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Differential sensitivity to capture stress assessed by blood acid–base status in five carcharhinid sharks

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Abstract Stress from fishing capture can incite potentially lethal physiological changes in fishes. Blood acid–base status has routinely been utilized to gauge the magnitude of the stress response, which is dependent on the nature of the capture event and metabolic capacity of the species in question. The mortality induced by demersal longline capture has been shown to vary among taxonomically similar carcharhinid elasmobranchs. In this study, we aimed to: (1) quantify and compare blood acid–base disturbances associated with longline capture in five carcharhinid species; (2) examine the extent to which these disturbances correspond with reported at-vessel mortality rates; and (3) investigate how interspecific differences in the physiological stress response could relate to life history, ecology, and phylogeny. Results showed that blood acid–base disturbances from longline-capture varied between species, with relative degrees of disturbance by species proportional to previously reported at-vessel mortality rates. In addition, the degree in which metabolic and respiratory acidoses influenced relative depressions in blood pH also differed by species. The differences in blood acid–base status point to discrepancies in the aerobic and anaerobic capacities among these taxonomically similar species, and are important when considering the effects of, and possible means to mitigate deleterious consequences from, longline fishing capture.

Keywords Carcharhinids · Longline fishing · Stress · Blood acid–base balance · Acidosis

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Introduction

Sharks, like many species of fish, are exploited by extensive recreational and commercial fisheries throughout the world. Off the east coast of the US, sharks are targeted by commercial vessels using demersal longlines. Although this fishery is strictly regulated through quotas and retention limits (NMFS 2006), this fishing method results in the capture and mortality of several closely related carcharhinoid species that may or may not be retained. According to historical shark fishery observer data, overall at-vessel mortality rates of sharks caught by demersal longline gear range from 9% in the tiger shark (*Galeocerdo cuvier*) to 94% in the great hammerhead shark (*Sphyrna mokarran*) (Morgan and Burgess 2007). These data also indicate that at-vessel mortality rates can differ markedly between closely related species. For example, Morgan and Burgess (2007) report that sandbar shark (*Carcharhinus plumbeus*) at-vessel mortality (36%) is considerably lower than the congeneric blacktip (*Carcharhinus limbatus*, 88%) and dusky (*Carcharhinus obscurus*, 81%) sharks.

Immediate and post-release mortality associated with capture stress is poorly known for most commercially and recreationally important shark species. In many species of fish, including a limited number of elasmobranchs, the acute stress associated with capture has been

shown to elicit a suite of potentially lethal physiological changes (e.g. Wood et al. 1983; Cliff and Thurman 1984; Hoffmayer and Parsons 2001; Manire et al. 2001; see Skomal 2007). Specifically, most fishes studied to date react to the acute stress of capture with more exaggerated disruptions to their physiology and biochemistry than higher vertebrates (reviewed by Pickering 1981; Adams 1990; Wood 1991; Milligan 1996; Wendelaar Bonga 1997; Kieffer 2000; Skomal 2007). Like most fishing techniques, longline capture restricts mobility and causes exhaustive anaerobic exercise. The typical duration of commercial demersal longline sets targeting sharks (and other species) is 9–16 h, and can exceed 20 h (Morgan and Burgess 2007), a potentially long period for animals to be subjected to capture stress. This can be particularly detrimental to elasmobranchs, many of which are obligate ram ventilators (Manire et al. 2001; Carlson et al. 2004).

Capture mortality, both immediate and delayed, appears to be tightly linked to the nature, severity, and duration of the stress imposed, as well as the metabolic capacity (aerobic and anaerobic) of the species and its ability to recover from homeostatic disruption (Wood et al. 1983; see Davis 2002; Skomal 2007). These latter attributes vary widely between fish species and, based on the aforementioned observer data, even between closely related sharks. Thus, the assessment of species-specific physiological responses to capture stress is vital to fisheries conservation and also provides insights into life history, ecology, and phylogeny.

Changes in blood biochemistry, particularly acid–base status, relative to the capture event have been routinely used to provide quantitative information about the magnitude and nature of capture stress (e.g. Wells et al. 1986; Skomal 2006). In general, exhaustive exercise typically causes the marked decrease in blood pH (acidemia) resulting from metabolic (increasing H^+ as indicated by rising blood lactate and decreasing blood bicarbonate) and respiratory (increasing pCO_2) acidoses (reviewed by Pickering 1981; Adams 1990; Wood 1991; Milligan 1996; Wendelaar Bonga 1997; Kieffer 2000; Skomal 2007). When coupled with estimates of mortality, these physiological indicators provide insights into causative factors as well potential mitigation measures (Skomal 2007). The objectives of the current study were: (1) to quantify acid–base disturbances associated with longline capture in five species of carcharhinid sharks; (2) to examine the extent to which the magnitude and nature of these disturbances correspond with observed at-vessel mortality rates; and (3) to examine the extent to which interspecific differences in the physiological stress response relate to life history, ecology, and phylogeny.

