Laboratory Investigation of the Physiological Effects of Multiple Forced Submergence in Loggerhead Sea Turtles (*Caretta caretta*)

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Abstract

Sea turtles are subjected to involuntary submergence and potential mortality due to incidental capture by the commercial fishing industry. Despite implementation of regulations requiring the use of turtle excluder devices (TEDs) to reduce at-sea mortality, dead stranded turtles continue to be found in near-record numbers along western Atlantic Ocean and northern Gulf of Mexico beaches. One plausible explanation for this continued mortality is that sea turtles are repetitively submerged in legal TEDs of one vessel following another. This study was designed, therefore, to examine the physiological effects of multiple enforced submergence in the loggerhead sea turtle (*Caretta caretta*). Pre- and post-submergence blood samples were collected from turtles submerged three times at 7.5 min per episode with a rest interval of 10, 42 or 180 min between successive submergences. No turtles died during the course of this study. Analyses of the pre- and post-submergence 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, P_{CO_2}, and lactate, although the magnitude of the acid-base imbalance was substantially reduced as the number of submergences increased. Increasing the interval between successive submergences permitted greater recovery of blood homeostasis. While most blood parameters had fully recovered 180 min after the final submergence, the [lactate] remained elevated, yet not significantly different, from pre-submergence values. These data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential provided the animal has an adequate rest interval at the surface between successive submergences.

Introduction

All five of the sea turtle species that inhabit the waters of the U.S. Gulf of Mexico and Atlantic Ocean are considered to be threatened or endanger of extinction. 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) has suggested that as many as 5,500 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). Due to the impact of trawl-related mortality on sea turtle populations, the U.S. government passed legislation 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 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, although the rate of decline was significantly less since 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. While there may be other man-related or natural causes for this continued sea turtle mortality, there are two plausible reasons for the mortality to be caused during shrimping activities. First, commercial shrimp fishermen are not carrying legally certified TEDs, which may occur

with improper installation or by purposely sewing them shut. Second, the shrimp fishermen are pulling legal TEDs; however, the turtles are repetitively involuntarily submerged as they are caught in the TEDs of vessels following each other. This successive submergence may compound the physiological effects experienced by sea turtles during an involuntary submersion, and thus, may limit their survival potential.

Sea turtles spend all but 1% 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 seen only after prolonged dives. Scant information is available on the physiological effects of voluntary submergence 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 respiratory acidosis that is ameliorated by hyperventilation at the surface. Obviously, voluntary diving does not limit sea turtle survival potential.

In contrast, involuntary submergence of Kemp's ridley and loggerhead sea turtles produces significant blood respiratory and metabolic derangements. Stabenau *et al.* (1991) reported that involuntary 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 P_{CO_2}, and decreases in blood pH. Moreover, several hours were required for forcibly submerged turtles to fully recover blood homeostasis (E. Stabenau, pers. observ.). These data suggest that repeated submergence may physiologically limit the survival potential of sea turtles. No information is available, however, on the acid-base disturbance

caused by multiple involuntary submergence. Therefore, the purpose of this experiment was to examine the physiological effects of multiple forced submergence in the loggerhead sea turtle. These data may offer insight into potential sea turtle mortality caused by multiple capture in commercial shrimping nets carrying legal TEDs.

Materials and Methods

Thirty-nine 2-year old loggerhead sea turtles were used in this study. Each turtle was randomly placed into the experimental (submerged) or control (non-submerged) treatment. Table 1 lists the straight standard carapace length and weight of the 21 turtles used in the submergence study, while Table 2 lists the morphometric data for the 18 control turtles. 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.

The study was initiated by collecting pre-submergence blood samples from the experimental turtles immediately prior to their individual confinement in a weighted canvass bag. Each turtle was then submerged for 7.5 min in seawater filled tanks. Post-submergence 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 pre-submergence blood sample was collected and the turtle was submerged a second time. A post-submergence 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 post-submergence blood samples were collected as described above. A final 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 affects associated with blood volume depletion.

Blood gases (Po_2 and Pco_2) and pH were analyzed immediately following collection using a clinical blood gas analyzer thermostatted at 37°C. Blood pH and Pco_2 were corrected to turtle cloacal temperature using requisite correction factors for sea turtle blood and plasma (Stabenau and Heming, 1994). Packed red cell volume (hematocrit) was determined following centrifugation of heparinized micro-capillary 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 using standard enzymatic techniques (Sigma, kit 826-B). 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), while plasma Cl^- was determined with electrometric titration (Haake-Bucher, Model 4425000). Plasma glucose was measured spectrophotometrically (Sigma, kit 16-20), and plasma osmolality was determined with a vapor pressure osmometer (Wescor, Model 5500). Plasma catecholamines (norepinephrine and epinephrine) were analyzed with HPLC (BAS, Model LC-300).

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 tests and a level of $P \leq 0.05$ was regarded as significant.

Results

Blood pH, Pco_2, and lactate. The initial submergence of loggerhead sea turtles produced a dramatic and severe acidosis in all experimental turtles with blood pH falling an average of 0.54 ± 0.03 (range 0.49 to 0.59 pH units) from pre-submergence values (Figure 1). It was evident that the blood acidosis was derived from respiratory and metabolic components. Blood Pco_2 and lactate increased significantly following the initial involuntary submergence (Figures 2-3). Significant increases in the plasma catecholamines, epinephrine and norepinephrine, were also measured following the first submergence (data not shown). In contrast, an average 0.05 ± 0.01 pH change was observed following collection of the first two blood samples in non-submerged control turtles (Tables 3-5).

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 turtles) permitted partial recovery of blood pH (Figure 1A) and Pco_2 (Figure 2A), with blood pH remaining significantly different from pre-submergence values. Washout of additional lactate caused by the submergence episode occurred during the first rest interval, whereby lactate increased higher than the post-submergence value (Figure 3A). Turtles with a 42 min interval (treatment 2 turtles) between the first and second submergence had partial recovery of blood pH (Figure 1B), complete recovery of blood Pco_2 (Figure 2B), and slight recovery of blood lactate (Figure 3B). Only the blood lactate remained significantly different from the initial pre-submergence value after the 42 min rest interval. Turtles with

a 180 min in-water interval (treatment 3 turtles) showed complete recovery of blood pH and Pco_2 (Figures 1C and 2C, respectively), although the [lactate] was slightly higher than baseline levels (Figure 3C). Non-submerged control turtles exhibited no 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 (Tables 3-5).

