

# Blood Biochemistry of Sea Turtles Captured in Gillnets in the Lower Cape Fear River, North Carolina, USA

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**ABSTRACT** Mortality due to fisheries interactions has been implicated as a contributor to population decline for several species of sea turtle. The incidental capture of sea turtles in the coastal gillnet fisheries of North Carolina, USA, has received much attention in recent years, and mitigation measures to reduce sea turtle mortality due to gillnet entanglement are a high priority for managers and conservationists. Efforts to evaluate effects of gillnet entanglement on sea turtle populations are complicated by the lack of information on health status of turtles released alive from nets and postrelease mortality. We obtained blood samples from green (*Chelonia mydas*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles captured in gillnets for 20–240 minutes to assess the impacts of gillnet entanglement on blood biochemistry and physiological status. We measured concentrations of lactate, corticosterone, ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{P}$ ,  $\text{Ca}^{2+}$ ), enzymes (lactate dehydrogenase [LDH], creatine phosphokinase [CPK], aspartate aminotransferase [AST]), protein, and glucose in the blood and also performed physical examinations of turtles to document external indicators of health status (injuries, lethargy, muted reflexes). We evaluated the effects of entanglement time on blood biochemistry and to look for correlations between blood biochemistry and results of the physical examinations. We observed a significant increase in blood lactate, LDH, CPK, phosphorus, and glucose with increased entanglement time. Alterations in blood biochemistry were generally associated with a decline in health status as indicated by results of the physical examination. Although entanglement time plays an important role in determining the health status of sea turtles upon release from a gillnet, our results suggest that factors such as the depth and severity of entanglement may also have an effect on health status of turtles and the probability of postrelease survival. We were unable to set a maximum unattended gillnet soak time to minimize impacts on captured sea turtles, and therefore recommend that fisheries managers continue to enforce the net attendance regulations currently in place in the lower Cape Fear River, North Carolina, during the summer months. (JOURNAL OF WILDLIFE MANAGEMENT 73(8):1394–1401; 2009)

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Commercial fishing operations frequently overlap with sea turtle habitat, and unintended capture of sea turtles in fishing gear has become a problem of increasing concern for fisheries managers and conservationists (Magnuson et al. 1990, National Marine Fisheries Service and United States Fish and Wildlife Service 1991, Santora 2003, Lewison et al. 2004, Read et al. 2004). All sea turtles are protected by the Endangered Species Act of 1973, and the federal government has a mandate to assess and mitigate the impacts of commercial fisheries interactions on sea turtle populations (National Marine Fisheries Service and United States Fish and Wildlife Service 1991). Interactions with gillnet fisheries have been implicated as a major cause of mortality for loggerhead (*Caretta caretta*), Kemp's ridley (*Lepidochelys kempii*), and green (*Chelonia mydas*) sea turtles in coastal North Carolina, USA (Gearhart 2001, Price 2005). Throughout spring, summer, and fall of 1999, 430 sea turtle carcasses washed up on the shores of North Carolina, accounting for 19% of the total sea turtle carcass strandings reported in the United States that year (Boettcher 2000). Coastal gillnet fisheries were identified as a primary contributor to the mass sea turtle stranding event because of the high fishing effort that year and injuries on sea turtle carcasses consistent with gillnet entanglement (Boettcher 2000, Gearhart 2001).

Since the mass stranding events of 1999, North Carolina Division of Marine Fisheries and the National Oceanic and

Atmospheric Administration (NOAA) Fisheries have implemented temporal and spatial fisheries closures to avoid interactions with sea turtles, as well as gear restrictions designed to minimize the impacts of entanglement on sea turtle health and survival (Federal Register 2004, Thorpe and Beresoff 2005). For example, use of gillnets is now restricted to shallow waters (<3 m), because the incidence of in-net mortality for turtles caught in shallow-set gillnets is low compared with deep-set gillnets (3–6 m; Gearhart 2001, Price 2005). Managers speculate that sea turtles entangled in shallow-set nets are still capable of reaching the surface to breathe and therefore the risk of drowning in the nets is reduced (Gearhart 2001). Although observer data and reports from fishermen indicate that sea turtles caught in shallow-set gillnets are typically released alive (Gearhart 2001), the ultimate fate of these sea turtles is not known. Severe disruptions to normal physiological function and injuries sustained as a result of entanglement in fishing gear could lead to undocumented postrelease mortality (Lutcavage and Lutz 1991, Harms et al. 2003, Stabenau and Vietti 2003).

