Fish Physiology and Biochemistry **25:** 277–285, 2001.

The physiological response to capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*

Eric R. Hoffmayer* & Glenn R. Parsons
*Department of Biology, University of Mississippi, University, MS 38677, USA; *Current address: Center for Fisheries Research and Development, Gulf Coast Research Laboratory, Ocean Springs, MS 39564, USA (Phone: (228)-872-4257; Fax: (228)-872-4204; E-mail: ehoff@olemiss.edu)*

Key words: blood, glucose, hematocrit, lactate, osmolality, pH, plasma, repeated measures

Abstract

Secondary effects of capture and handling stress in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, were investigated in this study. Twenty-four sharks were serially bled and changes in several hematological parameters were monitored over a 1-h time period, following capture by hook-and-line. Blood samples were obtained from each shark at 0, 15, 30, 45 and 60 min. All 0-min (initial) blood samples were obtained within 3 min of capture. Blood glucose (9.2–13.1 mmol l^{-1}), lactate (1.5–28.9 mmol l^{-1}), and plasma osmolality (871–929 mOsm kg^{-1}) all increased after capture, whereas blood pH (6.86–6.78) declined. Hematocrit values (initial = 25.1%) remained unchanged throughout the 1-h stress period. Due to the short amount of time it took to obtain the initial sample and the lack of a significant relationship between the initial time and the initial parameter levels, all initial samples are considered the best approximation of the predisturbance resting levels. The use of repeated measures in this study enables us to describe the dynamics of the secondary stress response in the Atlantic sharpnose shark.

Introduction

Generally, sharks respond to capture and handling stress in an exaggerated manner, by struggling dramatically, thrashing violently (Manire et al. 2001), and becoming hyperactive (Cliff and Thurman 1984); thus making it difficult to capture and handle these animals. In light of this, the majority of the studies addressing acute stress in sharks have been conducted using small species that are easily handled and maintained in captivity (Scott 1921; Jones and Price 1974; Martini 1974; Mandrup-Poulsen 1981; Hazon and Henderson 1985; Evans and Kormanik 1985). Only a few studies have investigated changes in the physiological state of sharks in the wild (Martini 1974; Cliff and Thurman 1984; Wells and Davie 1985; Wells et al. 1986). Unfortunately, the majority of studies suffer because the effects of acute stress are considered after obtaining either a single blood sample or multiple samples taken more than an hour after the initial stress event. This makes the actual onset of changes in several hematological parameters impossible to determine because these parameters can reach maximal levels within a short period of time and may already be in the recovery phase when measured (Cliff and Thurman 1984). The goal of this study was to fill this gap and describe the short-term profiles of several hematological parameters in the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, before and after the exposure to an acute stressor.

The Atlantic sharpnose shark is a common, small coastal species that inhabits the northern Gulf of Mexico from Florida to Texas and the Atlantic Ocean from North Carolina to Florida (Springer 1938). In the Mississippi Sound, Atlantic sharpnose sharks appear inshore in late spring, remain inshore throughout the summer, and then return to deeper waters offshore in the late fall (Parsons 1983). This species was chosen for this study because of its abundance and small size, making blood collection and handling much easier.

The objectives of the study were two-fold. First, we wanted to obtain baseline estimates for several hematological parameters. Second, we wanted to de-

scribe the physiological changes that occur in the Atlantic sharpnose during an acute stress event. This information is essential to an understanding of the effects of acute stress in sharks.

Materials and methods

Fish capture and blood sampling

Atlantic sharpnose sharks were caught by hook-and-line between May 1999 and August 2000 in the Mississippi Sound off the coasts of Mississippi and Alabama. The use of hook-and-line capture was the method chosen for this study for various reasons. It allowed us to determine the exact amount of time the sharks had been subjected to acute stress and made it possible to obtain various blood samples at selected intervals. Over the period of an hour, five blood samples were taken from each animal, with 15-minute intervals between samples (i.e., 0, 15, 30, 45 & 60 min). The time was recorded from the first sign of struggle (when the shark was first detected on hook-and-line), until the first blood sample was taken. This was done so that the best estimate of pre-disturbance levels of blood parameters could be determined. After the initial blood sample, each shark remained on hook-and-line and was allowed to freely swim around the boat until the next blood sample. At each interval, each shark was retrieved slowly to minimize further stress and was quickly bled. This repeated sampling protocol was modified from methodologies used to investigate acute stress in birds (Wingfield et al. 1992), amphibians (Coddington and Cree 1995), and reptiles (Cash et al. 1997).