The second 7.5 min submergence produced a drop in blood pH and an increase in Pco_2 (Figure 1-2) in all experimental treatments, although significant differences in these variable only occurred in treatment 2 and 3 turtles (Figures 1 and 2). 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 (Δ pH) between the second pre- and post-submergence ranged from 0.11 ± 0.03 in treatment 1 turtles to 0.50 ± 0.03 in treatment 3 turtles. The substantial drop in blood pH in treatment 2 and 3 turtles following the second submergence resulted from greater pre- to post-submergence increases in blood Pco_2 and lactate (Figures 1-3) than measured in treatment 1 turtles. This was due to a longer surface interval between the two submergence episodes. Collection of the fourth sample from non-submerged control turtles revealed no significant changes in blood pH, Pco_2, or lactate when compared to the third sample (Tables 3-5). However, the blood lactate in the fourth sample was significantly higher than the initial baseline [lactate] in all groups of control turtles (Tables 4 and 5).

Recovery of blood pH, Pco_2, and lactate following the second submergence was again dependent on the surface interval. The treatment 1 turtles (10 min surface interval)

had partial recovery of blood pH and complete recovery of Pco_2 (Figures 1A and 2A). Lactate continued to increase during this period (Figure 3A). Treatment 2 turtles (42 min surface interval) exhibited a complete recovery of blood pH (Figure 1B) and Pco_2 (Figure 2B), and a partial recovery of lactate (Figure 3B). Treatment 3 turtles (180 min surface interval) exhibited recovery of blood pH (Figure 1C), Pco_2 (Figure 2C) and lactate between the fourth and fifth blood samples. No significant changes in control turtle blood pH, Pco_2, or lactate were observed in the fifth serial sample (Tables 3-5).

The physiological effects of the final submergence were comparable to that measured following the second submergence, in that there were decreases in pH and increases in Pco_2 in all of the experimental turtles, with significant changes occurring in treatment 2 and 3 turtles (Figures 1-3). The mean lactate difference between the third pre- and post-submergence ranged from 1.0 ± 0.5 in treatment 1 ($P > 0.05$) turtles to 6.0 ± 1.2 in treatment 3 turtles ($P < 0.05$). Collection of the sixth serial blood sample from non-submerged, control turtles resulted in no significant changes in blood pH, Pco_2 or lactate (Tables 3-5).

The seventh serial sample collected 180 min after the final post-submergence sample revealed that blood pH, Pco_2, and lactate recovered completely for all experimental turtles (Figures 1-3). One group of control turtles exhibited a significant blood alkalosis following collection of the seventh serial blood sample when compared to the initial baseline value. In contrast, blood Pco_2 and lactate remained fairly constant in all of the control groups (Tables 3-5).

Ions, Glucose, and Osmotic Pressure. Post-submergence blood samples revealed elevations in plasma Na^+, K^+, and osmotic pressure when compared to the corresponding pre-submergence values (Tables 6-8). 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 (Tables 6-8). In contrast, the plasma ion concentrations, glucose and osmotic pressure of control turtles did not substantially change during serial blood sample collection (Tables 9-11). While most of the post-submergence changes in the blood parameters in experimental turtles were not significant (Tables 6-8), and minimal alterations in blood chemistry were observed in control turtles, the results suggest that there was a relationship between blood acid-base status and plasma ion concentration. Therefore, correlation analyses were used to determine the interdependence of these variables.

Figure 4 shows the results of the correlation analyses, whereby pH is plotted versus ion concentration (i.e., Na^+, K^+, and Cl^- concentration), osmolality, or hematocrit. Blood pH and plasma [Cl^-], and pH and hematocrit (packed red cell volume) were significantly correlated in non-submerged, control turtles (Figure 4). As pH declined, there were slight, yet significant, increases in the [Cl^-] and hematocrit. However, no correlation was detected between blood 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 (Figure 4). In each case, a decrease in blood pH led to an increase in the correlated variable.

Discussion

Acid-Base Status. Multiple submergence of 2-yr old loggerhead sea turtles produced a significant blood metabolic and respiratory disturbance. The first of the three forced submergences produced substantial changes in blood pH, Pco_2, and lactate in all experimental turtles. Table 12 shows the pre- to post-submergence acid-base status of turtles used in this study and comparable data from 5-16 kg Kemp's ridley and loggerhead sea turtles forcibly submerged during TED certification trials. Briefly, in certification tests, a turtle enclosed in a weighted mesh bag is sent to scuba divers on the headrope of a commercial shrimp trawl equipped with a TED. The turtle is released into the net and the animal is given up to 5 min to escape. At that time, divers remove the turtle if it has not escaped the trawl. The turtle is then recaptured at the surface. The maximum time underwater is 7.5 min, although it was 12.5 min for 3 turtles during the 1994 tests (Table 12). In 1993-94, one of the authors of this paper (EKS) collected blood samples prior to and following forced submergence in TED-equipped nets. Blood samples were processed as described in this paper. As shown in Table 12, forced submergence of turtles in TED-equipped shrimp nets produced pH changes ranging from 0.31 U in Kemp's ridleys to 0.52 U in loggerheads. The loggerhead turtles used in the study herein exhibited an average pH change of 0.54 U. The greater acidosis measured in the multiple forced submergence study resulted from substantial increases in blood Pco_2 following the forced submergence (Table 12). Post-submergence blood samples were collected within 30 s in this controlled laboratory study, whereas turtles in TED certification trails are returned to the vessel within 1-3 min for post-trawl blood sampling depending on sea state. This delay in

returning the turtle for post-submergence blood sampling permitted significant hyperventilation by the turtles, and thus, a lowering of blood Pco_2 (Table 12). However, as shown in Table 12, turtles forcibly submerged in TED-equipped nets exhibited a greater change in blood lactate (8.5-17.2 mM) than measured during the course of the multiple submergence study (7.6-9.6 mM). This difference in blood lactate levels reflected active swimming in the shrimp nets during the submergence episodes, whereas turtles in the multiple submergence study were enclosed in a canvass bag to limit anaerobic activity during submergence.

It is noteworthy that the second and third submergences of 2-yr old loggerheads sea turtles did not result in comparable changes in blood pH, Pco_2, and lactate as measured following the initial submergence (Table 12). To the authors knowledge, no information is available in the literature 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 turtles captured during trawling at Cape Canaveral, Florida exhibited a 16.8% decline in lactate 3 hr 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 hr^{-1}. However, in the current study, the rate of lactate decline was considerably higher than suggested by Lutz and Dunbar-Cooper (1987). Lactate declined 70.0% and 79.6% within 3 hr 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 (Figure 3A). 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 3 hr recovery period. Thus, the overall rate of lactate decline in final 3 h of this study was 2.6 ± 0.2 mM hr^{-1}. Finally, the elevated lactate concentration in turtles during the 3 hr post-submergence recovery time interval suggests that the samples were collected to soon to permit complete lactate recovery.