Previous studies have shown that sea turtles that experience hypoxia and restraint stress related to enforced submergence have significant alterations in blood biochemistry (Berkson 1966, Stabenau et al. 1991, Gregory et al. 1996). Harms et al. (2003) observed a decrease in blood pH in loggerhead turtles submerged in trawls for  $\leq 30$  minutes. The controlled release of ions into the blood may occur as a counteractive measure against blood acidosis. For example, a

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significant increase in blood  $K^+$  concentration has been observed following capture and restraint in Kemp's ridley turtles (Stabenau et al. 1991, Hoopes et al. 2000). It is possible that  $K^+$  ions are released by cells in exchange for  $H^+$  ions to buffer changes in blood pH, although a  $K^+-H^+$  exchanger has not yet been identified in the cells of sea turtles (Rose 1977, Lutz 1997, Hoopes et al. 2000, Stabenau and Vietti 2003). It is likely that the acidosis experienced by forcibly submerged sea turtles has both a respiratory and metabolic component. Stabenau et al. (1991) saw a 6-fold increase in lactate levels of Kemp's ridleys forcibly submerged in shrimp trawls for approximately 7.3 minutes. Kemp's ridley turtles captured in entanglement nets and temporarily restrained in holding tanks also experienced an increase in plasma lactate concentrations and alterations in blood ions indicative of acid-base adjustments (Hoopes et al. 2000). Increases in blood lactate concentrations and a concomitant decrease in blood pH suggest a shift towards reliance on anaerobic metabolic pathways, which could be the result of intense activity associated with escape attempts or hypoxia due to forced submergence. Prolonged anaerobiosis due to entanglement in fishing gear or restraint may leave sea turtles exhausted and vulnerable to other threats upon release from gear. There is also evidence that entanglement in fishing gear results in induction of a systemic stress response in sea turtles, which may persist following release depending on the extent of injuries suffered or stress experienced. Gregory et al. (1996) noted approximately a 3-fold increase above control values for plasma corticosterone (a hormone indicative of stress) in loggerheads that were forcibly submerged in a trawl for  $\leq 30$  minutes.

As with entanglement in trawls and other gear types, sea turtles entangled in gillnets may experience physiological disturbances related to restricted access to air, intense struggling, injuries to soft tissues, and induction of a systemic stress response (Lutz and Dunbar-Cooper 1987, Stabenau et al. 1991, Gregory et al. 1996, Boettcher 2000, Jessop et al. 2004). Our objectives were to 1) investigate the physiological impacts of gillnet entanglement on juvenile sea turtles, and 2) determine if entanglement time would be indicative of the degree of physiological disruption in gillnet-captured juvenile sea turtles. We predicted that the degree of physiological disruption, as indicated by blood biochemistry, would increase with increased entanglement time and that severe disruptions in blood biochemistry would be associated with poor health status as ascertained by a physical examination.

## STUDY AREA

We determined capture sites for our study based on where sea turtles had been previously and reliably sighted in the lower Cape Fear River, North Carolina, between  $33^{\circ}59'24''N$  and  $33^{\circ}51'0''N$  and  $78^{\circ}2'24''W$  and  $77^{\circ}55'12''W$ . This area consisted of 3 bays enclosed by marsh to the east and a man-made rock wall that divided the bays from the river to the west. We made 56% of captures along the rock wall, where green turtles were likely foraging on the algae that grows on the rocks. Average tide height at this location was approximately 1 m, and the rock wall was partially exposed at low tide. Average

salinity was 36 parts per thousand and average water temperature ( $T_W$ ) was  $27.6^{\circ}C$  during the study period.

## METHODS

We captured sea turtles in mesh gillnets set at depths of 1–2 m in the lower Cape Fear River, North Carolina, during daylight hours (0600–1600) from May through October of 2007. Gillnets remained in water for a maximum of 6 hours and were attended at all times so that we could record time when we captured turtles and length of time turtles spent in the net (entanglement time). We captured 14 green turtles and 4 Kemp's ridley turtles. Table 1 provides details on capture time and location, entanglement time, environmental conditions at the capture site, and morphometric data for each turtle. Turtles were entangled for an average of 82.3 minutes (range of 20–240 min) and we closely monitored them for signs of distress while in the net. If turtles remained submerged for  $>20$  minutes or appeared to be in danger of drowning due to airway or swimming restriction, we immediately removed them from the net.

Upon removal from nets, we brought turtles on board the boat and restrained them in a 16-cm  $\times$  43-cm padded plastic bin. We shaded turtles from direct sunlight and periodically sprayed them with seawater. We immediately obtained a 4-mL blood sample (INITIAL sample) from the cervical sinus using heparinized vacuum tubes and a 21-gauge  $\times$  3.8-cm needle (BD Vacutainer, Franklin Lakes, NJ) and stored samples on ice ( $n = 12$  for green turtles,  $n = 4$  for Kemp's ridley turtles). We did not obtain INITIAL blood samples from 2 of the 14 captured green turtles. Using calipers, we measured straight carapace length notch to notch (SCL<sub>nn</sub>) and straight carapace width (SCW), and calculated carapace area (cm<sup>2</sup>) using the formula for the area of an ellipse:  $\text{area (cm}^2\text{)} = \pi \times 1/2(\text{SCL}_{nn}) \times 1/2(\text{SCW})$ . We obtained cloacal body temperature ( $T_B$ ) using a digital thermometer (model 52 II; Fluke Corp., Everett, WA) with a flexible veterinary probe at a depth of 4–25 cm. We inserted a passive induced transponder tag above the left front flipper of each turtle we captured for future identification.

