The physiological effects of multiple forced submergences in loggerhead sea turtles (*Caretta caretta*)

Erich K. Stabenau

Kimberly R. N. Vietti

Department of Biology
Bradley University
1501 W. Bradley Ave.
Peoria, Illinois 61625

E-mail address (for E. K. Stabenau): eks@bradley.edu

Abstract—Sea turtles are subjected to involuntary submergence and potential mortality due to incidental capture by the commercial shrimp fishing industry. Despite implementation of turtle excluder devices (TEDs) to reduce at-sea mortality, dead stranded turtles continue to be found in near-record numbers along the coasts of the western Atlantic Ocean and northern Gulf of Mexico. Although this mortality may be due to an increase in the number of turtles available to strand, one alternative explanation is that sea turtles are repetitively submerged (as one fishing vessel follows the path of another) in legal TEDs. In the present study, laboratory and field investigations were undertaken to examine the physiological effects of multiple submergence of loggerhead sea turtles (*Caretta caretta*). Turtles in the laboratory study were confined during the submersion episodes, whereas under field conditions, turtles were released directly into TED-equipped commercial fishing nets. Under laboratory and field conditions, pre- and postsubmergence blood samples were collected from turtles submerged three times at 7.5 min per episode with an in-water rest interval of 10, 42, or 180 min between submergences. Analyses of pre- and postsubmergence blood samples revealed that the initial submergence produced a severe and pronounced metabolic and respiratory acidosis in all turtles. Successive submergences produced significant changes in blood pH, Pco_2, and lactate, although the magnitude of the acid-base imbalance was substantially reduced as the number of submergences increased. In addition, increasing the interval between successive submergences permitted greater recovery of blood homeostasis. No turtles died during these studies. Taken together, these data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential provided that the animal has an adequate rest interval at the surface between successive submergences.

Manuscript approved for publication 25 March 2003 by Scientific Editor.

Manuscript received 26 June 2003 at NMFS Scientific Publications Office.

Fish. Bull. 101:889–899 (2003).

The five sea turtle species inhabiting the waters of the U.S. Gulf of Mexico and Atlantic Ocean are considered to be threatened or endangered. One contributing factor to sea turtle mortality is incidental capture in the nets of commercial shrimping vessels. The National Research Council's Committee on Sea Turtle Conservation (1990) suggested that as many as 5500 to 55,000 loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempi*) sea turtles were killed annually during shrimping-related activities. More recently, two independent studies statistically confirmed the relationship between shrimping activity and the appearance of stranded sea turtles in the U.S. Gulf of Mexico and the Atlantic Ocean (Caillouet et al., 1991; Crowder et al., 1995). Because of the impact of trawl-related mortality on sea turtle populations, the U.S. government passed regulations in 1987 requiring that commercial shrimping vessels pull nets equipped with certified turtle excluder devices (TEDs). TEDs are designed to exclude any turtle that may enter into shrimping nets, while not affecting the catch of the target species. Crowder et al. (1995) reported that the sea turtle population off the coast of South Carolina continued to decline when TED regulations were implemented; however, the rate of decline decreased significantly after full-time TED use.

In spite of the TED regulations, near-record numbers of dead stranded sea turtles have been found on U.S. Gulf of Mexico and Atlantic Ocean beaches (Shaver-Miller[1]). Although there may be other man-related or natural causes for this continued sea turtle mortality, there are two plausible reasons for the increased mortality during shrimping activities. First, commercial shrimp fishermen generally do not carry legally certified TEDs in their trawl nets and the TEDs that are used are often installed incorrectly or purposely sewn shut. Second, the shrimp fishermen may pull legal TEDs; however, the turtles are repetitively submerged as they are caught in the TEDs of vessels that follow each other. These successive submergences may exacerbate the physiological effects experienced by sea turtles during a forced submersion, and thus, may limit their survival potential.

Sea turtles spend approximately 99% of their time under the surface of the water. During the brief period at the surface, the turtle will exhale and inhale a solitary breath and then dive under the surface (Jackson, 1985). In fact, multiple breaths by sea turtles are generally seen only after prolonged dives. Minimal information is available on the physiological effects of forced submergences of sea turtles. It has been suggested that voluntary dives by sea turtles are aerobic in nature (Wood et al., 1984), whereby oxygen availability minimizes the metabolic production of lactic acid. The turtles may accumulate carbon dioxide, resulting in a respira-

[1] Shaver-Miller, D. 2002. Personal commun. Texas coordinator, Sea Turtle Stranding and Salvage Network, USGS, Corpus Christi, Texas 78406.

tory acidosis that is ameliorated by hyperventilation at the surface. Therefore, voluntary diving in the absence of any other external stressor does not limit sea turtle survival potential.

In contrast, forced submergence of Kemp's ridley and loggerhead sea turtles produces significant blood respiratory and metabolic derangements. Stabenau et al. (1991) reported that forced submergence of Kemp's ridley sea turtles for less than 7.5 min in shrimp nets equipped with TEDs resulted in significant increases in blood lactic acid and Pco_2, and decreases in blood pH. Moreover, several hours were required for these turtles to fully recover blood homeostasis (National Marine Fisheries Service, unpubl. data[2]). However, the study by Stabenau et al. (1991) did not address the physiological effects of multiple forced submergences of sea turtles. It is plausible that repeated submergence induces progressive, significant blood acid-base disturbances, and limits sea turtle survival potential. Therefore, the present study examined the physiological effects of multiple forced submergences on loggerhead sea turtles.

This investigation was divided into two phases. First, a laboratory component was conducted to examine the feasibility of a multiple submergence study. This phase of the research permitted characterization of the magnitude of the acid-base disturbance under controlled conditions. Second, a field investigation was conducted to expose turtles to TED-equipped commercial fishing nets. Data from these studies may offer greater insight into potential sea turtle mortality caused by multiple capture in commercial shrimping nets carrying legal TEDs.

Materials and methods

Laboratory study

Thirty-nine headstarted 2-year-old loggerhead sea turtles reared in captivity at the National Marine Fisheries Service (NMFS) Galveston Laboratory were used in this phase of the study. Each turtle was randomly placed into experimental (submerged, 37.0 ±0.2 cm, 6.51 ±0.06 kg, n=21) or control (nonsubmerged, 36.9 ±0.2 cm, 6.45 ±0.10 kg, n=18) treatments. All turtles were of comparable size and weight and therefore any alterations in blood parameters between experimental and control turtles represented treatment effects rather than size effects. It should be noted that the turtles used in our study were representative of the average size of dead stranded turtles and those animals used in annual TED certification tests.