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 submergence. 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 submergence was suggested by the alteration in plasma ion concentration, osmolality, and hematocrit during the blood acidosis. As shown in Figure 4, 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 experiment and that a normal hematocrit was measured during the final serial blood sample. A more plausible explanation is that there was osmotically obliged water influx into the red blood cells, swelling the cells, leading to increases in hematocrit, plasma ion concentrations, and osmotic pressure. Red cell volume is regulated in animals through transport of intracellular and extracellular solutes. While there is scant 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* red cells, the mechanism of RVD is K^+_{out}/H^+_{in} counter-transport coupled with $Cl^-_{out}/HCO_3^-{}_{in}$ exchange (where the subscript 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 due to electroneutral KCl cotransport out of the cell and RVI occurs by 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 (Figures 1 and 4). In addition, hypoxic and acidotic conditions were absent in non-submerged control turtles which did not

experience substantial shifts in plasma ion concentrations, osmotic pressure, or hematocrit (Tables 3-5; Tables 9-11; Figure 4).

Effects of Handling. Significant changes in blood pH, Pco_2 and lactate were occasionally detected in non-submerged control turtles (Tables 3-5). However, it is impossible to determine if these changes resulted from repetitive handling during blood sampling or due to increased activity while free-swimming in a large circular tank following blood collection. Nevertheless, control turtle blood [lactate] was substantially less than the [lactate] measured following forced submergence in experimental turtles (Figure 3 and Table 3-5). In addition, the blood pH remained fairly constant in the control turtles during collection of the seven serial samples.

Conclusions. From the current study, the data suggest that forced submergence of 2-yr old loggerhead sea turtles produced a significant blood metabolic and respiratory acidosis. Repetitive submergence did not augment the acidosis; rather subsequent submergence resulted in a less severe acid-base disturbance. While the present study was conducted under controlled laboratory conditions, the physiological consequences of repetitive submergence of sea turtles in shrimp trawls equipped with legal TEDs can be inferred. Under trawl conditions, the turtle must recover from any physiological acid-base disturbance when it is freed from a TED-equipped net. This is accomplished, in part, by the turtle immediately surfacing and hyperventilating (Jackson, 1985, Stabenau *et al.*, 1991). This behavior was observed during the current study following each submergence episode. In addition, the amount of time at the surface became progressively less with

increased time between the submergence episodes (data not shown). Thus, turtles would resume normal voluntary diving behavior, presumably after partial to complete recovery from the acid-base disturbance. These data suggest that repetitive submergence of sea turtles in TEDs would not significantly affect their survival potential, provided that the turtles have an adequate recovery surface interval between successive submergences.

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Table 1. Straight standard carapace length, weight, and the interval between successive submergence episodes for experimental turtles.

Tag #	Submergence Interval	Length (cm)	Weight (kg)
sss-245	10 min	38.9	6.96
sss-217	10 min	38.5	6.61
sss-320	10 min	38.1	6.61
sss-228	10 min	37.5	6.75
sss-308	10 min	36.7	6.44
sss-204	10 min	37.5	6.18
sss-337	10 min	37.4	6.12
sss-351	10 min	36.7	6.51
sss-239	10 min	36.2	6.55
	mean	37.5	6.5
	SE	0.3	0.1
sss-362	42 min	37.4	6.53
sss-365	42 min	37.8	6.72
sss-242	42 min	36.2	6.18
sss-283	42 min	35.5	6.05
sss-329	42 min	36.5	6.47
sss-338	42 min	37.4	6.95
	mean	36.8	6.5
	SE	0.4	0.1
sss-322	180 min	37.0	6.55
sss-262	180 min	35.9	5.97
sss-339	180 min	36.7	6.92
sss-274	180 min	37.3	6.68
sss-289	180 min	36.6	6.56
sss-210	180 min	36.1	6.37
	mean	36.6	6.5
	SE	0.2	0.1

Table 2. Straight standard carapace length and weight of individual control turtles.

Tag #	Length (cm)	Weight (kg)
sss-273	36.7	6.27
sss-350	37.2	6.79
sss-330	37.8	6.72
sss-269	36.5	6.47
sss-215	38.0	6.62
sss-220	38.3	7.01
sss-268	38.1	6.79
sss-314	38.6	6.53
sss-259	37.0	6.65
sss-266	37.9	6.97
sss-277	36.6	6.60
sss-295	35.1	5.75
sss-264	35.5	5.79
sss-333	37.0	6.60
sss-254	36.5	6.63
sss-206	35.2	5.76
sss-281	36.4	6.18
sss-342	36.0	5.91
mean	36.9	6.4
SE	0.2	0.1

Table 3. Mean (± SE) blood pH, P_{CO_2}, and lactate from non-submerged control turtles to examine the effects of repetitive sampling. Serial blood samples were collected with a 7.5 min interval between samples 1-2, 3-4, and 5-6. A 10 min interval separated collection of samples 2-3, and 4-5. Sample 7 was collected 3 h recovery after sample 6. A significant difference between samples 1-2, 3-4, and 5-6 are indicated by an asterisk (*), whereas significant differences of samples from the initial blood sample (serial sample 1) are denoted by a pound sign (#).

Serial Sample	pH	P_{CO_2} (mm Hg)	Lactate (mM)	HCO_3^- (mM)
1	7.41 ± 0.03	43.1 ± 3.2	1.0 ± 0.4	29.2 ± 0.7
2	7.36 ± 0.02	45.3 ± 2.0	2.3 ± 0.6	27.3 ± 1.2
3	7.38 ± 0.04	43.5 ± 3.9	2.6 ± 0.9#	27.3 ± 1.5
4	7.40 ± 0.02	38.8 ± 1.6	2.4 ± 0.8#	26.4 ± 1.2
5	7.41 ± 0.01	38.6 ± 2.3	2.1 ± 0.7	26.5 ± 1.3
6	7.42 ± 0.02	38.1 ± 2.3	2.4 ± 0.8#	26.7 ± 1.1
7	7.49 ± 0.01#	39.1 ± 3.9	0.8 ± 0.3	32.9 ± 3.6

Table 4. Mean (± SE) blood pH, P_{CO_2}, and lactate from non-submerged control turtles to examine the effects of repetitive sampling. The rest of the legend is as in Table 3, with the exception that the interval between samples 2-3, and 4-5 was 42 min.

Serial Sample	pH	P_{CO_2} (mm Hg)	Lactate (mM)	HCO_3^- (mM)
1	7.47 ± 0.01	37.5 ± 0.9	0.7 ± 0.2	29.9 ± 1.2
2	7.39 ± 0.02*#	44.8 ± 2.9*#	1.4 ± 0.5	29.1 ± 1.2
3	7.49 ± 0.01	36.6 ± 1.6	1.2 ± 0.3	30.3 ± 1.4
4	7.46 ± 0.02	36.5 ± 1.3	2.1 ± 0.5#	28.6 ± 1.5
5	7.49 ± 0.02	35.4 ± 1.9	1.3 ± 0.2	29.6 ± 1.0
6	7.46 ± 0.02	37.8 ± 1.4	1.5 ± 0.3	29.2 ± 1.4
7	7.48 ± 0.01	40.2 ± 3.5	0.8 ± 0.1	32.8 ± 3.4

Table 5. Mean (± SE) blood pH, Pco_2, and lactate from non-submerged control turtles to examine the effects of repetitive sampling. The rest of the legend is as in Table 3, with the exception that the interval between samples 2-3, and 4-5 was 180 min.