Methods

Animal collection

Five carcharhinid shark species were blood sampled during five National Marine Fisheries Service (NMFS) Apex Predators Program longline surveys (1996, 1998, 2001, 2004, 2007): sandbar shark [$n = 61$, 62–180 cm fork length (FL)]; tiger shark ($n = 59$, 62–292 cm FL); dusky shark ($n = 37$, 78–248 cm FL); Atlantic sharpnose shark (*Rhizoprionodon terraenovae*; $n = 24$, 51–83 cm FL); and blacktip shark ($n = 9$, 111–147 cm FL). Fishing was conducted at fixed stations on the continental shelf off the US Atlantic coast from Key West, Florida to Maryland. The majority of fishing sets were carried out in coastal waters at depths of 20–37 m, with certain sets conducted in more shallow absolute water depths.

All sharks were captured using standard Florida style (Berkeley et al. 1981) commercial demersal longline fishing gear with 300 hooks per set at each station. The mainline and 3.6 m gangions were composed of 426 and 331 kg test monofilament, respectively. Each gangion had a 3/0 shark hook baited with chunks of spiny dogfish (*Squalus acanthias*). Gangions were affixed to the mainline at intervals of 18–21 m. The longline was weighted to the bottom with 2–3 kg sash weights at intervals of every 15 hooks: bullet floats and heavier (7 kg) sash weights were also attached intermittently. Each end of the mainline had 9–13 kg weights and was marked with a 5 m staff buoy equipped with a radar reflector and a flashing light.

Longlines were fished day and night with an average of 2.1 sets per day. Each longline set was fished ("soaked") for 3 h, which was defined as the period between when set deployment was complete and set retrieval had commenced. The collective time required for deployment (considered the time when the first baited hook entered the water pre-soak) and retrieval (considered the time when the last hook was brought in post-soak), however, made it conceivable that individual sharks could have been hooked for periods exceeding 3 h. As hook-timers were not employed, the specific duration that sharks were on hooks, an estimated range of 5–330 min, could not be determined.

Phlebotomy and blood gas analyses

Upon haul-back, live sharks were immediately brought on deck and $\sim$1 cc of blood was drawn via caudal venipuncture using 18–20 gauge 3.8 cm non-heparinized syringes (Skomal 2006; Mandelman and Farrington 2007a, b). Because access to the caudal vein in the hemal arch of fishes often impinges on the caudal artery (dorsal aorta) and it cannot be ensured samples were purely arterial or

venous, we hereby characterize our samples as mixed arterial/venous blood. To avoid coagulation and minimize compromises in blood gas accuracy following phlebotomy, sampled whole-blood was immediately (<30 s) analyzed for pH and pCO_2 in one of two portable blood gas analyzers (95 µl in the i-STAT, Heska Corporation, Fort Collins, CO, USA or 200 µl in the IRMA, International Technidyne Corporation, Edison, NJ, USA) thermostatted to 37°C. As in other vertebrates, a large disparity exists between venous and arterial pO_2 values in elasmobranchs (e.g. Cliff and Thurman 1984; Lai et al. 1990; Cooper and Morris 1998), thus we did not address this parameter herein. Since the IRMA does not measure blood lactate, it was necessary to secure 5 µl aliquots of the remaining whole-blood for assay in a portable blood lactate analyzer on survey cruises employing this machine (Lactate Pro, Fact Canada Consulting Performance Ltd, North Quesnel, BC, Canada). Prior to release, all sharks were measured and tagged.

