mobilize similar amounts of calcium/magnesium as pH falls (Fig. 4*B*), with a slope of 0.43 ($r^2 = 0.98$) for a [lactate] >60 mmol/L. This is close to the slope expected because each Ca or Mg can electrically neutralize two lactates, while the associated carbonate anion can neutralize two protons. The lower slope of the line before the break is due to the combined buffering of endogenous plasma [HCO_3^-] and released Ca and Mg carbonates.

In summary, we conclude that all subspecies of painted turtles, regardless of latitudinal origin, can tolerate periods of continuous submergence in normoxic water that is in excess of

that required in their natural habitats, with only minor perturbations of their acid-base and ionic statuses. *Chrysemys picta bellii* and *C. p. picta* at the northern limits of their ranges, if they overwinter in anoxic hibernacula (e.g., mud) for prolonged periods, will experience profound physiological challenges, which they are able to meet because of the extraordinary buffering abilities found in all painted turtles. Southern groups of *Chrysemys picta* have a reduced survival during anoxic submergence that may be partially accounted for by differences in lactate accumulation and buffering ability, but there may be additional factors that differentiate *C. p. dorsalis* from *C. p. bellii* and CT *C. p. picta* from GA *C. p. picta*.

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