Serial Sample	pH	Pco_2 (mm Hg)	Lactate (mM)	HCO_3^- (mM)
1	7.42 ± 0.02	42.5 ± 1.5	0.6 ± 0.1	29.6 ± 0.8
2	7.40 ± 0.01	42.7 ± 1.4	2.2 ± 0.5*#	28.0 ± 1.5
3	7.46 ± 0.03	40.3 ± 2.1	0.9 ± 0.4	30.4 ± 1.5
4	7.43 ± 0.02	41.1 ± 1.4	2.2 ± 0.5#	29.2 ± 2.4
5	7.45 ± 0.01	40.5 ± 1.2	0.5 ± 0.2	30.4 ± 1.3
6	7.46 ± 0.02	37.1 ± 1.6	1.7 ± 0.5	28.0 ± 1.3
7	7.47 ± 0.01	36.3 ± 1.0	1.0 ± 0.2	28.6 ± 0.9

Table 6. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 10 min. The rest of the legend as in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	144 ± 5	4.5 ± 0.3	116 ± 1	6.4 ± 1.0	319 ± 6
2	159 ± 6	5.9 ± 0.6	116 ± 4	6.6 ± 0.3	341 ± 4
3	145 ± 3	4.9 ± 0.2	116 ± 1	6.3 ± 0.5	330 ± 5
4	166 ± 7#	6.2 ± 0.3#	117 ± 1	6.5 ± 0.5	351 ±14#
5	158 ± 6	5.1 ± 0.1	116 ± 1	6.6 ± 0.6	335 ±11
6	154 ± 5	6.1 ± 0.3#	117 ± 2	6.9 ± 0.5	340 ±12
7	139 ± 3	4.8 ± 0.4	115 ± 2	7.3 ± 0.6	323 ± 8

Table 7. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 42 min. The rest of the caption is as described in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	158 ± 6	3.9 ± 0.2	116 ±2	6.1 ± 0.6	314 ± 11
2	163 ± 3	6.1 ± 0.6*#	120 ± 2	6.9 ± 0.5	364 ± 10*#
3	156 ± 2	4.1 ± 0.3	115 ± 2	6.6 ± 0.6	336 ± 8
4	160 ± 6	5.5 ± 0.2#	117 ± 1	6.8 ± 0.6	342 ±15
5	147 ± 6	4.1 ± 0.3	113 ± 2	6.9 ± 0.5	334 ±12
6	157 ± 9	4.8 ± 0.3	117 ± 2	6.8 ± 0.6	345 ±12#
7	149 ± 6	4.4 ± 0.3	118 ± 2	7.1 ± 0.4	331 ± 7

Table 8. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure prior to and following multiple forced submergence in loggerhead sea turtles. The interval between each submergence episode was 180 min. The rest of the caption is as described in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	162 ± 2	4.1 ± 0.1	116 ± 1	5.1 ± 0.2	296 ± 3
2	187 ± 2*#	6.9 ± 0.3*#	124 ± 5	5.7 ± 0.2	342 ± 5*#
3	160 ± 4	4.4 ± 0.1	116 ± 1	6.4 ± 0.0	308 ± 4
4	179 ± 4	6.7 ± 0.5*#	119 ± 2	7.0 ± 0.0	339 ± 4*#
5	158 ± 6	3.9 ± 0.2	111 ± 1	7.3 ± 0.1	305 ± 5
6	181 ± 2	5.7 ± 0.5*	116 ± 1	6.7 ± 0.9	323 ± 8#
7	158 ±10	4.4 ± 0.5	113 ± 2	7.1 ± 0.1	305 ± 3

Table 9. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure in non-submerged control turtles. Samples were taken following the 10 min blood collection protocol. Rest of the caption as in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	153 ± 3	4.0 ± 0.2	114 ± 3	6.0 ± 0.3	319 ± 3
2	160 ± 4	3.8 ± 0.3	117 ± 0	6.1 ± 0.3	328 ± 6
3	157 ± 3	3.8 ± 0.3	116 ± 3	6.0 ± 0.2	312 ± 9
4	159 ± 4	4.1 ± 0.6	117 ± 2	6.2 ± 0.3	317 ± 6
5	156 ± 2	4.0 ± 0.4	117 ± 1	6.2 ± 0.4	322 ± 6
6	157 ± 4	4.0 ± 0.6	116 ± 2	6.2 ± 0.4	326 ± 6
7	148 ± 3	4.0 ± 0.6	115 ± 2	5.9 ± 0.3	321 ± 4

Table 10. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure in non-submerged control turtles. Samples were taken following the 42 min blood collection protocol. The rest of the caption is as described in Table 3.

Serial Sampling	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	152 ± 4	3.9 ± 0.3	117 ± 2	5.1 ± 0.2	305 ± 2
2	154 ± 4	4.4 ± 0.1	120 ±1	5.2 ± 0.1	311 ± 8
3	155 ± 5	4.5 ± 0.1	115 ± 2	5.7 ± 0.3	314 ± 8
4	154 ± 6	4.6 ± 0.2	117 ± 0	5.8 ± 0.2	318 ± 9
5	161 ± 8	4.5 ± 0.4	117 ± 0	6.0 ± 0.3	317 ± 8
6	158 ± 6	4.5 ± 0.2	118 ± 0	6.1 ± 0.4	314 ± 8
7	168 ± 4	4.7 ± 0.6	117 ± 0	5.5 ± 0.7	314 ±11

Table 11. Mean (± SE) plasma Na^+, K^+, Cl^-, glucose concentrations, and plasma osmotic pressure in non-submerged control turtles. Samples were taken following the 180 min blood collection protocol. The rest of the caption is as described in Table 3.

Serial Sample	Na^+ (mM)	K^+ (mM)	Cl^- (mM)	Glucose (mM)	Osmotic pressure (mosmoles•kg^{-1})
1	158 ± 4	4.3 ± 0.4	116 ± 2	5.9 ± 0.3	322 ± 4
2	158 ± 4	4.7 ± 0.3	113 ± 2	6.1 ± 0.2	329 ± 5
3	164 ± 0	4.2 ± 0.4	112 ± 2	6.4 ± 0.3	321 ± 3
4	165 ± 1	4.5 ± 0.3	112 ± 3	6.6 ± 0.3	328 ± 4
5	173 ± 2	4.4 ± 0.3	112 ± 4	6.5 ± 0.6	326 ± 9
6	173 ± 6	4.5 ± 0.3	111 ± 3	6.6 ± 0.7	331 ± 5
7	175 ± 6	4.9 ± 0.4	113 ± 3	6.7 ± 0.7	326 ± 8

Table 12. 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 (post-pre) submergence values. In this study, the values are provided from the three submergence episodes for each treatment.