We examined turtles for net-inflicted external injuries and assessed reflex responses and activity levels using a protocol described by Sadove et al. (1998). We classified injuries as minor (scrapes to skin or shell), moderate (shallow cuts to skin, bruising of skin), or severe (deep cuts that exposed muscle). We classified reflex responses to a gentle touch to the tail, nose, and eyelid as good (immediate and strong flinch), delayed (slow or lethargic flinch), or absent. We classified activity as high if the turtle frequently and vigorously struggled in attempts to escape. We classified turtles as having moderate activity levels if they occasionally struggled vigorously with long periods of little to no movement in between. We classified activity level as low if turtles exhibited infrequent and weak struggling or no movement when on board. We assigned a physical grade (A, B, C, D) based on the reflex response level, activity level, and presence-absence and severity of net-inflicted external injuries to each turtle. The physical grade criteria are as follows: A, high activity level, all reflexes present and good,

**Table 1.** Descriptive information<sup>a</sup> for Kemp's ridley (Lk,  $n = 4$ ) and green (Cm,  $n = 14$ ) sea turtles captured in gillnets in the lower Cape Fear River, North Carolina, USA, from May to October 2007.

Turtle ID	Capture date	Capture time	Capture location	Salinity (ppt)	T <sub>W</sub> (°C)	T <sub>B</sub> (°C)	SCL (cm)	SCW (cm)	Carapace area (cm <sup>2</sup> )	Entanglement time (min)	Physical grade <sup>b</sup>
Lk 1	6 Jun 2007	1315	33°57'02"N, 77°56'26"W	32	27.3	27.6	29.9	32.0	751.5	45	A
Lk 2	30 Jun 2007	0829	33°54'03"N, 78°02'03"W	33	28.5	30.5	37.6	34.5	1,021.8	107	B
Lk 3	3 Jul 2007	0924	33°54'42"N, 77°59'52"W	34	26.2		22.4	19.9	350.1	30	A
Lk 4	31 Aug 2007	0920	33°55'25"N, 77°57'32"W	38	28.7	29.3	38.1	36.6	1,095.2	30	B
$\bar{x}$						29.1	32.0	30.8	804.7	53	
SD						1.5	7.4	7.5	337.2	36.7	
Cm 1	7 Jun 2007	0900	33°55'32"N, 77°57'48"W	33	26.9	27.5	32.2	25.0	632.2	63	C
Cm 2	8 Jun 2007	1057	33°55'42"N, 77°57'35"W	32	28.2	28.3	29.3	22.7	526.5	218	D
Cm 3	14 Jun 2007	1030	33°56'34"N, 77°56'52"W	34	27.0	26.3	28.6	22.7	512.1	132	C
Cm 4	4 Jul 2007	1219	33°56'32"N, 77°56'54"W	33	27.0	27.0	32.2	24.9	632.2	49	C
Cm 5	20 Aug 2007	1311	33°55'36"N, 77°57'14"W	35	32.3	33.0	27.6	23.0	498.6	143	D
Cm 6	2 Sep 2007	1258	33°55'03"N, 77°58'14"W	38	27.0		26.2	19.7	405.4	30	B
Cm 7	2 Sep 2007	1613	33°55'03"N, 77°58'14"W	38	27.0	28.3	35.7	27.4	768.3	30	C
Cm 8	8 Sep 2007	1311	33°55'05"N, 77°58'15"W	37	27.8	31.8	28.8	23.2	524.8	30	B
Cm 9	12 Sep 2007	1334	33°55'16"N, 77°58'05"W	37	28.7		26.9	20.0	522.5	70	C
Cm 10	19 Sep 2007	1535	33°53'39"N, 77°57'32"W	38	23.3	26.3	30.6	23.8	572.0	30	B
Cm 11	26 Sep 2007	1442	33°55'17"N, 77°58'04"W	35	28.3	30.2	29.6	23.0	529.3	20	A
Cm 12	19 Oct 2007	1311	33°53'24"N, 77°57'47"W	39	27.4	27.8	27.0	21.6	458.0	212	B
Cm 13	19 Oct 2007	1457	33°53'24"N, 77°57'47"W	39	27.4	28.0	32.2	25.7	652.5	88	C
Cm 14	19 Oct 2007	1813	33°53'24"N, 77°57'47"W	39	27.4		25.0	20.3	400.6	240	C
$\bar{x}$						28.6	29.4	23.1	544.6	96.8	
SD						2.2	2.9	2.2	100.2	78.4	

<sup>a</sup> ppt, parts per thousand; T<sub>W</sub>, average water temp; T<sub>B</sub>, cloacal body temp; SCL, straight carapace length; SCW, straight carapace width.

<sup>b</sup> Physical grade criteria: A, high activity level, all reflexes present and good, no injuries; B, medium activity level, all reflexes present and good, minor injuries; C, medium activity level, missing or delayed reflexes, moderate injuries; and D, low activity level, missing or delayed reflexes, severe injuries.

no injuries; B, medium activity level, all reflexes present and good, minor injuries; C, medium activity level, missing or delayed reflexes, moderate injuries; and D, low activity level, missing or delayed reflexes, severe injuries. We assigned each grade based on exhibition of at least 2 of the 3 criteria.