Reference control values

To generate minimally stressed controls for comparison to field-based samples and to quantify potential differences between the two blood gas analyzers, blood samples from ten captive spiny (66–88 cm FL) and ten captive smooth (*Mustelus canis*) (62–90 cm FL) dogfish were analyzed on both machines at 37°C, as well as on the Lactate Pro. Dogfish acquired by otter trawl were acclimated for 7 days at 15°C prior to phlebotomy at the Marine Biological Laboratory's (Woods Hole, MA) Marine Resources Center; seawater treatment and holding conditions are described in Mandelman and Farrington (2007a). Trawl caught spiny dogfish have previously been shown to rapidly begin feeding and restore what is presumed to be a minimally stressed physiological status upon arrival at this holding facility (Mandelman and Farrington 2007a). Dogfish of both species were deprived of food for 24 h prior to being sampled. Animals were removed from the holding tanks by hand and restrained and sampled according to protocols described in Mandelman and Farrington (2007a). The elapsed time from tank removal to the onset of blood withdrawal never exceeded 10 s. Samples were immediately processed in the same fashion as previously discussed.

Conversions

To more closely estimate in vivo blood gas and pH values, it was necessary to correct for temperature (Reeves 1977). Although the acquisition of bottom seawater temperatures was not possible, sea surface temperatures (SSTs) were collected by associated research vessels before each set during each cruise. The mean SST across all sets where sharks in the study were sampled was 22°C. We thus estimated that 20°C more realistically approximated ambient bottom seawater conditions. For comparative purposes, blood gas and pH data from both dogfish control species were corrected as well. For all temperature corrections, we used the following equations, where *M* and TC refer to measured and corrected values, respectively:

$$\text{Experimental and control sharks}: \Delta T = 37 - 20 = 17$$

$$pH_{TC} = pH_M - \Delta pH/\Delta T(T - 37)$$

(Heisler and Neumann 1980; Ashwood et al. 1983; Heisler 1988) (1A)

This function assumes a lack of linearity in regard to temperature and pH. However, the designated temperature of 20°C (all experimental and control sharks) and the pH range observed allowed the use of this equation without discernable effects to the pH data (raw and converted data is presented in "Results"). Further, based on the effects of temperature on pH in the extracellular space of the larger spotted dogfish (*Scyliorhinus stellaris*) in vivo, $\Delta pH/\Delta T$ was changed from 0.015 (Ashwood et al. 1983) to 0.011 (Heisler and Neumann 1980; Heisler 1988) to reflect that of an elasmobranch. This equates to:

$$pH_{TC} = pH_M - 0.011(T - 37) \quad (1B)$$

$$pCO_{2TC} = pCO_{2M}\left(10^{-0.019\Delta T}\right)$$

(Nunn et al. 1965; Ashwood et al. 1983) (2)

$HCO_3^-{}_{TC}$ was calculated using the Henderson–Hasselbalch equation from pCO_{2TC} and pH_{TC}. Values for the constants pK' and αCO_2 [mmol $(mmHg^{-1})^{-1}$] were derived with the following formulas from investigations on the ventilatory responses to hypercapnia in the lesser spotted dogfish (Randall et al. 1976)[1]:

$$\text{Experimental } pK'(\text{at } 20^\circ C) = -0.1003(pH_{TC}) + 6.67 \quad (3)$$

$$\text{Experimental } \alpha CO_2 = 0.0414 \pm 0.001 \quad (4)$$

Paired samples *t* tests on control dogfish revealed a close similarity in side-by-side values generated by the IRMA and i-STAT analyzers (blood gases and pH). There was also presumed to be a close correspondence in lactate anion

[1] This study was selected on the basis that pK' and αCO_2 values were presented for temperatures (10, 15, and 20°C) corresponding with those in the present study. Additional studies on elasmobranchs present alternative experimentally derived, though slightly divergent, values for pK' and αCO_2 (e.g. Albers and Pleschka 1967; Pleschka and Wittenbrock 1971; Boutilier et al. 1984; and see Weber et al. 1983). It should be noted that the variability in these constants when comparing fish, reptiles and mammals is quite small, where if used interchangeably in the Henderson–Hasselbalch equation at a given temperature and pH, will not yield considerable differences in HCO_3^-.

Table 1 Mean and median blood acid–base values from spiny and smooth dogfish controls at measured (37°C), temperature-corrected (TC) to 20°C, and TC to 15°C

	Spiny and smooth dogfish ($n = 20$)		
	$\bar{X}(\tilde{X}) \pm$ SD		
	M (37°C)	TC (20°C)	TC (15°C)
pH	7.37 (7.35) ± 0.08	7.56 (7.54) ± 0.08	7.61 (7.6) ± 0.08
Lactate (mmol l^{-1})	0.42 (0.3) ± 0.33	N/A	N/A
pCO_2 (torr)	11.22 (11.2) ± 1.94	5.33 (5.32) ± 0.92	4.29 (4.28) ± 0.74
HCO_3^- (mmol l^{-1})	N/A	7.92 (7.68) ± 1.04	10.21 (9.9) ± 1.34

Values are considered minimally stressed in relation to those from experimental species

values between the i-STAT and Lactate Pro.[2] This allowed the comprehensive analysis of data generated in previous survey cruises independent of analyzer type. The i-STAT values from the samples obtained from the control dogfish spp were those subjected to manual temperature correction and assessed herein.