The study was initiated by collecting presubmergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted canvas bag. Each turtle was then submerged for 7.5 min in seawater filled tanks. Postsubmergence blood samples were collected within 30 s of bringing the turtle out of the water to minimize blood acid-base changes. Following an in-water rest interval of 10 (treatment 1), 42 (treatment 2), or 180 (treatment 3) min, a presubmergence blood sample was collected and the turtle was submerged a second time. A postsubmergence blood sample was then collected immediately upon surfacing. The turtle was then submerged a third time, following the same rest interval between the first and second submergence episodes, and pre- and postsubmergence blood samples were collected as described above. The seventh serial blood sample was collected 180 min after the final submergence in all turtles. Blood samples were also collected from control turtles over the same time intervals to ensure that repetitive handling and blood sampling did not alter blood homeostasis. All blood samples were collected into heparinized vacutainers from the dorsal cervical sinus as described by Owens and Ruiz (1980). No more than 4–6% of blood volume was collected during the serial sampling to minimize potential physiological effects associated with blood volume depletion.

Field study

Thirty-six headstarted 2-year-old loggerhead sea turtles reared in captivity from the NMFS Galveston Laboratory were used in this phase of the study. The turtles were transported from Galveston, TX, to Panama City, FL, where they were placed into large pens in St. Andrews Bay. The submergence study was initiated after a minimum of 21 days of natural conditioning in the in-water pens. Each turtle was randomly placed into experimental (submerged, 35.9 ±0.2 cm, 6.77 ±0.09 kg, n=24) or control (nonsubmerged, 35.4 ±0.3 cm, 6.46 ±0.12 kg, n=12) treatments. As in the laboratory study, all experimental and control turtles were of comparable size and weight.

The study was initiated by collecting presubmergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted mesh bag. Each turtle was then submerged using the standard protocol for TED certification tests. Briefly, the mesh bag containing a turtle was placed onto a line connecting the trawl vessel to the headrope on the shrimp net. Divers then released the turtle (without handling the animal) into the mouth of the trawl. Often, turtles were observed vigorously swimming in the trawl until being overcome by the net. Although the shrimp net was equipped with a TED, divers held the escape door closed for 5 min. The turtle was then permitted to leave the trawl and surface. Thus, the total submergence time was approximately 7.5 min, including the time for the weighted mesh bag containing the turtle to reach the headrope for release into the trawl, the 5 min within the trawl, and the time for the turtle to surface. Turtles were immediately captured at the surface and returned to the trawl vessel for postsubmergence blood sampling. Typically, postsubmergence blood samples were collected within 1–2 min of the turtle surfacing. Following a rest interval of 10 (treatment 4), 42 (treatment 5), or 180 (treatment 6) min in water-filled containers on the trawl vessel, a presubmergence blood sample was collected and the turtle was submerged a second time. A postsubmergence blood sample was then collected immediately upon surfacing. The turtle was then submerged a third time,

[2] National Marine Fisheries Service. 1994. Unpubl. data. [Available from E. K. Stabenau, Bradley University, 1501 W. Bradley Ave., Peoria, IL 61625.]

following the same rest interval between the first and second submergence episodes, and pre- and postsubmergence blood samples were collected as described above. A seventh serial blood sample was collected 180 min after the final submergence in all turtles. Blood samples were also collected from nonsubmerged control turtles over the same time intervals to ensure that repetitive handling and blood sampling did not alter blood homeostasis. The blood sampling technique and volume collected was identical to that described for the laboratory component of the study.

Blood and plasma analyses

In the laboratory study, blood Pco_2 and pH were analyzed immediately following collection by using a clinical blood gas analyzer with electrodes thermostatted at 37°C. Both variables were corrected to turtle cloacal temperature using requisite correction factors for sea turtle blood and plasma (Stabenau and Heming, 1994). In the field study, blood gases (Po_2 and Pco_2) and pH were analyzed on the trawl vessel immediately following collection using a blood gas analyzer with electrodes thermostatted to turtle body temperature (27–28.5°C). The remaining analyses were comparable for both the laboratory and field components of the submergence study. Packed red cell volume (hematocrit) was determined by following centrifugation of heparinized microcapillary tubes. Two hundred microliters of whole blood were then added to 10% trichloroacetic acid for lactate analysis. The deproteinized samples were centrifuged, and the supernatant removed and stored at –70°C. Lactate was determined spectrophotometrically by using standard enzymatic techniques (Sigma, kit 826-B, Saint Louis, MO). The remaining whole blood was then centrifuged, the plasma removed and stored at –70°C. Plasma Na^+ and K^+ were measured with flame photometry (Jenway, model PFP7, Essex, England), and plasma Cl^- was determined with electrometric titration (Haake-Bucher, model 4425000, Saddle Brook, NJ). Plasma glucose was measured spectrophotometrically (Sigma, kit 16-20), and plasma osmolality was determined with a vapor pressure osmometer (Wescor, model 5500, Logan, UT). For the laboratory study, plasma norepinephrine was analyzed with HPLC (BAS, model LC-300, West Lafayette, IN).

All data are expressed as means ±SE. Where appropriate, the data was analyzed with one-way ANOVA. *Post-hoc* comparisons between means were analyzed with Tukey's multiple comparison test. A fiduciary level of $P \leq 0.05$ was regarded as significant.

Results

Blood pH, Pco_2, and lactate

The initial submergence of loggerhead sea turtles under laboratory and field conditions produced a dramatic and severe acidosis in all experimental turtles. Blood pH fell an average of 0.54 ±0.03 (range 0.49 to 0.59 pH units) and 0.63 ±0.06 (range 0.53 to 0.73 pH units) in laboratory turtles and field turtles, respectively, following initial submergence (Figs. 1A and 2A). The blood acidosis was derived from respiratory and metabolic components as evident from a positive proton-lactate deficit (Buffer capacity×ΔpH–Δ[lactate]), and from significant increases in blood Pco_2 and lactate (Figs. 1 and 2). The initial submergence also produced significant decreases in blood Po_2 and increases in plasma norepinephine ($P \leq 0.05$, $n=24$ for Po_2 and $n=11$ for norepinephrine). In contrast, minimal changes in blood pH, Pco_2, and lactate were observed following collection of the first two blood samples in nonsubmerged control turtles (Figs. 1 and 2).