Turtles were on board the boat for an average of 57.7 minutes (range of 10–110 min), and we subsequently released them within 10 m of the capture site. Immediately prior to release, we obtained a second 4-mL blood sample (PRERELEASE sample) from 7 green turtles so that we could assess the effect of onboard restraint on blood parameters. We stored INITIAL and PRERELEASE blood samples on ice for 30–240 minutes before centrifuging at 7,000 rpm for 10 minutes using a portable field centrifuge (Zip Spin; LW Scientific Inc., Lawrenceville, GA). We stored plasma in cryogenic tubes on dry ice and ultimately

transferred it to a  $-80^{\circ}$  C freezer. We analyzed blood biochemistry within 4 months of sample collection.

All procedures used for this study were approved by University of North Carolina Wilmington Institutional Animal Care and Use Committee (protocol 2006-12) and the NOAA Fisheries Office of Protected Resources (permit 1572).

We analyzed plasma concentrations of lactate dehydrogenase (LDH), creatine phosphokinase (CPK), aspartate aminotransferase (AST), Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, P, Ca<sup>2+</sup>, total protein, albumin, globulin, uric acid, urea nitrogen, and glucose by spectrophotometry at a veterinary diagnostic laboratory (Antech Diagnostics, Southaven, MS). We determined plasma lactate concentrations using a commercially available 2-step lactate reagent kit (Pointe Scientific Inc., Canton, MI) and standard spectrophotometric techniques (Lambda 25 UV/Vis; PerkinElmer, Waltham, MA).

We used lactate standards of 5 millimoles per liter (mmol/L), 10 mmol/L, 15 mmol/L, and 50 mmol/L to generate a regression equation to describe the relationship between absorbance (abs) and lactate concentration ( $[\text{Lactate}]_{\text{mmol/L}} = [\text{abs} - 0.0309]/0.0299$ ,  $r^2 = 0.9995$ ).

We ran all plasma samples in duplicate, and we used the mean of duplicate absorbance values to estimate plasma lactate concentrations using the standard regression. We assayed buffer solutions and 15-mmol/L standard solutions simultaneously with plasma samples as a quality control measure.

We analyzed corticosterone levels by radioimmunoassay, as previously described by Valverde (1996). For each sample, we extracted 25–250  $\mu\text{L}$  of plasma with 4 mL of anhydrous diethyl ether, dried the tubes under nitrogen gas, and resuspended them with 1 mL of gel buffer (pH 7.0). We pipetted 2 400- $\mu\text{L}$  aliquots from the 1 mL of gel buffer and placed the tubes at 4° C overnight. The following day, we incubated all tubes in a water bath for 30 minutes at 37° C. At this point, we prepared tubes containing 400  $\mu\text{L}$  of corticosterone standard solution with concentrations ranging from 0.0625 ng/mL to 8 ng/mL in duplicate. Following incubation, we added 100  $\mu\text{L}$  of corticosterone antibody (no. B3-163, lot 163-077, purchased from Esoterix Laboratory Services, Calabasas Hills, CA) to all tubes (standards and samples), as well as 100  $\mu\text{L}$  of tritiated corticosterone (approx. 10,000 counts/min [cpm]; PerkinElmer Life and Analytical Sciences, Inc., Boston, MA). We incubated the tubes overnight at 4° C. The next day, we placed all tubes in an ice bath and added 500  $\mu\text{L}$  of dextran-coated charcoal to each assay tube except those used to determine total counts. We incubated all tubes for 15 minutes at 4° C, and centrifuged them at 2,300 rpm at 4° C for 15 minutes. We then poured the supernatant into scintillation vials and added 5 mL of Ecolume scintillation cocktail (MP Biomedicals, Solon, OH) to each vial. We counted the vials for 60 seconds with a Wallac 1409 liquid scintillation counter (Pegasus Scientific, Inc., Rockville, MD). We then calculated corticosterone concentrations in nanograms per milliliter from the counts using the standard curve. We corrected the values by multiplying the volume extracted by the extraction efficiency and the fraction aliquoted from the reconstituted sample (40%). We calculated the extraction efficiency for each sample individually by extracting the same volume of plasma used to determine corticosterone concentration as described above (25–250  $\mu\text{L}$ ), and by adding 100  $\mu\text{L}$  of tritiated corticosterone (approx. 10,000 cpm) prior to the ether-extraction. Extraction efficiencies ranged from 92.4% to 100%. We used a loggerhead sea turtle control sample and extracted 4–5 times to evaluate intra- and inter-assay variability, which was 4.3% and 12.9%, respectively.