Species	Size (kg)	Submergence Duration (min)		Δ pH	Δ Pco_2 (mm Hg)	Δ lactate (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[a]
CC	5-6	4.3		0.33	ND	13.4	TED certification tests[a]
	5-6	12.5		0.52	ND	17.2	
CC	6.5	7.5	treatment 1	1) 0.49	61.1	7.6	This Study
				2) 0.11	16.3	-0.1	
				3) 0.10	15.3	1.1	
		7.5	treatment 2	1) 0.57	70.8	9.3	
				2) 0.20	20.9	2.3	
				3) 0.23	21.1	1.1	
		7.5	treatment 3	1) 0.59	98.7	9.6	
				2) 0.50	68.6	7.2	
				3) 0.46	67.3	5.9	

[a] Data were collected by one of the authors (EKS) during standard TED certification tests during 1993-1994. Samples were collected from the cervical sinus of Kemp's ridley and loggerhead sea turtles prior to and following forced submergence in a commercial shrimp net equipped with a TED. ND is not determined.

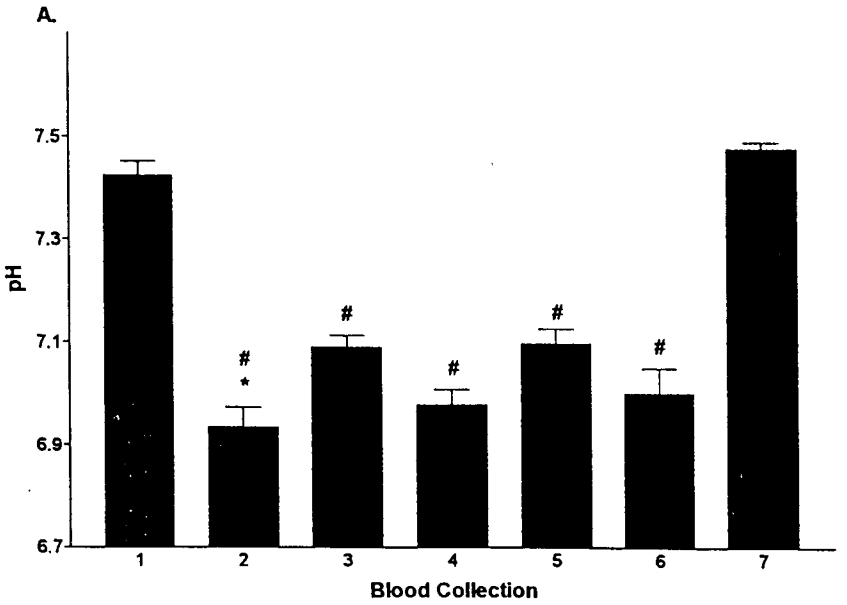
Figure Legends

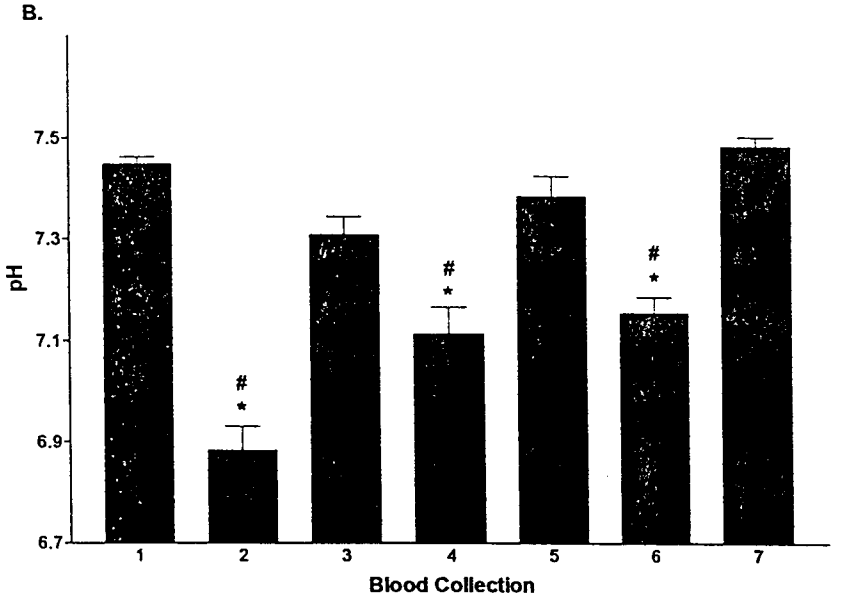
1. Blood pH measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. Samples 1, 3 and 5 are pre-submergence, whereas samples 2, 4, and 6 are post-submergence. Sample 7 was collected three hours after the final submergence. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Significant differences from each pre-submergence value is denoted by an asterisk (*), whereas significant differences of samples from the first pre-submergence value are denoted with a pound sign (#).

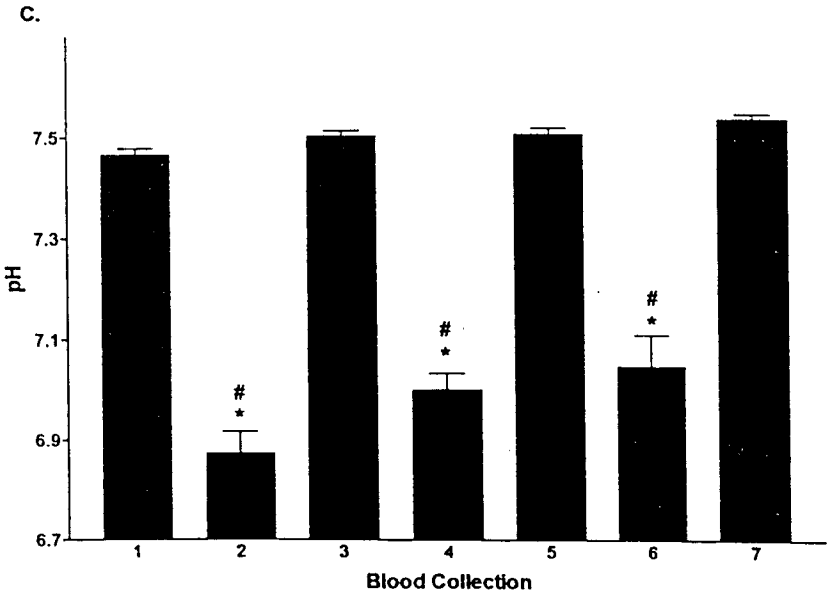
2. Blood Pco_2 measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Rest of the legend as in Figure 1.