Recovery of the respiratory and metabolic derangements in submerged turtles was dependent on the interval between successive submergences. A 10-min in-water rest interval between the first and second submergence (treatment-1 and -4 turtles) permitted partial recovery of blood pH (Figs. 1A and 2A) and Pco_2 (Figs. 1B and 2B), but blood pH remained significantly different from presubmergence values. Washout of additional lactate was also detected in these animals, whereby blood lactate concentration increased higher than the postsubmergence value (Figs. 1C and 2C). Turtles with a 42-min surface interval (treatment-2 and -5 turtles) between the first and second submergence had partial to complete recovery of blood pH (Figs. 1A and 2A), complete recovery of blood Pco_2 (Figs. 1B and 2B), and slight recovery of blood lactate (Figs. 1C and 2C). Only the blood lactate remained significantly different from the initial presubmergence value after the 42-min rest interval. Turtles with a 180-min in-water recovery interval (treatment-3 and -6 turtles) showed complete recovery of blood pH and Pco_2, although the lactate concentration was slightly higher than baseline levels (Figs. 1 and 2). Blood Po_2 and plasma norepinephrine recovered completely regardless of the surface interval ($P>0.05$, $n=24$ and $n=11$ for Po_2 and norepinephrine, respectively). Nonsubmerged control turtles in the laboratory and the field exhibited few significant changes in blood pH, Pco_2, or lactate, whether the interval between the second and third serial blood sample was 10, 42, or 180 min (Figs. 1 and 2).

The second 7.5-min submergence produced a drop in blood pH and an increase in Pco_2 (Figs. 1 and 2) in all of the experimental animals, and significant differences occurred in treatment 2–6 turtles. It is noteworthy, however, that the severity of the acid-base imbalance was not as drastic as the acidosis measured following the first submergence. The mean pH difference (ΔpH) between the second pre- and postsubmergence ranged from 0.11 and 0.16 in treatment-1 and -4 turtles (animals with a 10-min interval between submergences), respectively, to 0.50 in treatment-3 turtles and 0.66 in treatment-6 turtles (animals with a 180-min interval between submergences). The acidosis in treatment-1 and -4 turtles resulted, in part, from the continual elevation in blood lactate. In contrast, the longer surface interval between the two submergence episodes resulted in enhanced recovery of acid-base variables. Therefore, the turtles with a surface interval of 42 or 180 min had increased production of CO_2 and lactate in relation to turtles with a brief surface interval (Figs. 1 and 2). Comparable changes in blood Po_2 and norepinephrine were measured following the second submergence ($P \leq 0.05$, $n=24$ and $n=9$

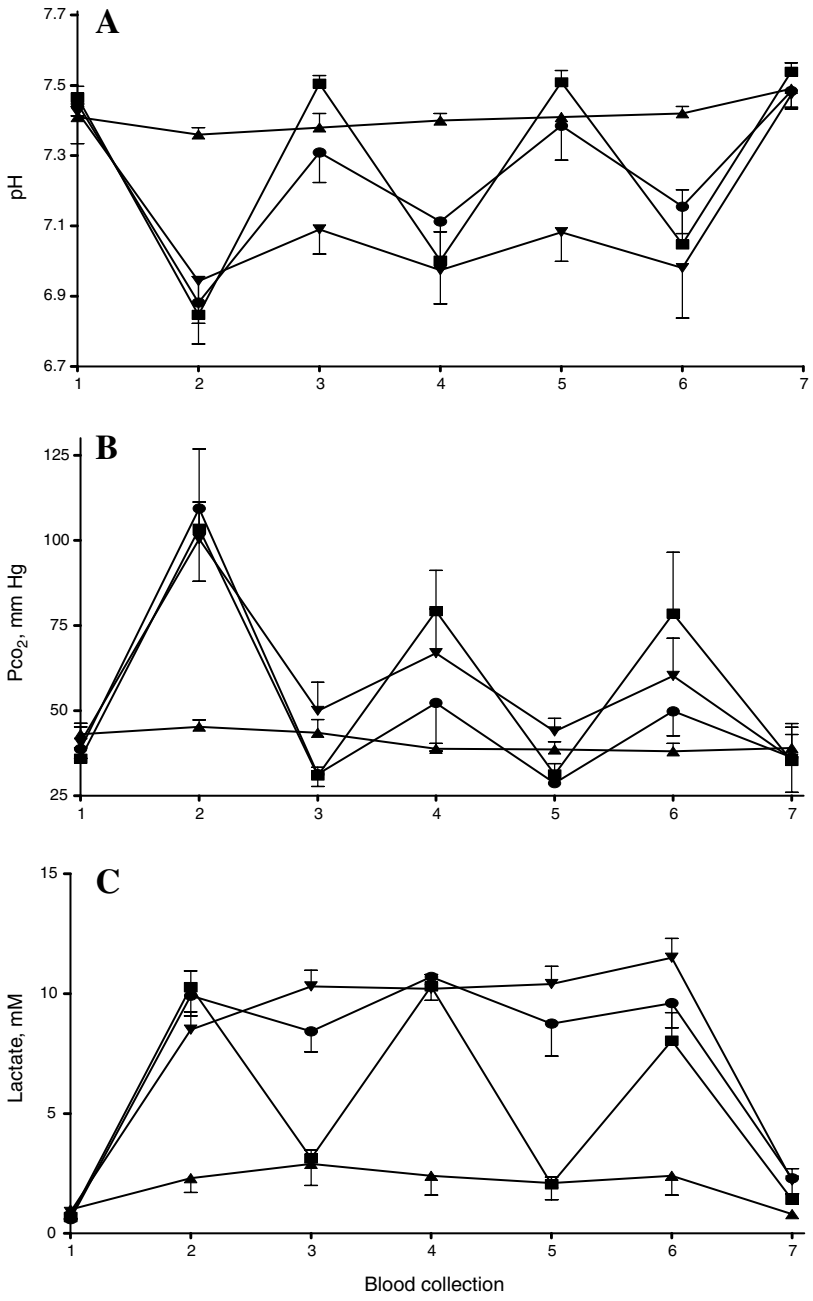

Figure 1

Blood pH (**A**), Pco_2 (**B**), and lactate (**C**) measured prior to and after three successive forced submergence episodes in loggerhead sea turtles in the laboratory. Blood collection 1, 3, and 5 are presubmergence samples, whereas blood collection 2, 4, and 6 are postsubmergence samples. Blood collection 7 was taken 180 min after the final submergence. The surface interval between the submergences was 10 min (▼), 42 min (●), or 180 min (■). Data from control sea turtles (▲) are shown for comparison.

for Po_2 and norepinephrine, respectively). Collection of the fourth sample from nonsubmerged control turtles revealed no significant changes in blood pH, Pco_2, or lactate when compared to the third sample (Figs. 1 and 2).