In order to assess the effects of entanglement time and physical grade on each blood parameter for the INITIAL samples collected from green turtles, we used analysis of covariance (ANCOVA). We used only INITIAL samples ( $n = 12$ ), as opposed to PRERELEASE samples, for these analyses because this allowed us to assess the physiological disturbances attributable to gillnet entanglement without

the potentially confounding effects of onboard restraint. Due to a small sample size ( $n = 4$ ), we did not perform statistical analyses of Kemp's ridley data. We examined the relationship between each blood parameter and predictors such as  $T_B$ ,  $T_W$ , carapace area (as an indicator of body size), and salinity using Pearson's correlation. For predictors that were determined to be strongly ( $r > 0.50$ ) and significantly ( $P < 0.05$ ) correlated with a particular blood parameter, we initially included them in the ANCOVA model for that parameter. As a result, we used  $T_B$  as a covariate in the ANCOVA model for LDH, and we used carapace area as a covariate in the ANCOVA models for  $\text{Na}^+$ ,  $\text{Cl}^-$ , and glucose. Ultimately, carapace area contributed significantly to the ANCOVA model for glucose, but none of the other covariates contributed significantly to ANCOVA models.

We used a paired *t*-test and applied a Bonferroni correction to compare INITIAL and PRERELEASE values for blood parameters in green sea turtles ( $n = 7$ ). We set significance at  $P < 0.003$  using the Bonferroni correction. We performed all analyses using Statistical Analysis Software (SAS) version 9.1 (SAS Institute, Cary, NC).

## RESULTS

We classified most turtles that were entangled in gillnets for <4 hours as physical grades of B and C (Table 1). Of the 18 turtles that we captured, we classified 17% as physical grade A, 33% as physical grade B, 39% as physical grade C, and 11% as physical grade D. We found that the values for several blood parameters were elevated above baseline values (Table 2). Increased entanglement time and decreased physical grade accounted for an increase in plasma lactate ( $F_6 = 25.91$ ,  $P = 0.001$ ), LDH ( $F_7 = 7,611.39$ ,  $P = 0.009$ ), CPK ( $F_5 = 8.53$ ,  $P = 0.017$ ), phosphorus ( $F_6 = 10.61$ ,  $P = 0.010$ ), and glucose ( $F_7 = 8.44$ ,  $P = 0.028$ ). The models for CPK and LDH were largely driven by the values for 2 turtles. When we removed these turtles from the analysis, the ANCOVA model testing the effects of entanglement time and physical grade on LDH concentration was still statistically significant ( $P = 0.039$ ), but the model for CPK concentrations was not significant ( $P = 0.915$ ). We also found that entanglement time and physical grade did not account for trends in plasma albumin ( $F_5 = 5.01$ ,  $P = 0.051$ ), AST ( $F_6 = 0.25$ ,  $P = 0.939$ ),  $\text{Na}^+$  ( $F_7 = 0.58$ ,  $P = 0.750$ ),  $\text{K}^+$  ( $F_6 = 2.41$ ,  $P = 0.177$ ),  $\text{Cl}^-$  ( $F_7 = 2.39$ ,  $P = 0.209$ ),  $\text{Ca}^{2+}$  ( $F_6 = 3.67$ ,  $P = 0.088$ ), total protein ( $F_6 = 3.83$ ,  $P = 0.081$ ), globulin ( $F_6 = 2.11$ ,  $P = 0.216$ ), uric acid ( $F_6 = 4.83$ ,  $P = 0.052$ ), urea nitrogen ( $F_6 = 0.86$ ,  $P = 0.577$ ), or corticosterone ( $F_6 = 1.34$ ,  $P = 0.383$ ). Although the trend in increased corticosterone with increased time in net and decreased physical grade was not significant, we recorded very high corticosterone in gillnet-entangled turtles (Table 2). We did not find any significant trend in any blood parameter between INITIAL and PRERELEASE samples from gillnet-entangled green turtles (Table 3).

## DISCUSSION

Our objective was to investigate the effect of gillnet entanglement time on blood biochemistry and health status of sea turtles. We found that longer entanglement times

**Table 2.** INITIAL blood parameters of green and Kemp's ridley sea turtles captured in the lower Cape Fear River, North Carolina, USA, May–October 2007.