Statistical analyses

In this study, we defined the magnitude of physiological disturbance as proportional to relative blood chemistry measurements. Hence, sharks were considered more physiologically disturbed with decreasing blood pH (increasing acidemia), increasing pCO_2 (respiratory acidosis), increasing blood lactate (metabolic acidosis) and decreasing $HCO_3^-{}_{TC}$ (metabolic acidosis). Since we had no account of precise hook-times for sharks, and a highly unequal distribution of sharks of given species were caught across cruises and sets that varied in conditions, we utilized a conservative Meta analysis (see Lau et al. 1997) to assess interspecific differences in blood acid–base chemistry. Data were pooled from all sets and cruises, and the five field-sampled species and the control (spiny and smooth dogfish aggregate sample) were ranked (1–6, from least to most disturbed) according to mean values of three blood parameters: blood pH_{TC} (lowest mean was considered most acidemic); pCO_{2TC} (highest mean inferred most extensive respiratory acidosis); and lactate (highest mean indicated most extensive metabolic acidosis). As a calculated value, $HCO_3^-{}_{TC}$ was also excluded. To establish whether a difference in the metadata existed between species, cumulative ranks were analyzed using a nonparametric Kruskal–Wallis One-Way ANOVA on Ranks, with individual species relationships assessed using post hoc Tukey pairwise comparisons.

[2] The low end of the readable range for lactate differs on the i-STAT (0.3 mmol l^{-1}) and Lactate Pro (0.8 mmol l^{-1}). Thus, concluding a close correspondence in side-by-side values for this parameter between the two instruments (on control dogfish spp) assumed that i-STAT lactate values (mean value of 0.42 mmol l^{-1}) were closely aligned with corresponding Lactate Pro values, although values of the latter read as "<0.8 mmol l^{-1}".

In each species, forward stepwise regressions were used to ascertain how pCO_{2TC} and lactate, as indicators of respiratory and metabolic acidoses, respectively, predicted for the variance in pH_{TC}. Since it was calculated according to pCO_{2TC} and pH_{TC} values, we did not include $HCO_3^-{}_{TC}$ in this analysis. A set of values from a shark was only included in this analysis if values were obtained for all three parameters. Linear regressions were used to test whether ontogenetic (fork length) differences could predict for intraspecific variation in values of respective blood parameters. Values are presented as mean (±SD). However, 10–90th percentile distributions are also presented to lend a broader scope of the data range for each parameter/species. Although raw (for field-sampled species) and 15°C (control dogfish) data are presented, all statistical analyses were conducted on data corrected to 20°C. Where applicable, results are reported as significant at $\alpha = 0.05$. All analyses were performed using JMP 4.04 Software (SAS Institute, Cary, NC).

Results

Blood biochemistry values from the spiny and smooth dogfish controls were similar (independent samples t tests, $P > 0.05$) and thus pooled for subsequent analyses. When corrected for temperature, these values at both 15°C (tank temperature) and 20°C (to be consistent with temperature corrections of field-sampled species) (Table 1) were less disturbed than all experimental species corrected to 20°C (Tables 2, 3).

The meta analysis revealed a significant difference in acid–base status between species (Kruskal–Wallis One Way ANOVA on Ranks: $H_5 = 15.394$; $P = 0.009$) (Table 4). Only the species ranked as most and least disturbed (dogfish spp vs. blacktip) differed significantly according to post hoc pairwise tests. Because, however, the two dogfishes (dogfish spp), tiger, and sandbar were consistently ranked 1–3, respectively, from least-to-most disturbed overall and for each individual parameter constituting the metadata (highest to lowest mean pH_{TC}; lowest to highest mean pCO_{2TC}; highest to lowest mean

Table 2 Mean measured (*M*, 37°C) and temperature-corrected (TC, 20°C) blood acid–base chemistry values from five carcharhinid shark species captured by demersal longline or alternative hook gear