The remaining serial blood samples revealed comparable patterns in the blood pH, Pco_2, Po_2, lactate, and norepinephrine. Turtles given a longer rest interval at the surface (after the second submergence) had enhanced recovery of

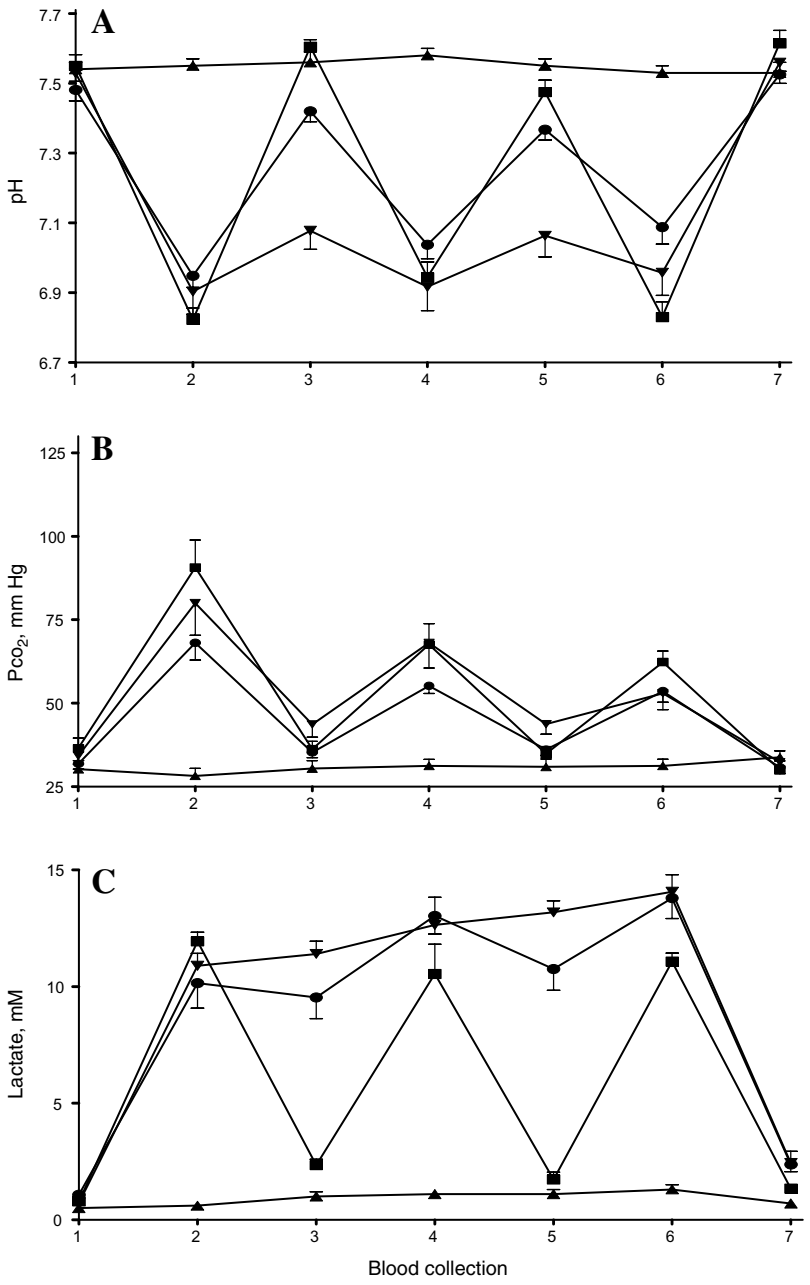

Figure 2

Blood pH (**A**), Pco_2 (**B**), and lactate (**C**) measured prior to and after three successive forced submergence episodes in loggerhead sea turtles in TED-equipped nets. Blood collection 1, 3, and 5 are presubmergence samples, whereas blood collection 2, 4, and 6 are postsubmergence samples. Blood collection 7 was taken 180 min after the final submergence. The surface interval between the submergences was 10 min (▼), 42 min (●), or 180 min (■). Data from control turtles (▲) are shown for comparison.

blood acid-base variables, whereas a brief surface interval permitted minimal recovery of blood homeostasis (Figs. 1 and 2). Submersion of experimental turtles a third time resulted in similar changes in blood pH, Pco_2, and lactate to that measured following the second submersion, and the length of the at-surface rest interval affected the magnitude of recovery of blood acid-base status. The seventh serial sample collected 180 min after the final postsubmer-

Table 1

Mean (±SE) plasma Na^+, K^+, and plasma osmotic pressure (OP) prior to and following laboratory multiple forced submergences of sea turtles with a 10-min, 42-min, or 180-min rest interval. Serial blood sampling regime is described in the "Materials and methods" section. Significant differences between samples 1 and 2, 3 and 4, and 5 and 6 are indicated by an asterisk (*), whereas significant differences of samples from the initial blood sample (serial sample 1) are denoted by a pound sign (#).

	10 min			42 min			180 min		
Treatment	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)
Control	153 ±3	4.0 ±0.2	319 ±3	152 ±4	3.9 ±0.3	305 ±2	158 ±4	4.3 ±0.4	322 ±4
Serial sample									
1	144 ±5	4.5 ±0.3	319 ±6	158 ±6	3.9 ±0.2	314 ±11	162 ±2	4.1 ±0.1	296 ±3
2	159 ±6	5.9 ±0.6	341 ±4	163 ±3	6.1 ±0.6*#	364 ±10*#	187 ±2*#	6.9 ±0.3*#	342 ±5*#
3	145 ±3	4.9 ±0.2	330 ±5	156 ±2	4.1 ±0.3	336 ±8	160 ±4	4.4 ±0.1	308 ±4
4	166 ±7#	6.2 ±0.3#	351 ±14#	160 ±6	5.5 ±0.2#	342 ±15	179 ±4	6.7 ±0.5*#	339 ±4*#
5	158 ±6	5.1 ±0.1	335 ±11	147 ±6	4.1 ±0.3	334 ±12	158 ±6	3.9 ±0.2	305 ±5
6	154 ±5	6.1 ±0.3#	340 ±12	157 ±9	4.8 ±0.3	345 ±12#	181 ±2	5.7 ±0.5*	323 ±8#
7	139 ±3	4.8 ±0.4	323 ±8	149 ±6	4.4 ±0.3	331 ±7	158 ±10	4.4 ±0.5	305 ±3

gence sample revealed that blood pH, Pco_2, and lactate recovered completely for all experimental turtles (Figs. 1 and 2). Minimal changes in blood pH, Pco_2 and lactate were detected in laboratory and field control turtles during collection of the 5–7 serial blood samples (Figs. 1 and 2).