Blood parameter	Green turtles Initial ( <i>n</i> = 12)			Kemp's ridley turtles Initial ( <i>n</i> = 4)			Mean normal blood values	
	Range	$\bar{x}$	SD	Range	$\bar{x}$	SD	Green turtles	Kemp's ridley turtles
Lactate dehydrogenase (units[U]/L)	187–6,420	897.8	1,795.6	1–2,045	1,164.3	913.0	135 <sup>a</sup> , 109.4 <sup>b</sup>	1,298.7 <sup>c</sup>
Creatine phosphokinase (U/L)	1,629–31,000	7,148.3	9,007.2	80–4,734	2,412.3	2,235.4	425 <sup>d</sup>	
Aspartate aminotransferase (U/L)	227–702	311.6	129.6	59–183	108.8	54.9	178 <sup>a</sup> , 141.0 <sup>b</sup>	144.7 <sup>c</sup>
Na <sup>+</sup> (mEq/L)	159–173	165.1	5.3	158–332	202	86.7	172 <sup>a</sup> , 152.2 <sup>b</sup>	140.5 <sup>e</sup> , 153.3 <sup>c</sup>
K <sup>+</sup> (mEq/L)	4.8–8.5	6.4	1.2	5.2–8.8	6.3	1.7	5.3 <sup>a</sup> , 5.0 <sup>b</sup>	6.3 <sup>e</sup> , 3.6 <sup>c</sup>
Cl <sup>-</sup> (mEq/L)	103.0–131.0	116.5	7.0	118–380	187	128.7	113 <sup>a</sup> , 109.0 <sup>b</sup>	112.2 <sup>e</sup> , 115.2 <sup>c</sup>
P (mg/dL)	5.3–14.5	9.7	2.6	3.8–13.2	7.5	4.0	6.7 <sup>a</sup> , 7.9 <sup>b</sup>	6.8 <sup>c</sup>
Ca <sup>2+</sup> (mg/dL)	8.2–15.7	11.2	2.1	8.5–28.0	13.5	9.7	9.1 <sup>a</sup> , 8.4 <sup>b</sup>	7.4 <sup>c</sup>
Total protein (g/dL)	2.4–5.0	3.8	0.7	1.5–3.6	2.6	0.9	5.1 <sup>a</sup> , 4.3 <sup>b</sup>	3.1 <sup>c</sup>
Albumin (g/dL)	0.8–1.7	1.5	0.3	0.5–1.1	0.9	0.3	1.5 <sup>a</sup> , 0.9 <sup>b</sup>	1.3 <sup>c</sup>
Globulin (g/dL)	1.6–3.3	2.3	0.5	1.0–2.5	1.7	0.6	3.6 <sup>a</sup> , 3.0 <sup>b</sup>	1.8 <sup>c</sup>
Uric acid (mg/dL)	0.6–4.3	2.2	1.3	0.2–2.9	1.3	1.2	1.5 <sup>a</sup> , 0.8 <sup>b</sup>	
Urea nitrogen (mg/dL)	2–28	9.8	9.1	39–86	68.3	20.7	7 <sup>a</sup> , 1.0 <sup>b</sup>	73.7 <sup>c</sup>
Glucose (mg/dL)	89–192	136.3	34.9	60–178	112.3	48.8	114 <sup>a</sup> , 86.6 <sup>b</sup>	115.2 <sup>c</sup>
Lactate (mmol/L)	13.1–50.2	30.6	10.2	16.0–19.4	17.5	1.4	0.5 <sup>d</sup> , 1.1 <sup>f</sup>	0.7 <sup>c</sup>
Corticosterone (ng/mL)	0.29–51.8	20.8	16.5	3.5–19.3	7.8	7.7	0.7 <sup>b</sup>	3.1 <sup>g</sup>

<sup>a</sup> Bolten and Bjorndal 1992.

<sup>b</sup> Aguirre et al. 1995.

<sup>c</sup> Carminati et al. 1994.

<sup>d</sup> Butler et al. 1984.

<sup>e</sup> Stabenau et al. 1991.

<sup>f</sup> Berkson 1966.

<sup>g</sup> Gregory and Schmid 2001.

resulted in pronounced disruptions in blood biochemistry and were associated with lower physical grades (Tables 1 and 2). In general, blood parameters that did not vary significantly with increased gillnet entanglement time fell within the range of published values for healthy, wild-caught green sea turtles (Bolten and Bjorndal 1992, Aguirre et al. 1995, Hasbún et al. 1998), whereas parameters that were significantly impacted by gillnet entanglement time were in agreement with literature values for sea turtles exposed to stressors (Lutz and Dunbar-Cooper 1987, Gregory et al. 1996, Hoopes et al. 2000, Jessop et al. 2002, Harms et al. 2003).

Entanglement times of as little as 30 minutes resulted in elevated plasma lactate. The highest lactate value recorded in

our study (50.6 mmol/L) was 2–8 times higher than maximum values for blood lactate reported in other studies of sea turtle entanglement in fishing gear (6.2–20 mmol/L; Lutz and Dunbar-Cooper 1987, Hoopes et al. 2000, Harms et al. 2003). The average blood lactate concentration for green turtles in our study ( $30.6 \pm 10.2$ ,  $n = 12$ ) was 9 times higher than average blood lactate concentration of rehabilitated captive green turtles not exposed to a stress protocol and just prior to release ( $3.4 \pm 1.1$ ,  $n = 10$ ; C. Harms, NC State University Center for Marine Sciences and Technology, and J. Beasley, Karen Beasley Sea Turtle Rescue and Rehabilitation Center, unpublished data). Although sea turtles have a high aerobic capacity to support sustained, long-distance swimming

**Table 3.** Results of paired *t*-test analysis for INITIAL and PRERELEASE blood parameters of green sea turtles captured in the lower Cape Fear River, North Carolina, USA, May–October 2007 ( $n = 6$  for all parameters except for lactate and corticosterone, where  $n = 7$ ). Time between INITIAL and PRERELEASE samples ranged from 45 minutes to 79 minutes.