Sharks were restrained in the water and about 0.5 ml of blood was obtained from the caudal artery with a vacutainer treated with EDTA. Samples were immediately placed on ice and returned to the laboratory for analysis. After sampling, total length (cm) and wet weight (kg) were measured and the shark was released. Sharks that were hooked poorly, either in the stomach or in the gills, were not used in this study.

Hematocrit and pH were determined from whole blood, while lactic acid, glucose and osmolality levels were determined from plasma. Micro-centrifuge tubes were filled with 0.5 ml of whole blood and centrifuged at 1,286 *g* for 30 sec. The plasma was removed with a 1-cc syringe, placed into a second micro-centrifuge tube, and frozen at $-20\,^{\circ}$C until analyzed.

Analytical procedures

The pH of the blood sample was determined using a micro pH meter (Fisher Accumet pH meter model 610A). Whole blood was centrifuged for 5 min at 14,000 *g* in 100 μl micro-hematocrit tubes, and then hematocrit (Hct) was estimated on a Critocaps Micro-Hematocrit Capillary Tube Reader. The determination of pH and centrifugation of the whole blood were performed immediately upon returning to the laboratory, usually within 4–5 h after sample collection. Plasma lactate and glucose were estimated with Sigma Chemical Co. test kit 735-10 and kit 17-25, respectively. Both of these tests were enzymatic endpoint determinations performed on a Benchmark microplate reader (spectrophotometer). Both plasma glucose and lactate concentrations were converted to whole blood concentrations using hematocrit in order to make comparisons with the relevant literature. Osmolality was measured using an Osmette freezing point depression osmometer (Precision Systems).

Data analysis

Hematocrit (%), pH, lactic acid (mmol l^{-1}), glucose (mmol l^{-1}) and osmolality (mOsm kg^{-1}) were each compared over time (0, 15, 30, 45 & 60 min) with a repeated measures one-way analysis of variance (ANOVA) followed by a Tukey test to separate significant mean values. If any variable failed tests of normality or homogeneity of variance, the data were then transformed (i.e., Hct: arcsine). If the assumptions were still violated, data were analyzed with the nonparametric Friedman Repeated Measures ANOVA on ranks followed by a Student-Newman-Keuls multiple pairwise comparison test to separate mean ranks. All data were analyzed using Sigmastat 2.0 (Jandel Corp., San Mateo, CA), and all values were considered significant if $P \leqslant 0.05$. For simplicity, all data were graphed using arithmetic means and standard errors.

Results

Twenty-four sharpnose sharks were sampled during this study. It was found that shark length (40–99.5 cm total length) had no significant (all $P > 0.05$) effect on the stress response with any variable, so all data were pooled for analysis. Sex differences were not investigated due to the small number (3) of female sharks used in this study. In addition, all initial blood samples were obtained within 3 minutes of the shark being

hooked (87 ± 6.9 s). There was no statistically significant relationship between initial levels of plasma glucose, lactate or pH and the time (0.3 to 3 min) it took to obtain the initial blood sample (Glucose: $P = 0.765$, $R^2 = 0.03$, Lactate: $P = 0.401$, $R^2 = 0.06$, pH: $P = 0.923$, $R^2 = 0.004$).

Plasma glucose

Plasma glucose increased 40% in concentration from 12.6 to 17.6 mmol l^{-1} over a 60-min stress period (Figure 1A). A significant difference in plasma glucose among time intervals was apparent (FRM ANOVA: $\chi^2_4 = 21.9$, $P \leqslant 0.001$). Glucose levels increased during the first 15 min, remained constant at the 30-min and 45-min intervals, and then increased again at the 60 min interval. A significant increase in glucose level above initial values was identified only at the 15-min and the 60-min intervals.

Plasma lactate

Plasma lactate rose in a linear fashion (2.0 to 38.3 mmol l^{-1}) throughout the 60-min stress period (Figure 1B), with a statistically significant increase apparent at each time interval (RM ANOVA:$F_{4,23} = 133.9$, $P \leqslant 0.001$).

pH

Blood pH changed significantly (RM ANOVA: $F_{4,13} = 2.83$, $P = 0.034$) during the 60-min stress period, decreasing from 6.86 at the time of capture to 6.78 at the end of the hour (Figure 2A). The power for this test was low ($\beta = 0.513$). Blood pH remained constant at the 15-min interval, then decreased at the 30, 45 and 60-min intervals. Tukey's post hoc test revealed that none of the time intervals were significantly different from each other.

Plasma osmolality

Plasma osmolality increased significantly during the 60-minute stress period (FRM ANOVA: $\chi^2_4 = 26.6$, $P \leqslant 0.001$, Figure 2B) from 871 mOsm kg^{-1} initially to 936 mOsm kg^{-1} at 30 min, and then stabilized to 929 mOsm kg^{-1} for the last two time periods. The greatest increase occurred during the first 15 min of the stress period, and all subsequent blood samples were significantly higher than the initial sample.