Ions, glucose, and osmotic pressure

Postsubmergence blood samples from laboratory turtles revealed elevations in plasma Na^+, K^+, and osmotic pressure when compared to the corresponding presubmergence values (Table 1). Significant increases in the plasma Na^+, K^+, and osmotic pressure were observed more frequently in turtles with a longer in-water rest interval between successive submergences (Table 1). In contrast, the plasma ion concentrations and osmotic pressure of control turtles did not substantially change ($P>0.05$, $n=9$) during serial blood sample collection. In addition, no significant differences in plasma glucose and Cl^- ($P>0.05$, $n=10$) were measured in any of the experimental turtles. Although most of the postsubmergence changes in the blood parameters in experimental turtles were not significant (Table 1), and minimal alterations in blood chemistry were observed in control turtles, the results suggested that there was a relationship between blood acid-base status and plasma osmolality and ion concentration. Therefore, correlation analyses were used to determine the interdependence of these variables.

Figure 3 shows the results of the correlation analyses, where pH is plotted versus ion concentration (i.e. Na^+, K^+, and Cl^- concentration), osmolality, or hematocrit. Nonsubmerged control turtles had a significant correlation between blood pH and plasma chloride, and pH and hematocrit (Fig. 3). As pH declined, there were slight, yet significant, increases in the $[Cl^-]$ and hematocrit. However, no correlation was detected between pH and plasma $[Na^+]$, $[K^+]$, or osmolality in these animals. In contrast, a significant correlation was detected between blood pH and plasma $[Na^+]$, $[K^+]$, $[Cl^-]$, osmolality, and hematocrit in experimentally submerged turtles (Fig. 3). In each case, a decrease in blood pH led to an increase in the correlated variable. These data are consistent with significant water movement into and out of the red blood cells during and after forced submersion.

Brief forced submergence of loggerhead turtles in trawl-equipped fishing nets had a profound effect on the plasma ionic status (Table 2). Plasma $[K^+]$ increased significantly immediately following submergence in all experimental turtles. Significant increases were also observed in the plasma $[Na^+]$ and osmotic pressure, although these changes did not occur in turtles from all of the experimental treatments (Table 2). Turtles partially to completely recovered from the ionic imbalances, although subsequent submergences caused significant increases in plasma K^+ and nonsignificant increases in plasma Na^+ and osmolality in most experimental turtles (Table 2). Ionic homeostasis in forcibly submerged turtles was achieved within 180 min of the final submergence, whereby plasma ion concentrations were comparable to the initial presubmergence values (Table 2). The plasma ion concentrations and osmotic pressure in nonsubmerged control turtles were unaffected by serial blood sampling. Thus, ionic changes in experimental turtles resulted from the forced submergence and not from handling and repetitive blood sampling.

Discussion

Acid-Base status

Multiple submergences of 2-year-old loggerhead sea turtles under laboratory and field conditions produced sig-