	Tiger ($n = 53$)		Sandbar ($n = 55$)		Dusky ($n = 25$)		Atlantic sharpnose ($n = 24$)		Blacktip ($n = 9$)	
	$\bar{X}_M$	$\bar{X}_{TC}$	$\bar{X}_M$	$\bar{X}_{TC}$	$\bar{X}_M$	$\bar{X}_{TC}$	$\bar{X}_M$	$\bar{X}_{TC}$	$\bar{X}_M$	$\bar{X}_{TC}$
pCO_2 (torr)	12.01	5.71	14.82	7.04	17.91	8.52	18.22	8.69	24.32	11.57
			9.4[a]	4.5	*11.3*[b]	5.3				
pH	7.24	7.43	7.11	7.30	6.87	7.06	6.92	7.11	6.80	6.98
			7.42[a]	7.60	*7.29*[b]	7.48				
Lactate (mmol l^{-1})	4.32		11.78		15.26		15.26		14.82	
			1.3[a]		*1.00*[b]					
HCO_3 (mmol l^{-1})		7.75		7.13		5.18		5.29		5.03
				9.42[a]		*8.17*[b]				

Italicized values associated with given blood parameters represent minimally stressed values from the literature (temperature corrections were also made on pCO_2 and pH values from those studies

For lactate only: $n = 59$ (tiger); 61 (sandbar) 37 (dusky)

[a] Spargo (2001)

[b] Cliff and Thurman (1984)

Table 3 Meta analysis ranking (in ascending order) degree of blood acid–base disturbance in five carcharhinid shark species caught by demersal longline, and two dogfish control species

Species	Median rank		At-vessel mortality rank (rate)[a]	
Dogfish spp	1[b]	Least disturbed	N/A (%)	Lowest mortality
Tiger	2 (0)		9	
Sandbar	3 (0)		36	
Dusky	5 (0.58)		81	
Atlantic sharpnose	5 (0.58)		N/A	
Blacktip	6 (1.16)[b]		88	

Degree of disturbance comparatively based on highest lactate and pCO_2 values, and most depressed pH. Where available for a species, corresponding published at-vessel mortality data is documented

[a] Data obtained from Morgan and Burgess (2007)

[b] Significant (Tukey < 0.05) differences between species ranks

(lactate) (Table 3), we are considering these species as distinct in their respective blood acid–base statuses. In contrast, the order of the remaining species—ranked 4–6 dependent upon the blood parameter—was not consistent, implying that the overall blood acid–base status was less distinguishable among these species. Indeed, the actual physiological values presented (Tables 1, 2, 4) reflect a much closer resemblance in blood acid–base status between the dusky, Atlantic sharpnose and blacktip sharks, all more disturbed than the consistently ranked 1–3 species.

For a given species, fork length was never a significant predictor of the values of any particular blood parameter (linear regressions, $P > 0.05$ for each).

The variance in pH_{TC} explained by metabolic (as indicated by lactate) and respiratory (as indicated by pCO_{2TC}) acidoses was assessed in the five field-sampled species. In tiger, blacktip, and sandbar sharks, forward stepwise regression analyses showed that pCO_{2TC} was the first (or only) parameter to enter the model and thus explained a larger proportion of the variation in pH_{TC} than did lactate (Table 5). This implies a more pronounced respiratory component to the acidemia observed in these species. Conversely, lactate was the first (or only) parameter to enter the model in dusky and Atlantic sharpnose sharks, and explained the higher proportion of variation in pH_{TC} (Table 5). This implies a more pronounced metabolic component relative to the overall acidemia exhibited in these species. As a further indicator of metabolic acidosis, calculated bicarbonate levels were highest in the more physiologically tolerant tiger and sandbar sharks, but were comparatively depressed in a uniform manner across the remaining species (Table 4). Collectively, our analyses revealed the relative contributions of metabolic and respiratory acidoses to the overall variance in pH in a given species and we found four types of responses to longline capture: strictly metabolic in origin (dusky shark); mixed with greater metabolic component (Atlantic sharpnose shark); mixed with greater respiratory component (sandbar and tiger sharks); and strictly respiratory (blacktip shark).

Discussion

Sampling strategy and precision of blood gas values

The majority of previous studies assessing blood acid–base balance in fishes have relied upon in-dwelling catheterization to obtain blood samples specific to an artery or vein. This was not possible in the field component of our study.