Hematocrit

The mean hematocrit level in the Atlantic sharpnose shark was 25.1 ± 1.2%, and no significant change (FRM ANOVA: $\chi^2_4 = 2.98$, $P = 0.561$) occurred throughout the 60-min stress period.

Discussion

Glucose

Hyperglycemia is an expected result of stress or exhaustive exercise in fishes (Mazeaud et al. 1977; Wells and Davie 1985; Barton and Iwama 1991; Barton 1996). In this study, significantly elevated blood glucose levels were apparent within 15 min of the initial struggle. Scott (1921) reported significant elevations in blood glucose in the smooth dogfish, *Mustelus canis*, in response to asphyxia. Cliff and Thurman (1984) reported the same phenomenon in the dusky shark, *Carcharhinus obscurus*, in response to capture and handling stress. This secondary stress response appears to be, in part, the result of the release of catecholamines (epinephrine, norepinepherine) in the blood (Barton and Iwama, 1991; Wendeleer Boga, 1997). Patent (1970) and Deroos & Deroos (1978) reported increases in blood glucose in the spiny dogfish, *Squalus acanthias*, after injections of epinephrine and norepinephrine. In addition, Deroos and Deroos (1992) reported significant elevations in plasma glucose as early as 45 min after the infusion of mammalian ACTH. Blood glucose levels may be elevated immediately by catecholamines, however, further increases are usually the result of corticosteriods, which facilitate gluconeogenesis (Munck et al. 1984; Barton and Iwama 1991). This response provides the muscle tissue with large amounts of glucose very rapidly to assist the organism during an acute stress event.

Typically, blood glucose levels taken from sharks at the time of their capture have been reported to range from 60–90 mg/dl (3.3–5.0 mmol l^{-1}) (Scott 1921; Patent 1970; Martini 1974; Cliff and Thurman 1984; Manire et al. 2001). The initial blood glucose level (9.2 mmol l^{-1}) in the Atlantic sharpnose shark was relatively high when compared to other shark species and was nearly double the concentration observed in the dusky shark, *C. obscurus* (5.0 mmol l^{-1}). However, this initial level was similar to that reported for another small carcharhinid species, the bonnethead shark, *Sphryna tiburo*, captured in Florida waters