Blood parameter	INITIAL		PRERELEASE		df	<i>t</i>	<i>P</i>
	$\bar{x}$	SD	$\bar{x}$	SD			
Lactate dehydrogenase (units[U]/L)	534.0	623.2	652.2	783.2	5	-1.53	0.188
Creatine phosphokinase (U/L)	6,790.5	6,601.7	8,306.5	8,213.6	5	-1.97	0.105
Aspartate aminotransferase (U/L)	327.7	185.2	324.3	147.8	5	0.18	0.864
Na <sup>+</sup> (mEq/L)	162.0	2.3	158.8	10.5	5	0.72	0.502
K <sup>+</sup> (mEq/L)	6.3	1.5	5.9	1.4	5	1.02	0.356
Cl <sup>-</sup> (mEq/L)	112.8	5.3	110.5	5.0	5	0.73	0.497
P (mg/dL)	9.1	2.3	8.2	2.4	5	1.68	0.153
Ca <sup>2+</sup> (mg/dL)	11.0	2.7	10.9	3.9	5	0.24	0.819
Total protein (g/dL)	3.8	1.0	3.7	0.9	5	0.86	0.428
Albumin (g/dL)	1.4	0.4	1.4	0.3	5	0.25	0.809
Globulin (g/dL)	2.4	0.6	2.3	0.6	5	1.07	0.332
Uric acid (mg/dL)	2.5	1.6	3.3	2.1	5	-2.68	0.044
Urea nitrogen (mg/dL)	8.2	6.6	7.8	5.6	5	0.60	0.576
Glucose (mg/dL)	134.0	32.1	142.7	36.6	5	-1.93	0.112
Lactate (mmol/L)	30.8	12.9	32.4	11.6	6	-0.85	0.430
Corticosterone (ng/mL)	19.9	16.0	24.0	12.5	6	-1.82	0.119

(Butler et al. 1984), they resort to anaerobic pathways during intense burst activity. Intense struggling and forced submergence during entanglement likely result in a shift from aerobic to anaerobic metabolic pathways due to an imbalance between oxygen supply and demand. Increased reliance on glycolysis and lactic acid fermentation results in lactate accumulation in blood and tissues.

Oxygen is required in order for lactate to be metabolized and cleared from the bloodstream. Previous studies have noted that sea turtles subjected to enforced submergence may require extended periods of time at the surface to rest, recover, and repay the oxygen debt incurred while forcibly submerged (Lutz and Bentley 1985, Stabenau and Vietti 2003). Extended time at the surface may leave recovering sea turtles vulnerable to other threats, such as boat strikes or shark predation. Studies investigating lactate loads and clearance rates for sea turtles captured in trawls or restrained in in-water cages have demonstrated that lactate clearance rates can vary between 0.25 mmol/L/hour and 3.3 mmol/L/hour for blood lactate concentrations of 5–14 mmol/L (Lutz and Bentley 1985, Stabenau and Vietti 2003). If studied sea turtles cleared lactate at the fastest rates documented in the literature, it would take 4–15 hours to remove accumulated lactate from the bloodstream. It is likely that full clearance of the high lactate loads we observed would actually require more time, because clearance rates tend to decline with declining blood lactate concentrations. Lutz and Dunbar-Cooper (1987) calculated that clearance of only 3–4 mmol/L blood lactate could take as long as 20 hours due to the decline in clearance rates at low blood lactate concentrations.

Although low to moderate levels of circulating lactate (<5 mmol/L/hr) may not impose a great physiological challenge to sea turtles, this situation may be problematic for turtles that experience repeat captures in fishing gear. The limited home range of juvenile green and Kemp's ridley sea turtles in nearshore coastal waters (Mendonça 1983, Brill et al. 1995, Avens et al. 2003, Avens and Lohmann 2004, Makowski et al. 2006) predisposes them to multiple encounters with fishing gear set within their home range. Additional enforced submergence events greatly increase the chance of in-net or postrelease mortality, particularly if the turtle has not fully recovered from the physiological disruptions of the first entanglement. Stabenau and Vietti (2003) noted severe metabolic disturbances in juvenile loggerhead turtles forcibly submerged in trawls multiple times, and found that a surface recovery interval of 42 minutes following the first submergence was inadequate for blood lactate clearance.

Although the ANCOVA model to assess the effects of entanglement time and physical grade on blood corticosterone levels was not statistically significant, we feel this blood variable warrants further comment given the widespread effects that corticosterone may have on the physiology of sea turtles (Gregory et al. 1996; Jessop et al. 2002, 2004; Jessop and Hamann 2005). Corticosterone is a glucocorticoid that is released into the blood by the adrenal glands as a response to various stressors. The release of corticosterone triggers behavioral and physiological adjustments to promote survival while curtailing other nonessential processes, preferentially

partitioning energy stores towards survival (Jessop 2001, Jessop et al. 2002). Continued stress associated with injuries sustained during entanglement or behavioral alterations may delay clearance of corticosterone and impact postrelease survival, but we were not able to address this possibility in our study.