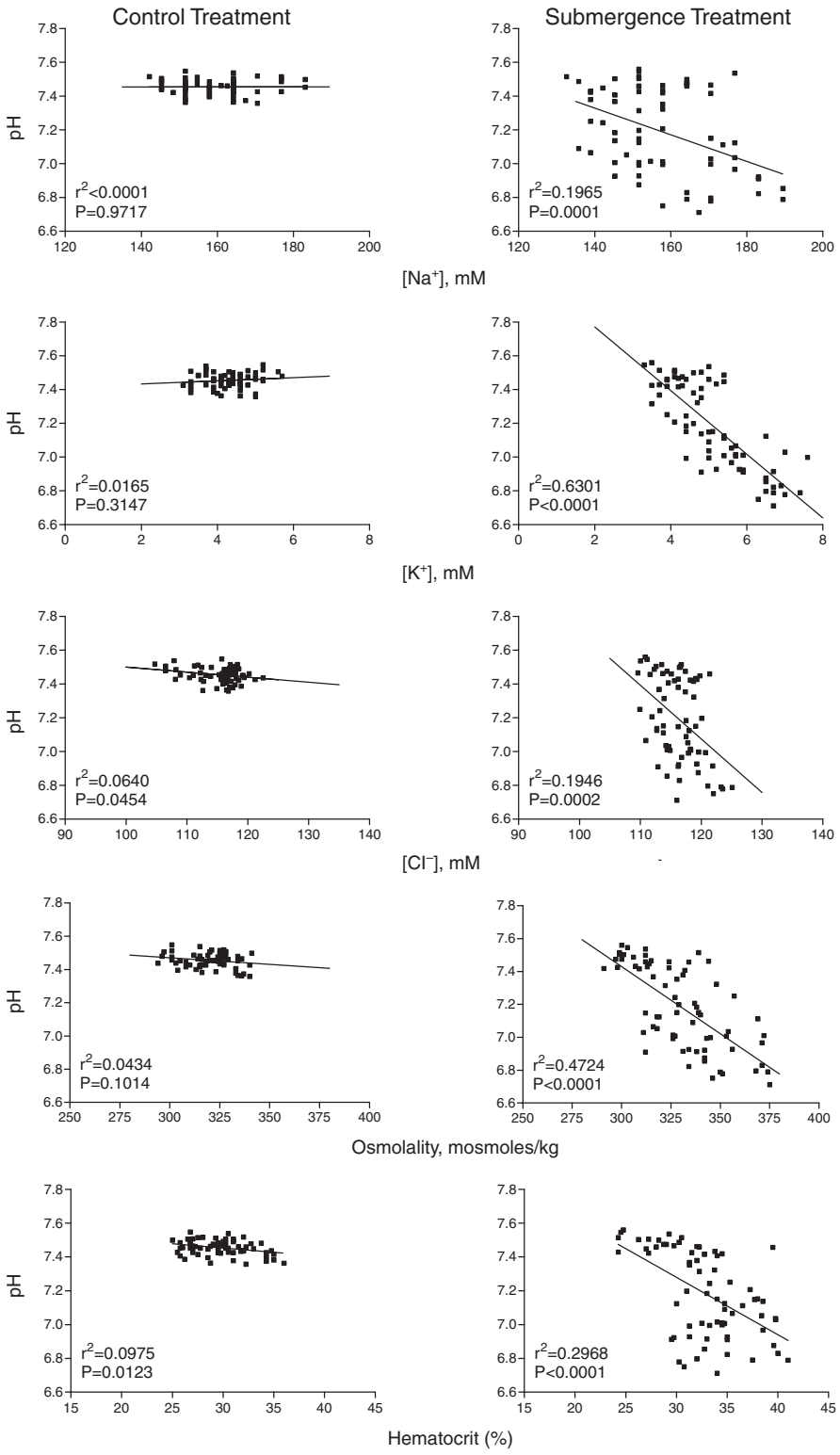

Figure 3

Relationship between blood pH and plasma [Na^+], [K^+], [Cl^-], osmolality, and hematocrit in control (left column) and submerged (right column) loggerhead sea turtles. The lines are best fits to the data. Significance of the correlated variables is noted on each figure.

Table 2

Mean (±SE) plasma Na^+, K^+, and plasma osmotic pressure (OP) prior to and following multiple forced submergences of sea turtles in TED-equipped nets with a 10-min, 42-min, or 180-min rest interval. Serial blood sampling regime is described in the "Materials and methods" section. Significant differences between samples 1 and 2, 3 and 4, and 5 and 6 are indicated by an asterisk (*), whereas significant differences of samples from the initial blood sample (serial sample 1) are denoted by a pound sign (#).

	10 min			42 min			180 min		
Treatment	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)	Na^+ (mM)	K^+ (mM)	OP (mosm/kg)
Control	150 ±3	3.0 ±0.2	313 ±8	139 ±6	3.4 ±0.2	321 ±7	151 ±1	3.1 ±0.1	310 ±5
Serial sample									
1	153 ±2	3.3 ±0.3	318 ±4	160 ±4	3.1 ±0.2	331 ±12	164 ±2	4.5 ±0.7	325 ±9
2	171 ±8	5.5 ±0.3*#	345 ±4*#	186 ±8*#	5.0 ±0.4*#	368 ±10	188 ±4	7.0 ±0.6*#	355 ±3*#
3	156 ±6	4.3 ±0.0#	332 ±4	163 ±3	2.8 ±0.1	338 ±11	163 ±10	3.6 ±0.3	314 ±3
4	171 ±8	5.3 ±0.1*#	349 ±1#	181 ±3	4.9 ±0.3*#	361 ±13	176 ±10	6.2 ±0.3*#	352 ±9*
5	166 ±4	4.3 ±0.1#	334 ±2	160 ±8	2.9 ±0.2	332 ±9	173 ±10	4.0 ±0.2	323 ±3
6	166 ±12	5.1 ±0.1#	335 ±11	185 ±4*#	4.5 ±0.4*#	343 ±14	175 ±18	5.3 ±0.0	333 ±11
7	157 ±4	3.7 ±0.1	325 ±2	161 ±6	2.6 ±0.2	326 ±9	159 ±11	3.6 ±0.6	320 ±4

nificant blood metabolic and respiratory disturbances. The most dramatic changes in blood pH, Pco_2, and lactate occurred following the first of the three forced submergences in all of the experimental turtles (Table 3). Under laboratory conditions, the turtles exhibited an average pH change of 0.54 U following the first submergence, whereas initial submergence of 2-year-old loggerhead sea turtles in TED-equipped commercial fishing nets induced a pH decrease of 0.63 U. The initial acid-base disturbances measured in our study were comparable in magnitude to those measured in Kemp's ridley and loggerhead sea turtles in standard TED certification trials (Table 3).

The second and third submergences of 2-year-old loggerheads sea turtles did not result in similar changes in blood pH, Pco_2, and lactate, as was measured following the initial submergence (Table 3). To our knowledge, no information is available in the literature on the physiological effects of multiple submergences in sea turtles for comparison. Obviously, the interval between the submergence episodes directly influenced the magnitude of the blood acid-base imbalance during successive submergences. A longer time interval at the surface led to enhanced recovery of blood pH, Pco_2, and lactate. Lutz and Dunbar-Cooper (1987) reported that loggerhead sea turtles captured during trawling at Cape Canaveral, Florida, exhibited a 16.8% decline in lactate 180 min following submergence. Those authors proposed that the rate of lactate decline was dependent on the magnitude of the lactate concentration, so that 10 mM of lactate would decline at a rate of 1.25 mM lactate/h. However, in the present study, the rate of lactate decline was considerably higher than that suggested by Lutz and Dunbar-Cooper (1987). Lactate declined 70.0% and 79.6% within 180 min of the submergence episodes in treatment 3 turtles, whereas no decline was measured in treatment 1 turtles (10 min interval) between submergences. In fact, it was apparent that lactate continued to washout into the bloodstream during the 10-min recovery phases in these turtles (Fig. 1, Table 3). Thus, turtles with a brief period between the submergence episodes would have a limited ability to release the CO_2 retained during submersion or to break down lactic acid produced during the course of the forced dive. Lactate declined 15.2% and 18.7% during the 42-min interval between submergences in treatment-2 turtles. Blood lactate declined 80.9%, 76.0%, and 82.5% in treatment-1, -2, and -3 turtles, respectively, during the final 180-min recovery period. Thus, the overall rate of lactate decline in the final 180 minutes of the laboratory study was 2.6 ±0.2 mM/h. Finally, the elevated lactate concentration in sea turtles during the 180-min postsubmergence recovery time interval suggests that the samples were collected too soon to permit complete recovery of blood lactate.

Comparable rates of lactate clearance measured in the laboratory submergence study were detected following forced submergences of loggerhead sea turtles in TED-equipped fishing nets. Substantial retention of CO_2 and additional washout of lactate occurred during the 10-min postsubmergence recovery interval in treatment-4 turtles. Treatment-5 turtles exhibited a 6% drop in the blood lactate concentration during the first 42-min postsubmergence recovery interval and a 17.5% decrease in the blood lactate during the second recovery interval. Thus, the 42-min postsubmersion recovery interval permitted recovery of blood gases, but was inadequate to clear the blood lactate (Fig. 2, Table 3). Lactate declined 80.4% and 83.8%, respectively, during the first two 180-min postsubmergence recovery intervals in treatment-6 turtles. As was the case for laboratory submerged sea turtles, a longer surface interval ultimately resulted in an increased ability to recover from the submersion episodes. In fact, lactate declined 82.7%, 82.8%, and 87.9%, respectively, in treatment-4, -5, and -6 turtles 180 minutes after the final submersion episode (Fig. 2, Table 3).