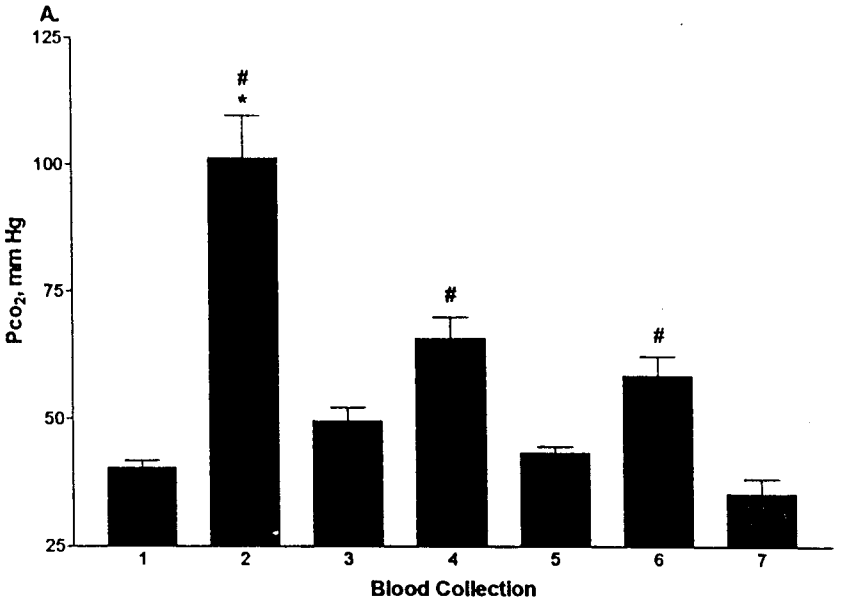
3. Blood lactate concentration measured prior to and after three successive forced submergence episodes in loggerhead sea turtles. The interval between the submergences was 10 min (A), 42 min (B) or 180 min (C). Rest of the legend as in Figure 1.

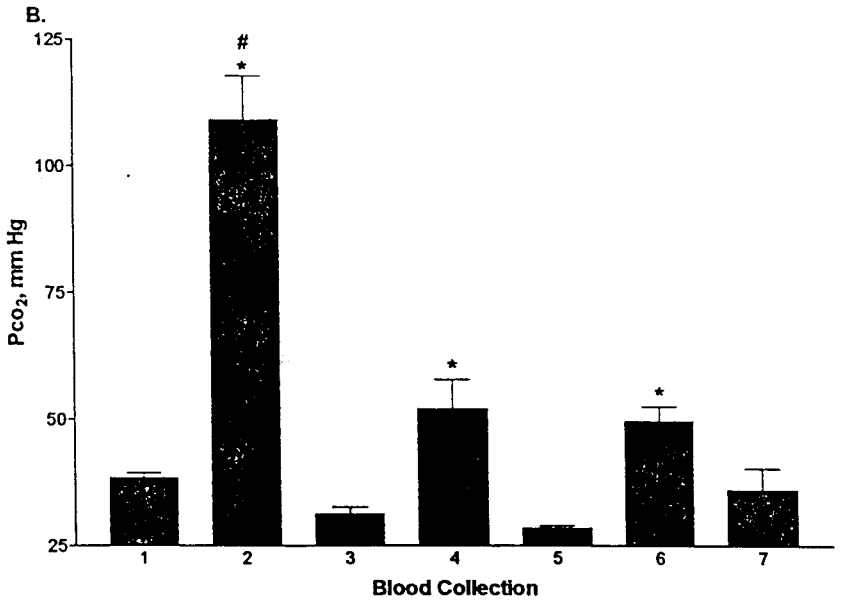
4. Relationship between blood pH and plasma [Na^+], [K^+], [Cl^-], osmolality, and hematocrit in control (left column) and submerged (right column) loggerhead sea turtles. The line is a best fit to the data. Significance of the correlated variables is noted on each figure.