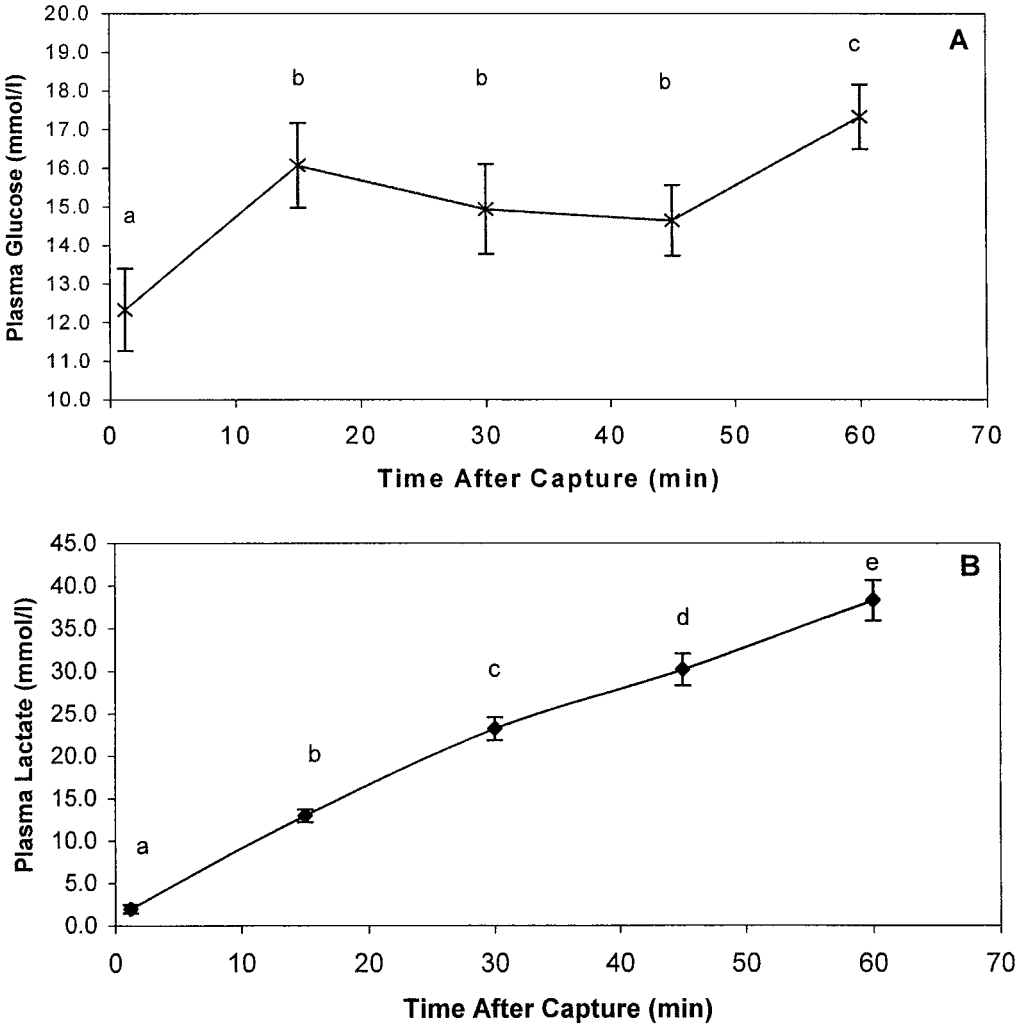

Figure 1. (A) Mean (± SEM) plasma glucose concentration (mmol l^{-1}) in the Atlantic sharpnose shark during a 60-min stress series (n = 24). (B) Mean (± SEM) plasma lactate concentration (mmol l^{-1}) in the Atlantic sharpnose shark during a 60-min stress series (n = 24). Letters not in common indicate values significantly different ($P < 0.05$) from each other.

(Manire et al. 2001). In the present study, the elevated initial glucose level may be a result of recently ingested food items. In addition, the blood glucose level only increased 40% over the 60-min stress event, whereas in the juvenile dusky shark it increased 77% in a 70-min stress event (Cliff and Thurman 1984). According to Menton (1927), cited in Love (1970), the degree to which blood glucose rises depends on the initial level, which is largely determined by the amount of food recently ingested. The sharks in this study exhibited a large variation in initial blood glucose levels, which may reflect variation in feeding success.

Lactate

Atlantic sharpnose sharks became hyperactive upon capture, which increased their energetic demands. Blood lactate concentrations were significantly elevated within 15 min, suggesting that the sharks entered oxygen debt, a result of meeting increased energy demands by anaerobic respiration in the white muscle (Black 1957). This rise in blood lactate is attributed to extremely low aerobic capacity compared to higher vertebrates (Black 1957; Schmidt-Nielsen 1997). Lactate levels in sharks are known to rise quickly when they are subjected to acute stress (Murdaugh et al. 1965; Rasmussen and Rasmussen 1967; Piiper and