We recorded a maximum corticosterone concentration of 51.8 ng/mL for INITIAL samples from green turtles (Table 2), and we observed levels as high as or higher than values reported in the literature for stressed turtles (2–25 ng/mL; Gregory et al. 1996; Jessop et al. 2002, 2004; Jessop and Hamann 2005). Previous studies of induction of the stress response in sea turtles demonstrated that maximum concentrations of blood corticosterone were reached within 60–180 minutes of stress exposure (Jessop 2001, Jessop et al. 2004, Jessop and Hamann 2005). In our study, corticosterone appeared to level off at maximum concentrations within 60–120 minutes of capture. This may explain why our ANCOVA model, which covered entanglement times that ranged from 20 minutes to 240 minutes, failed to detect a significant effect of entanglement time on blood corticosterone concentrations.

The trend towards increased corticosterone with increased time in net was accompanied by an increase in blood glucose concentration, a classic signature of induction of a systemic stress response. Elevated blood glucose has been documented in previous studies of sea turtles exposed to capture and handling stress (Aguirre et al. 1995, Hoopes et al. 2000). Blood glucose levels of juvenile green turtles in our study ranged from 89 mg/dL to 192 mg/dL ( $\bar{x} \pm SD = 136.3 \pm 34.9$  mg/dL), which is consistent with levels noted by Aguirre et al. (1995) for green turtles exposed to a capture stress protocol (87–195 mg/dL).

The physiological stress response induced by gillnet entanglement may be exacerbated by injuries incurred while in the net. The significant increases in plasma LDH and CPK seen in gillnet-entangled turtles are indicative of muscle or tissue damage, as these enzymes may leak from ruptured cells into the blood stream (Aguirre et al. 1995, Killen et al. 2003, Morrissey et al. 2005). Several of the studied turtles incurred soft tissue damage from the nets, as documented during the physical examination, and possible cardiac muscle damage due to struggling and overexertion. We found average LDH in juvenile green turtles of 897.8 units per liter, which was approximately 7 times the amount noted by Aguirre et al. (1995) in juvenile green turtles exposed to acute capture and handling stress for up to 4 hours.

We observed high concentrations of phosphorus in the blood that may indicate tissue damage, as inorganic phosphates leak out of damaged cells into the bloodstream (Bishop et al. 2004). Increased blood phosphorus may also indicate decreased kidney function and filtration. Previous studies on rabbits (*Oryctolagus cuniculus*; Nastuk 1947) and rats (*Rattus norvegicus*; Goranson et al. 1948) have shown an increase in blood inorganic phosphate levels associated with shock, potentially the result of an increase in the rate of high-energy phosphate bond hydrolysis in the face of increased energy demands (McShan et al. 1945, Nastuk 1947).

Although our study focused on effects of entanglement time on health status of sea turtles, other factors may also contribute to physiological stress during gillnet entanglement. For example, turtles entangled at the bottom of the gillnet (depths >0.5 m) or turtles that have net tightly wrapped around their neck or flippers may be prevented from reaching the surface to breathe and experience severe respiratory and metabolic disruptions after only a short entanglement time. Green turtle Cm 9 was entangled in the net for only 70 minutes but at a depth >0.5 m, which made it difficult to reach the surface. This turtle had a physical grade of C, very high lactate levels (38.2 mmol/L), and the highest corticosterone levels observed in our study (51.8 ng/mL). In contrast, green turtle Cm 12 was lightly entangled for 212 minutes at the top of the net (<0.5 m). Although this turtle had one of the longest entanglement times in the study, it had a physical grade of B and low lactate (15.0 mmol/L) and corticosterone (7.0 ng/mL) levels compared with other turtles entangled for similar amounts of time. Turtles entangled at the top of the net, or only lightly entangled, may be able to endure long entanglement times with only mild to moderate disruptions in blood biochemistry due to relatively unimpeded access to air.

## MANAGEMENT IMPLICATIONS

Currently, the North Carolina Division of Marine Fisheries enforces a mandatory gillnet attendance regulation on gillnet fisheries in the lower Cape Fear River, North Carolina, during the summer months in an effort to minimize sea turtle entanglements and mortalities. This has essentially closed the fishery during this time, because fishermen are unlikely to remain with their nets during the typical soak period of 12 hours or more. We hoped our investigation of effects of entanglement time on physiology of sea turtles would allow us to determine a maximum unattended gillnet soak time to be implemented to minimize impacts on captured sea turtles. Because variables other than entanglement time contribute to the severity of entanglement impact, it is very difficult to propose a safe soak time to reliably minimize detrimental effects on captured turtles. We therefore recommend that the current seasonal gillnet restrictions in the lower Cape Fear River continue to be enforced to minimize impacts on captured sea turtles. If sea turtles are incidentally captured in gillnets, their health status at the time of removal from the nets can be easily assessed using the onboard protocol described in this paper. Physical examinations to assess behavior, injuries, and reflexes provided valuable insight into the physiological impacts of entanglement on sea turtles. Data obtained through a simple physical examination may help determine whether to release a turtle or take it to a rehabilitation facility following a gillnet encounter, thereby minimizing the potential for postrelease mortality.

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