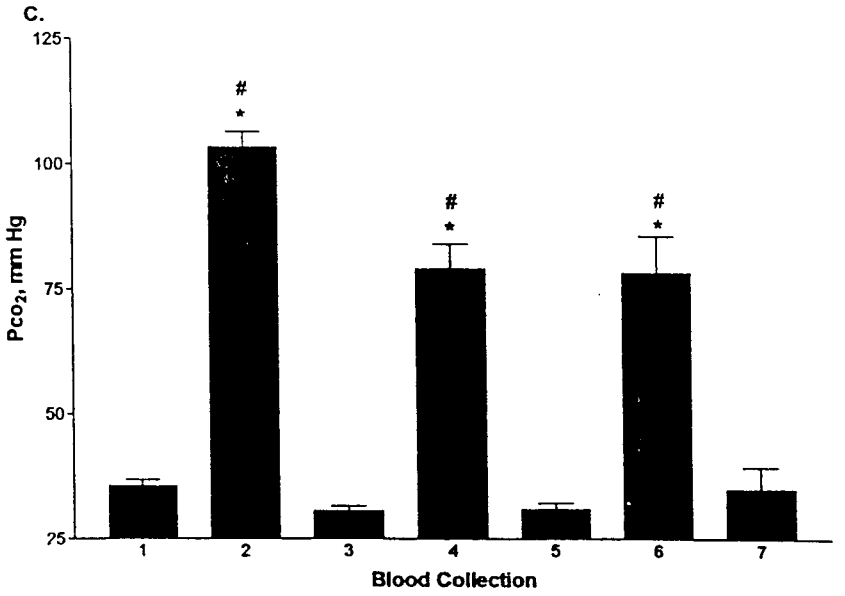

A.
pH
7.5
7.3
7.1
6.9
6.7
#
*
#
#
#
#
1
2
3
4
5
6
7
Blood Collection

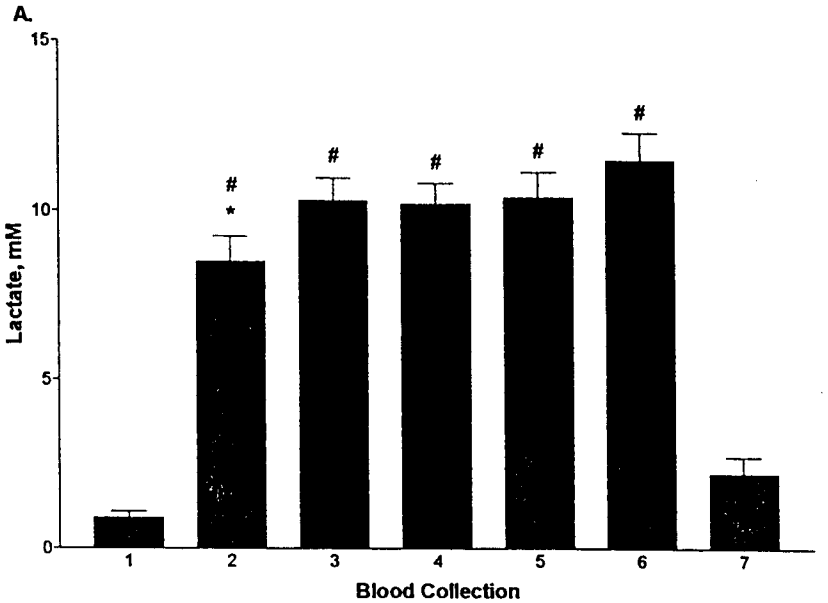

B.
pH
7.5
7.3
7.1
6.9
6.7
#
*
#
*
#
*
1
2
3
4
5
6
7
Blood Collection

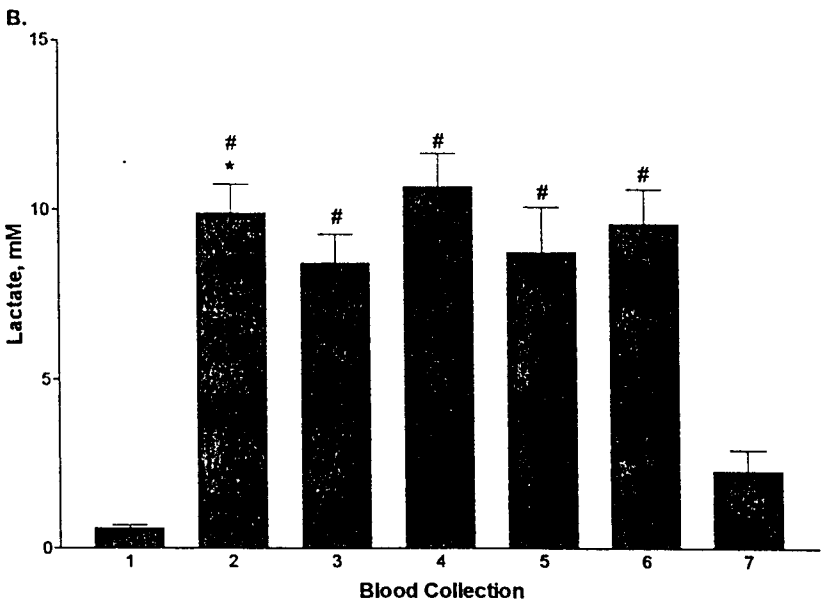

C.
pH
7.5
7.3
7.1
6.9
6.7
#
*
#
*
#
*
1
2
3
4
5
6
7
Blood Collection

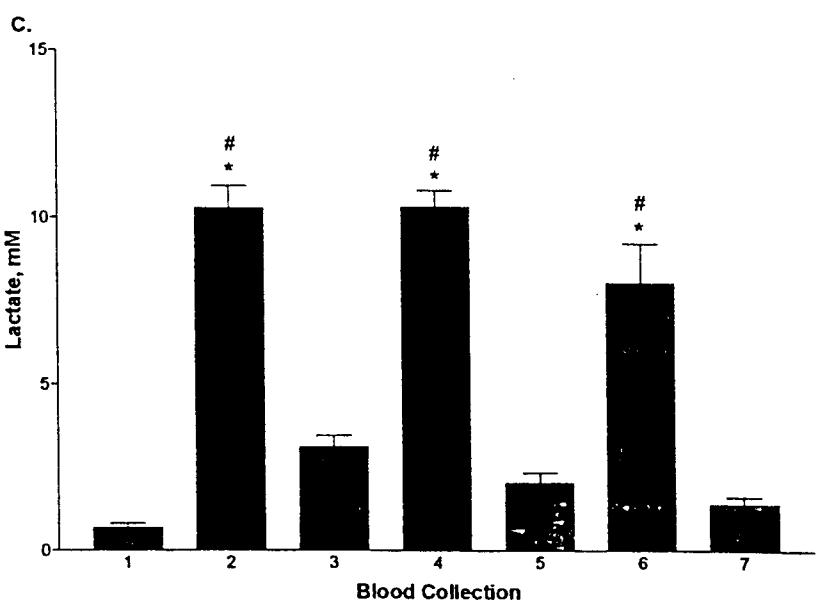

A.
125
100
75
50
25
Pco2, mm Hg
#
*
#
#
1
2
3
4
5
6
7
Blood Collection


B.
125
100
75
50
25
Pco2, mm Hg
#
*
*
*
1
2
3
4
5
6
7
Blood Collection


C.
125
100
75
50
25
Pco2, mm Hg
#
*
#
*
#
*
1
2
3
4
5
6
7
Blood Collection

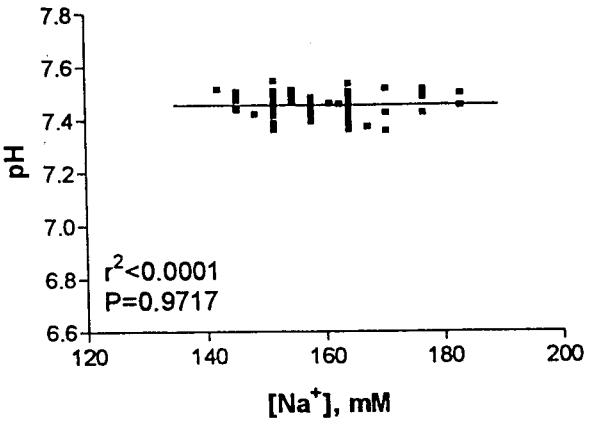

A.
Lactate, mM