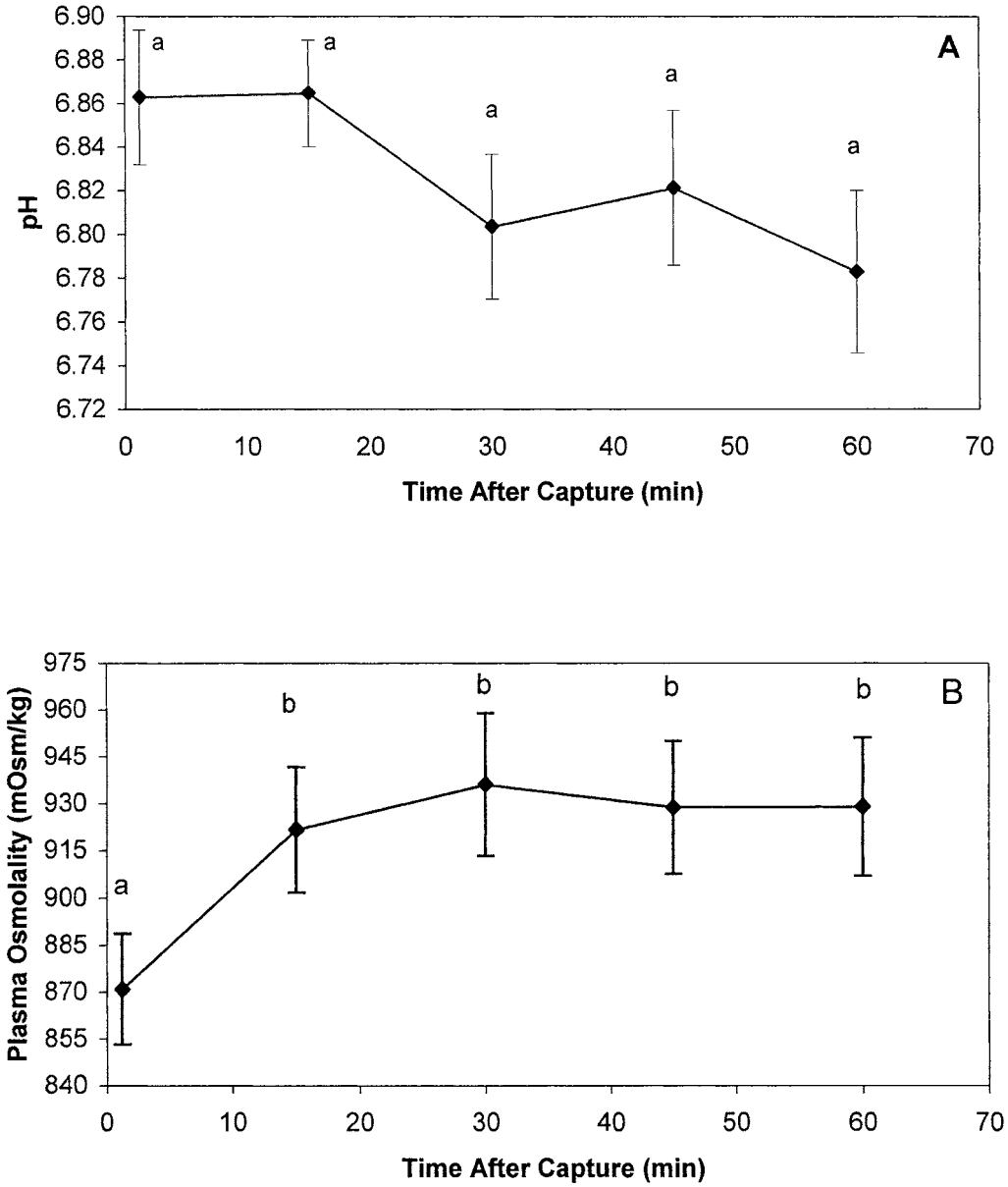

Figure 2. (A) Mean (± SEM) blood pH levels in the Atlantic sharpnose shark during a 60-min stress series (n = 14). (B) Mean (± SEM) plasma osmolality (mOsm kg^{-1}) in the Atlantic sharpnose shark during a 60-min stress series (n = 24). Letters not in common indicate values significantly different ($P < 0.05$) from each other.

Baumgarten 1969; Piiper et al. 1972; Martini 1974; Cliff and Thurman 1984). Blood lactate levels never reached an asymptote during the 1-h stress period, which is consistent with the findings of other researchers. Both Piiper et al. (1972) and Cliff and Thurman (1984) found that several hours were required for lactate to peak after exhaustive exercise in *Scyliorhinus stellaris* and *C. obscurus*, respectively.

In the present study, the blood lactate concentration reached 28.9 $mmol^{-1}$ after 1 h, a concentration higher than any previously reported in elasmobranchs or teleost fishes. The maximum reported blood lactate concentrations for *C. obscurus* after 6 h (18 $mmol^{-1}$) was significantly lower than reported in this study (Cliff and Thurman 1984). In addition, the high lactate concentrations reported herein are similar to those

Wells et al. (1986) found for striped marlin, *Tetrapturus audax*, that were fought on hook-and line for an average of 50 min. These extremely elevated blood lactate levels may be a result of a higher metabolic rate due to warmer water temperatures when compared to sharks from other studies. In the present study sharks were collected in water temperatures ranging from 25 to 31 °C, which is 6 to 14 °C higher than the majority of other studies on shark stress (Rasmussen and Rasmussen 1967; Piiper and Baumgarten 1969; Piiper et al. 1972; Cliff and Thurman 1984; Wells and Davie 1985; Wells et al. 1986; Manire et al. 2001). Since the sharpnose shark occurs in comparably warmer waters, one would expect them to have a higher metabolic rate and, therefore, higher blood lactate concentration. In addition, Atlantic sharpnose sharks increase their swimming speed and struggle dramatically when captured, and may engage in much greater levels of activity during capture when compared with other less active carcharhinid species found in the Mississippi Sound (pers. obs.).

Blood pH

In poikilotherms, lactate anions and lactic acid are not always found in the blood at equimolar concentrations (Wood et al. 1983). This suggests that other metabolic acids and not primarily lactic acid may affect pH. The blood pH in the Atlantic sharpnose shark declined slowly over time, and the values observed fell within the reported range for elasmobranchs (Piiper and Baumgarten 1969; Piiper et al. 1972; Cliff and Thurman 1984; Wells and Davie 1985).