Table 3

Effects of forced submergence on blood pH, Pco_2, and lactate in Kemp's ridley (LK) and loggerhead (CC) sea turtles. Data are expressed as the mean difference (Δ) between post- and presubmergence values. Data from this study are provided from the three submergence episodes of treatment 1–3 turtles under the laboratory protocol and treatment 4–6 turtles in the field protocol. ND = not determined.

Species	Turtle size (kg)	Submergence duration (min)		ΔpH	ΔPco_2 (mm Hg)	Δlactate (mM)	Reference
LK	5–16.5	≤7.3		0.37	12.8	8.5	Stabenau et al. (1991)
	5–6	≤7.3		0.31	24.5	15.1	TED certification tests[1]
CC	5–6	4.3		0.33	ND	13.4	TED certification tests[1]
	5–6	12.5		0.52	ND	17.2	
CC	6.5–7.0	7.5	treatment 1	1) 0.49	61.1	7.6	Laboratory study
				2) 0.11	16.3	–0.1	
				3) 0.10	15.3	1.1	
			treatment 2	1) 0.57	70.8	9.3	
				2) 0.20	20.9	2.3	
				3) 0.23	21.1	1.1	
			treatment 3	1) 0.59	98.7	9.6	
				2) 0.50	68.6	7.2	
				3) 0.46	67.3	5.9	
			treatment 4	1) 0.63	45.8	10.2	Field study
				2) 0.16	24.5	1.9	
				3) 0.11	9.3	0.9	
			treatment 5	1) 0.53	36.3	9.1	
				2) 0.38	19.9	3.5	
				3) 0.28	17.5	3.0	
			treatment 6	1) 0.73	54.2	11.2	
				2) 0.66	31.3	9.2	
				3) 0.65	27.5	9.3	

[1] Data were collected by one of the authors (EKS) during standard TED certification tests in 1993–94. Samples were collected from the cervical sinus of Kemp's ridley and loggerhead sea turtles prior to and following forced submergences in a commercial shrimp net equipped with a TED. Turtles in these studies were permitted to exit the TED-equipped net.

It must be noted that any discussion on lactate production and recovery following submersion is applicable to environmental conditions comparable to those reported in this study. For example, lactate formation and recovery rates of lactate build-up would be significantly influenced by water temperature. Longer recovery rates may take place in cold water, whereas warmer waters may lead to additional lactate production thereby influencing the rate of lactate elimination. In addition, the blood lactate concentrations measured in this study may underestimate the true lactate burden. Lactate has been shown to partition into other tissues, including the shell, following submersion of freshwater turtles (Jackson et al., 1999). Finally, sea turtle size could potentially alter lactate production and elimination. Results from submersion experiments conducted in our laboratory indicate that smaller animals exhibit a significant acidosis and lactate build-up in comparison to larger sea turtles. Whether less acidosis and lactate build-up is due to additional lactate buffering by the larger sea turtles warrants further investigation.

Ions, osmolality, and hematocrit

There are three primary mechanisms for recovery of blood pH following an acid-base disturbance: cellular buffering, and respiratory and renal compensation. Cellular responses occur immediately following the disturbance, whereas respiratory and renal adjustments occur within minutes to hours, respectively. Previously, Stabenau et al. (1991) reported that Kemp's ridley sea turtles exhibited a significant increase in plasma $[K^+]$ following trawl submergences. However, those authors reported that trawl stress had no effect on plasma $[Cl^-]$, $[Na^+]$, or hematocrit. In the present study, a cellular response to the severe acid-base disturbance caused by the multiple forced submergences was suggested by alterations in plasma ion concentrations, osmolality, and hematocrit during the blood acidosis. As shown in Figure 3, decreases in blood pH were correlated with increases in $[K^+]$, $[Na^+]$, $[Cl^-]$, osmolality, and hematocrit.

Hematocrit (percent packed red blood cells) changes may result from washout of additional red blood cells into the bloodstream, from areas such as the spleen, in order to provide more red blood cells during the hypoxic phases of the forced submergence. This explanation, however, is unlikely given that substantial fluctuations in hematocrit were observed during the course of the submergence experiments and that a normal hematocrit was measured in the final serial blood sample. A more plausible explanation is that there was an osmotically obliged influx of water into the red blood

cells, swelling the cells, and leading to increases in hematocrit, and in plasma ion concentration and osmotic pressure. Red cell volume is regulated in animals through transport of intracellular and extracellular solutes. Although there is minimal information available in the literature concerning regulatory volume transport in reptiles, the mechanisms of regulatory volume increase (RVI) and regulatory volume decrease (RVD) are known in other lower vertebrates. For example, Cala (1983) reported that in *Amphiuma* (amphiuma [common name]) red cells, the mechanism of RVD is K^+_{out}/H^+_{in} counter-transport coupled with $Cl^-_{out}/HCO_3^-_{in}$ exchange (where the subscripts in and out represent transport into and out of the cell, respectively), whereas RVI is accomplished by Na^+_{in}/H^+_{out} transport coupled with $Cl^-_{in}/HCO_3^-_{out}$ exchange (Cala, 1983). Other studies have suggested that red cell RVD occurs because of electroneutral KCl cotransport out of the cell and RVI occurs because of electroneutral NaK2Cl or NaCl cotransport into the cell (Haussinger and Lang, 1991). It is impossible to determine which of these mechanisms, if any, were involved in regulating red cell volume in sea turtles during and following forced submergence. These transporters, however, have been shown to be sensitive to cellular hypoxia (i.e. low Po_2) and low blood pH (Cossins and Gibson, 1997)—conditions present in the experimental turtles following submergence. In addition, hypoxic and acidotic conditions were absent in nonsubmerged control turtles which did not experience substantial shifts in plasma ion concentrations, osmotic pressure, or hematocrit.

Effects of handling

Significant changes in blood pH, Pco_2, and lactate were occasionally detected in nonsubmerged control turtles. However, it is impossible to determine if these changes resulted from repetitive handling during blood sampling or from increased activity while free-swimming in a large circular tank following blood collection. Nevertheless, control turtle blood lactate concentration was substantially less than the lactate measured following forced submergence in experimental turtles (Figs. 1 and 2). In addition, the blood pH remained fairly constant in the control turtles during collection of the seven serial samples.