15
10
5
0
#
*
#
#
#
#
1
2
3
4
5
6
7
Blood Collection

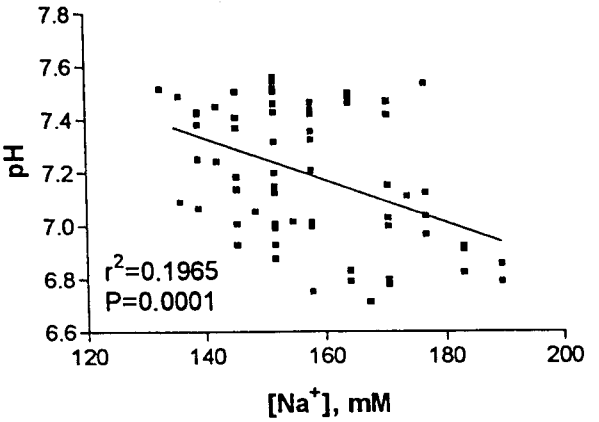

B.
Lactate, mM
15
10
5
0
#
*
#
#
#
#
1
2
3
4
5
6
7
Blood Collection

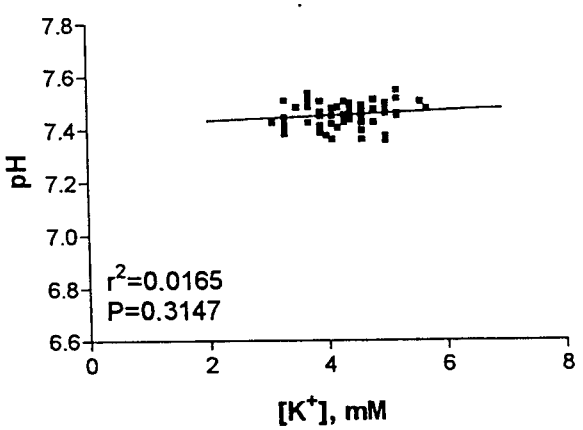

C.
Lactate, mM
15
10
5
0
#
*
#
*
#
*
1
2
3
4
5
6
7
Blood Collection

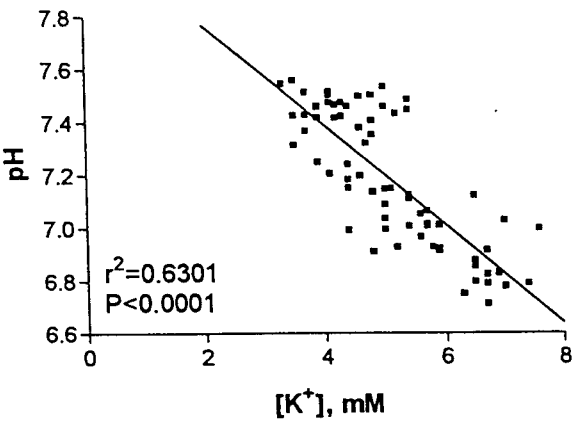
Control Treatment

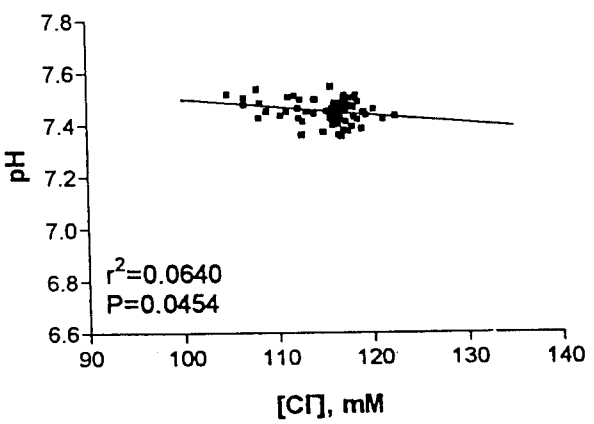
Submergence Treatment

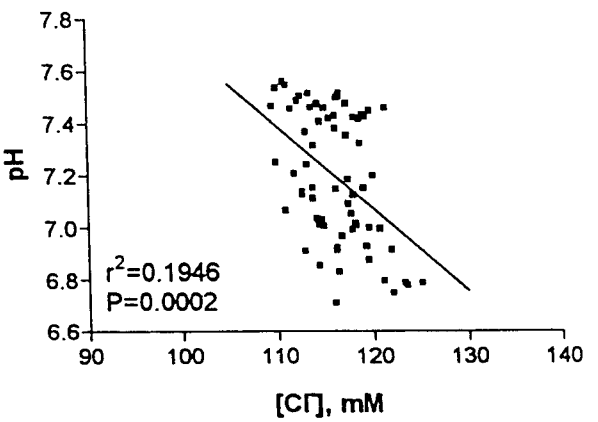


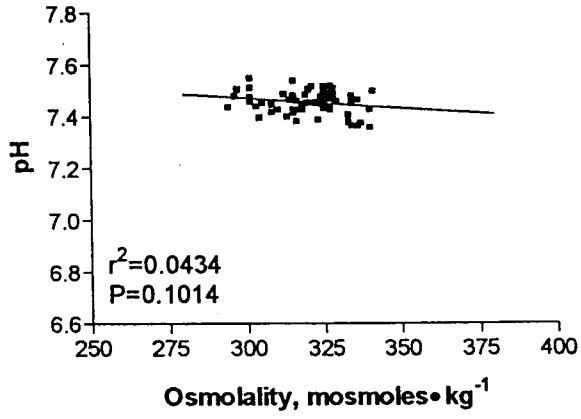
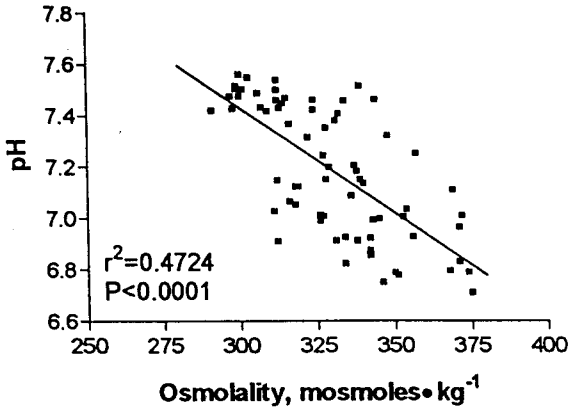
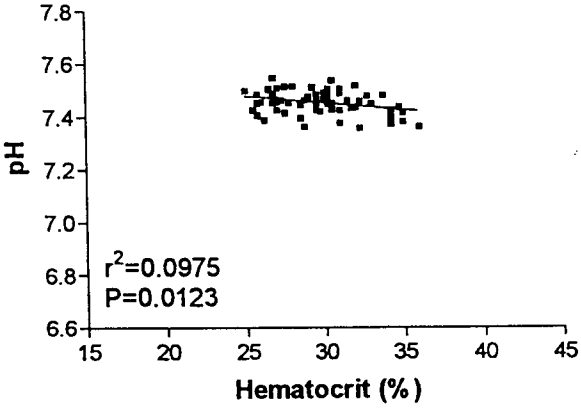
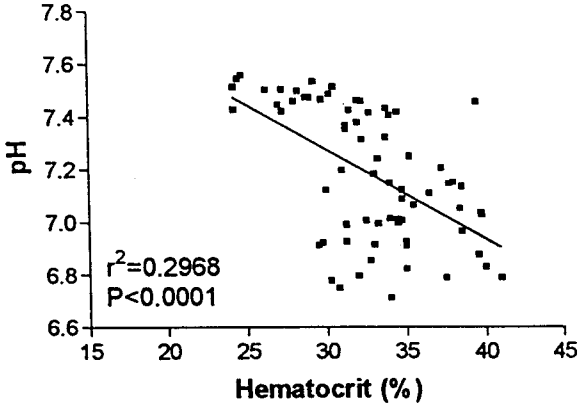










Control Treatment

Submergence Treatment