Following severe exercise in *S. stellaris* (Piiper et al. 1972) and *C. obscurus* (Cliff and Thurman 1984), the acid-base balance was immediately disrupted, resulting in acidosis well before a rise in blood lactate occurred. This initial disruption in acid-base balance was not evident in the sharpnose shark in this study. Blood lactate levels were significantly elevated as early as 15 min after the disturbance; however, the blood pH did not decline until after 30 min into the stress period. Butler et al. (1979) have shown that the blood pH changed very little in the face of a rising blood lactate concentration in *Scyliorhinus canicula* when exposed to moderate hypoxia. They suggested that an increase in ventilation volume resulted in respiratory alkalosis and counteracted the increased lactate concentration, which resulted in very little change in blood pH. This may also have occurred in the sharks in the present study and may be responsible for the discrepancy between blood pH and lactate concentrations.

Osmolality

Initially, water balance in these sharks was affected by capture and handling, but within 30 to 45 min plasma osmolality stabilized. It is well known that osmolality is affected by acute stress in both freshwater and marine fishes (Love 1970; Mazeaud et al. 1977; Wells et al. 1986). Cliff and Thurman (1984) reported a 6% increase in plasma osmolality after a 70-min stress period, which was similar to the 7% increase seen in the Atlantic sharpnose shark. A possible explanation for this is that in stressed elasmobranchs, water shifts out of the vascular compartment in response to raised intracellular lactate or increased sodium influx into the blood (Piiper et al. 1972).

Hematocrit

In the present study there was no significant change in hematocrit during the 60-min stress event. Similarly, Manire et al. (2001) did not find a significant change in hematocrit in the blacktip, bonnethead, and bull sharks when subjected to gill net capture and restraint. There is no evidence of hemoconcentration occurring in the Atlantic sharpnose shark, which has been observed in some teleost fishes as a response to acute stress (Miles et al. 1974; Mazeaud et al. 1977; Beggs et al. 1980; Wells et al. 1986; Iwama et al. 1989).

Repeated sampling protocol

The initial increase in hematological parameters at the 15-min interval was a result of the original hook-and-line capture. However, subsequent increases in these parameters many be compounded by the cumulative effects of maintaining the sharks on hook-and-line and handling them several times. It has been shown that when fish are subjected to multiple stressors, they respond with greater disruptions in their physiology and biochemistry (Barton et al. 1980; Barton et al. 1986; Schreck 2000). Chinook salmon, *Oncorhynchus tshawytscha*, when exposed to multiple stress events at 3-h intervals, exhibited increases in the rate and magnitude of plasma cortisol, glucose, and lactate when compared to fish that were exposed to a single stress event (Barton et al. 1986). In addition, Barton et al. (1980) found that in fingerling rainbow trout, *Oncorhynchus mykiss*, the magnitude of

the stress response was directly correlated to the duration of the stress event. Furthermore, Schreck (2000) suggests that the physiological response to multiple, concurrent stressors follows a general pattern to that of fish stressed by a single continuous challenging situation; however, the magnitude of the response may be greater than if only one stressor were present. Cumulative effects of repeated measures in this study may account for the elevated plasma lactate and glucose concentrations.

Initial blood parameters

There was no significant relationship between the amount of time it took to obtain the initial blood sample and the blood pH, lactate and glucose levels. This indicates that the initial values for these hematological parameters are similar to basal levels in the Atlantic sharpnose shark. In light of this, blood samples that are obtained within 3 min after disturbance can be considered best estimates of resting levels for the parameters measured in this study. In addition, initial blood glucose, lactate, pH and hematocrit values were very similar to values reported for other elasmobranchs that had fully recovered from various stressors (Piiper et al. 1972; Butler et al. 1979; Cliff and Thurman 1984; Deroos and Deroos 1992; Cooper and Morris 1998).

Conclusions

The methodology used in this study allowed us to describe the short-term plasma profiles of several hematological parameters in the Atlantic sharpnose shark. The profiles of these parameters showed the sharks' response to capture and handling stress was rapid, with fluctuations in the blood occurring within 15 min of the initial disturbance. Immediately upon capture, the sharks became hyperactive, which resulted in the accumulation of lactate in the tissues and eventually the blood. Elevated blood lactate levels have two potential consequences. A high lactate concentration in the blood may disrupt the acid-base balance leading a decrease in blood pH over time. In addition, a high lactate concentration in the tissues may also cause hemoconcentration (Piiper et al. 1972). Wood et al. (1983) also suggested that 80% of the lactate load in the white muscle is never released into the blood, but removed by metabolic processes. Hemoconcentration, a typical result of this process, would explain the increased lactate, glucose and osmolality levels; however, there was no increase in hematocrit, suggesting that there was no hemoconcentration taking place.