Laboratory versus field experimentation

It should be noted that conducting the study under laboratory and field conditions provided unique benefits for analyzing the physiological effects of submersion. For example, the laboratory conditions permitted collection of blood samples immediately upon termination of the submersion period, whereas in the field, sea turtles had to be transported back to the trawl vessel for postsubmersion blood sampling. Turtles forcibly submerged under laboratory or field conditions hyperventilated upon surfacing. Stabenau et al. (1991) reported a 9- to 10-fold increase in the breathing frequency of trawled Kemp's ridley sea turtles. Comparable breathing rates were observed in the present study after submersion and, thus, it is plausible that the blood Pco_2 measured in turtles under field conditions underestimated the actual buildup in blood CO_2 (see Table 3 for a comparison of the blood Pco_2 under laboratory and field conditions). In contrast, the field experiment permitted examining the physiological stress of semiwild turtles in TED-equipped commercial fishing nets following a minimum of 21 days of in-water conditioning. The greater acidosis measured in forcibly submerged turtles resulted from increased swimming activity during the forced submergence. This is confirmed by a postsubmergence increase in blood lactate of 10.1 mM under trawling conditions versus 8.8 mM following laboratory submergence. Under laboratory and field conditions, the behavior of the turtles following submergence was monitored up to their release. It is unclear, however, if the acid-base and ionic imbalance caused by forced submersions would alter long-term normal physiology and behavior. It is plausible that repetitive alteration of blood pH by the magnitude measured in the present study may have pathological consequences. For example, no information is available on whether turtles resume normal diving and feeding behavior following prolonged or multiple forced submersions, or whether turtles become more susceptible to repeated submersions in TED-equipped nets.

Use of turtles reared in captivity

Two-year-old loggerhead sea turtles reared in captivity were used for all of the submergence experiments. It was assumed that these animals were adequate surrogates for wild sea turtles. In fact, similar-size animals from the NMFS Galveston Laboratory are used in annual TED certification trials. Nevertheless, there may be differences in the physiology of captive and wild turtles subjected to forced submergences. For example, it is possible that wild sea turtles would be exposed to forced submergences following lengthy, voluntary dives. No information is available in the literature on the acid-base and ionic status of wild sea turtles following prolonged voluntary dives or forced multiple submergences. If dives are anaerobic, then subjecting wild sea turtles to multiple forced submergences may adversely affect survival potential.

Conclusions

The data suggest that forced submergences of 2-year-old loggerhead sea turtles reared in captivity produce significant blood metabolic and respiratory acidosis. Repetitive submergences did not augment the acidosis, rather subsequent submergences resulted in less severe acid-base disturbances. Under trawl conditions, the turtle must recover from any physiological acid-base disturbance when it is freed from a TED-equipped net. Recovery is accomplished, in part, by the turtle immediately surfacing and hyperventilating (Jackson, 1985; Stabenau et al., 1991). This behavior was observed following each submergence episode. Turtles would then resume normal voluntary diving behavior, presumably after partial-to-complete recovery from the acid-base disturbance. These data suggest that repetitive submergences of sea turtles in TED-equipped

nets would not significantly affect their survival potential, provided that the turtles have a recovery interval between successive submergences. However, it should be noted that the latter statement is based on comparable-size turtles that may be submerged in shrimp nets equipped with legally certified and installed turtle excluder devices. Poor installation or lack of use of legal TEDs would result in augmenting the acid-base imbalance in the turtles. Increasing the magnitude of the blood acid-base and ionic disturbance during each submersion would increase the length of time necessary to achieve partial or complete recovery.

Acknowledgments

Grateful appreciation is expressed to personnel from the National Marine Fisheries Service Galveston and Pascagoula Laboratories for their assistance in turtle husbandry and in conducting the submersion protocol under laboratory and very difficult field conditions. These studies were conducted under appropriate threatened and endangered species permits issued by the U.S. Fish and Wildlife Service, Texas Park and Wildlife Department, and the Florida Department of Natural Resources.

Literature cited

Caillouet, C. W., M. J. Duronslet, A. M. Landry, D. B. Revera, D. J. Shaver, K. M. Stanley, R. W. Heinly, and E. K. Stabenau.
1991. Sea turtle strandings and shrimp fishing effort in the Northwestern Gulf of Mexico, 1986–89. Fish. Bull. 89:712–718.

Cala, P. M.
1983. Volume regulation by red blood cells: mechanisms of ion transport. Mol. Physiol. 4:33–52.

Cossins, A., and J. Gibson.
1997. Volume-sensitive transport systems and volume homeostasis in vertebrate red blood cells. J. Exp. Biol. 200:343–352.

Crowder, L. B., S. R. Hopkins-Murphy, and J. A. Royle.
1995. Effects of turtle excluder devices (TEDs) on loggerhead sea turtle strandings with implications for conservation. Copeia 1995:773–779.

Haussinger, D., and F. Lang.
1991. The mutual interaction between cell volume and cell function: a new principle of metabolic regulation. Biochem. Cell Biol. 69:1–4.

Jackson, D. C.
1985. Respiration and respiratory control in the green turtle, *Chelonia mydas*. Copeia 1985:664–671.

Jackson, D. C., Z. Goldberger, S. Visuri, and R. N. Armstrong.
1999. Ionic exchanges of turtle shell *in vitro* and their relevance to shell function in the anoxic turtle. J. Exp. Biol. 202(part 5):513–520.

Lutz, P. L., and A. Dunbar-Cooper.
1987. Variations in blood chemistry of the loggerhead sea turtle *Caretta caretta*. Fish. Bull. 85:37–44.

National Research Council.
1990. Decline of sea turtles: causes and prevention, 259 p. National Academy Press, Washington, DC.

Owens D. W., and G. J. Ruiz.
1980. New methods of obtaining blood and cerebrospinal fluid from marine turtles. Herpetologica 36:17–20.

Stabenau, E. K., and T. A. Heming.
1994. The *in vitro* respiratory and acid-base properties of blood and tissue from the Kemp's ridley sea turtle, *Lepidochelys kempi*. Can. J. Zool. 72:1403–1408.

Stabenau, E. K., T. A. Heming, and J. A. Mitchell.
1991. Respiratory, acid-base and ionic status of Kemp's ridley sea turtles (*Lepidochelys kempi*) subjected to trawling. Comp. Biochem. Physiol. 99A:107–111.

Wood, S. C., R. N. Gatz, and M. L. Glass.
1984. Oxygen transport in the green sea turtle. J. Comp. Physiol. B. 154:275–280.