Elevated blood glucose levels may also be a result of the neuro-endocrine response of the Atlantic sharpnose shark, which provides necessary energy substrates to aid the organism when dealing with an acute stress event. It was also suggested by Cliff and Thurman (1984) that low blood glucose levels (< 2.7 mmol l^{-1}) were the possible cause of death in *C. obscurus* following exhaustive exercise resulting from capture and handling. If this is the case, the majority of sharks released in this study had a good chance of survival because of the high glucose concentration when released. This is also supported by the small fluctuation in blood pH, which indicated the sharks were able to continue normal acid-base balance.

Blood glucose, lactate, and pH are very useful indicators of capture and handling stress in the Atlantic sharpnose shark and other sharks as long as baseline data is available for comparisons. Plasma osmolality, on the other hand, is more difficult to interpret because sharks are iso-osmotic to slightly hyper-osmotic to their environment and their osmolality changes with the salinity of the water. The Atlantic sharpnose sharks in this study exhibited the highest plasma osmolality during the summer when the salinity was also the highest.

The methodology used in this study allowed us to obtain initial blood samples that can be considered the best estimates of wild, unstressed levels, and will be very useful when making comparisons with other shark species. In addition, this methodology can be used to elucidate seasonal, ontogenic, and gender differences in the stress response of the Atlantic sharpnose shark. This repeated measures approach is similar to those used in studies on other vertebrate taxa (Wingfield et al. 1992; Coddington and Cree 1995; Cash et al. 1997) and will enable us to make comparisons of shark responses to stress with those of other vertebrates.

Acknowledgments

Financial support for this project was provided, in part, by the University of Mississippi and by a grant to the secondary author from the National Marine Fisheries Service. We are greatly indebted to J. Bartlett, J. Horton, R. Seybert, A. Lee, S. Schieffer, A. Haggard, M. Sandrene and D. Martinovic for their assistance

in the field. We would also like to thank M. Peterson, J. Bartlett, S. Curran, J. Franks and two anonymous reviewers for providing critical comments and suggestions on earlier drafts of this manuscript.

References

Barton B.A., Peter, R.E. and Paulencu, C.R. 1980. Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Can. J. Fish. Aquat. Sci. 37: 805–811.

Barton, B.A., Schreck, C.A. and Sigismondi, L.A. 1985. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Trans. Am. Fish. Soc. 115: 245–251.

Barton, B.A. and Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. 1: 3–26.

Barton, B.A. 1996. Stress in finfish: past, present and future – a historical perpective. *In:* Fish Stress and Health in Aquaculture. pp. 1–33. Edited by A.D. Pickering and J. Sumpter. Cambridge University Press.

Beggs, G.L., Holeton, G.F. and Crossman, E.J. 1980. Some physiological consequences of angling stress in muskellunge, *Esox masquinongy* Mitchill. J. Fish Biol. 17: 649–659.

Black, E.C. 1957. Alterations in the blood level of lactic acid in certain salmonoid fishes following muscular activity. III. Sockeye salmon, *Oncorhynchus nerka.* J. Fish. Res. Bd. Can. 14: 807–814.

Butler, P.J., Taylor, E.W. and Davison, W. 1979. The effects of long term, moderate hypoxia on acid-base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula.* J. Comp. Physiol. 132: 297–303.

Cash, W.B., Holberton, R.C. and Knight, S.S. 1997. Corticosterone secretion in response to capture and handling in free-living red-eared slider turtles. Gen. Comp. Endocrinol. 108: 427–433.

Cliff, G. and Thurman, G.D. 1984. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus.* Comp. Biochem. Physiol. 78A: 167–173.

Coddington, E.J. and Cree, A. 1995. Effects of acute captivity stress on plasma concentrations of corticosterone and sex steroids in female whistling frogs (*Litoria ewingi).* Gen. Comp. Endocrinol. 100: 33–38.

Cooper, A.R. and Morris, S. 1998. The blood respiratory, hematological, acid-base and ionic status of the Port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anaesthesia and surgery: a comparison with sampling by direct caudal puncture. Comp. Biochem. Physiol. 119A: 895–903.

DeRoos, R. and DeRoos, C.C. 1978. Elevation of plasma glucose by catecholamines in elasmobranch fish. Gen. Comp. Endocrinol. 34: 447–452.

DeRoos, R. and DeRoos, C.C. 1992. Effects of mammalian ACTH on potential fuels and gluconeogenic substrates in the plasma of the spiny dogfish shark, *Squalus acanthias.* Gen. Comp. Endocrinol. 87: 149–158.

Evans, D.H. and Kormanik, G.A. 1985. Urea efflux from the *Squalus acanthias* pup: the effect of stress. J. Exp. Biol. 119: 375–379.

Hazon, N. and Henderson, I.W. 1985. Factors affecting the secretory dynamics of 1α-hydroxycorticosterone in the dogfish, *Scyliorhinus canicula.* Gen. Comp. Endocrinol. 59: 50–55.

Iwama, G.K., McGeer, J.C. and Pawluk, M.P. 1989. The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Can. J. Zool. 67: 2065–2073.

Jones, R.T. and Price, K.S. 1974. Osmotic responses of spiny dogfish *Squalus acanthias* embryos to temperature and salinity stress. Comp. Biochem. Physiol. 47A: 971–979.

Love, R.M. 1970. The Chemical Biology of Fishes, Vol. 1. Academic Press, New York, NY.

Mandrup-Poulsen, J. 1981. Changes in selected blood serum constituents, as a function of salinity variations, in the marine elasmobranch, *Sphryna tiburo.* Comp. Biochem. Physiol. 70A: 127–131.

Manire, C., Hueter, R., Hull, E. and Spieler, R. 2001. Serological changes associated with gill-net capture and restraint in three species of sharks. Trans. Am. Fish. Soc. 130: 1038–1048.

Martini, F.H. 1974. Effects of capture and fasting confinement on an elasmobranch, *Squalus acanthias.* Ph.D thesis, Cornell University, New York.

Mazeaud, M.M., Mazeaud, F. and Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 106: 201–212.

Menton, M.L. 1927. Changes in the blood sugar of the cod, sculpin, and Pollock during asphyxia. J. Biol. Chem. 72: 249–253.

Miles, H.M., Loehner, S.M., Michaud, D.T. and Salivar, S.L. 1974. Physiological responses of hatchery reared muskellunge *Esox masquinongy* to handling. Trans. Am. Fish. Soc. 2: 336–342.

Munck, A., Guyre, P.M. and Holbrook, N.J. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5: 25–44.

Murdaugh, H.V., Jr., Robin, E.D, Theodore, J. and Drewry, W. 1965. Studies of lactate metabolism in *Squalus acanthias.* Bull. Mt Des. Is. Biol. Lab. 5: 30–31.

Parsons, G.R. 1983. The reproductive biology of the Atlantic sharpnose shark, *Rhizoprionodon terraenovae*, Richardson. Fish. Bull. 81: 61–73.

Patent, G.J. 1970. Comparison of some hormonal effects on carbohydrate metabolism in an elasmobranch *Squalus acanthias* and a holocephalan *Hydrolagus colliei.* Gen. Comp. Endocrinol. 14: 215–242.

Piiper, J. and Baumgarten, D. 1969. Blood lactate and acid-base balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Publ. Staz. Zool. Napoli 37: 84–94.

Piiper, J., Meyer, M. and Drees, F. 1972. Hydrogen ion balance in the elasmobranch, *Scyliorhinus stellaris*, after exhausting activity. Resp. Physiol. 16: 290–303.

Rasmussen, R.A. and Rasmussen, L.E. 1967. Some observations on the protein and enzyme levels and fractions in normal and stressed elasmobranchs. Trans. N.Y. Acad. Sci. 29: 397–413.

Schreck, C.B. 2000. Accumulation and long-term effects of stress in fish. *In: The Biology of Animal Stress.* Pp. 147–158. G. Moberg (ed). CABI Publishing, Wallingford, United Kingdom.

Schmidt-Nielson, K. 1997. Animal Physiology: Adaptation and the Environment. Cambridge University Press, Cambridge, MA.

Scott, E.L. 1921. Sugar in the blood of the dogfish and of the sand shark. Am. J. Physiol. 55: 349–355.

Springer, S. 1938. Notes on the sharks of Florida. Proc. Florida Acad. Sci. 3: 9–19.

Wells, R.M.G. and Davie, P.S. 1985. Oxygen binding by the blood and hematological effects of capture stress in two big gamefish:

mako shark and striped marlin. Comp. Biochem. Physiol. 81A: 643–646.

Wells, R.M.G., McIntyre, R.H., Morgan, A.K. and Davie, P.S. 1986. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comp. Biochem. Physiol. 84A: 565–571.

Wendelaar Bonga, S.E. 1997. The stress response in fish. Physiol. Rev. 77: 591–616.

Wingfield, J.C., Vleck, C.M. and Moore, M.C. 1992. Seasonal changes of the adrenocortical response to stress in birds of the Sonoran Desert. J. Exp. Zool. 264: 1–10.

Wood, C.M., Turner, J.D. and Graham, M.S. 1983. Why do fish die after severe exercise? J. Fish Biol. 22: 189–201.