Table 4 Blood acid–base chemistry in five carcharhinid shark species caught by demersal longline

	Tiger ($n = 53$)		Sandbar ($n = 55$)		Dusky ($n = 25$)		Atlantic sharpnose ($n = 24$)		Blacktip ($n = 9$)	
	$\bar{X}(\tilde{X}) \pm$ SD	10–90th percentile	$\bar{X}(\tilde{X}) \pm$ SD	10–90th percentile	$\bar{X}(\tilde{X}) \pm$ SD	10–90th percentile	$\bar{X}(\tilde{X}) \pm$ SD	10–90th percentile	$\bar{X}(\tilde{X}) \pm$ SD	10–90th percentile
pH_{TC}	7.43 (7.43) ± 0.11	7.30–7.57	7.30 (7.36) ± 0.2	6.98–7.54	7.06 (7.06) ± 0.29	6.61–7.41	7.11 (7.08) ± 0.2	6.78–7.40	6.98 (7.04) ± 0.22	6.60–7.22
Lactate (mmol l^{-1})	4.32 (3.4) ± 3.02	1.44–8.07	11.78 (11.09) ± 6.72	2.69–22.40	15.26 (15.4) ± 8.07	4.20–27.68	15.26 (17.58) ± 5.59	4.02–20.00	14.82 (15.23) ± 4.1	7.29–23.30
pCO_{2TC} (torr)	5.71 (5.7) ± 1.41	3.84–7.66	7.04 (7) ± 1.78	4.64–9.30	8.52 (8) ± 2.73	5.94–12.12	8.69 (7.75) ± 3.18	5.40–14.80	11.57 (10.4) ± 4.3	8.30–20.90
$HCO_3^-{}_{TC}$ (mmol l^{-1})	7.75 (7.73) ± 1.93	5.52–10.49	7.13 (7.37) ± 2.46	3.52–10.38	5.18 (4.61) ± 2.8	1.68–8.72	5.29 (4.98) ± 1.5	2.63–9.06	5.03 (4.75) ± 1.5	2.66–6.59

Mean (median) values and 10–90% values are presented

For lactate only: $n = 59$ (tiger); 61 (sandbar) 37 (dusky)

TC = adjusted to 20°C

Table 5 Stepwise regression results table documenting the degree of variation in blood pH_{TC} explained by pCO_{2TC} and lactate in five carcharhinid shark species caught by demersal longline

Parameter	Cumulative *R*-squared	*df*	Sum of squares (SS)	*F*	*P*
Tiger shark					
pCO2	0.29	1	0.19	23.95	<0.0001
Lactate	0.37	1	0.05	6.66	0.013
Error		50	0.008		
Sandbar shark					
pCO2	0.38	1	0.65	41.17	<0.0001
Lactate	0.54	1	0.28	17.61	0.0001
Error		52	0.016		
Dusky shark					
Lactate	0.74	1	1.46	65.02	<0.0001
Error		23	0.022		
Atlantic sharpnose shark					
Lactate	0.47	1	0.24	16.87	0.0005
pCO_2	0.67	1	0.18	12.86	0.002
Error		21	0.014		
Blacktip shark					
pCO_2	0.75	1	0.3	20.59	0.003
Error		7	0.014		

For each species, blood parameters are listed according to the order in which they entered the model

As caudal puncture, the phlebotomy technique used herein, has been cited as a less physiologically taxing method of phlebotomy (Cooper and Morris 1998), there has been an increase in studies using this method to obtain samples for investigations that include blood acid–base analysis (e.g. Brill et al. 2008). Cooper and Morris (1998) found no discrepancy in pCO_2, pH, and additional parameters when comparing blood acid–base values derived from caudal puncture with those from the arterial cannulation of Port Jackson sharks (*Heterodontus portusjacksoni*). Studies on both large pelagic teleosts (Bushnell and Brill 1992) and elasmobranchs (Cooper and Morris 1998) have found no significant difference in pH and pCO_2 values between arterial and venous blood. In addition, blood gas values from mixed arterial/venous blood in our study were comparable with previously reported values in fishes. For example, when corrected for temperature, pCO_2 values herein were directly in line with other studies on both teleosts (Jensen et al. 1983; Schwalme and Mackay 1985; Iwama et al. 1989; Thompson et al. 2002) and elasmobranchs (Cliff and Thurman 1984; Heisler 1988; Randall et al. 1976) under variable types of stress, and independent of sampling technique and/or whether derived from venous, arterial or mixed arterial/venous blood.

There has been a recent increase in the number of studies assessing the physiological stress response in both teleosts (Thompson et al. 2002; Suski et al. 2007) and elasmobranchs (Mandelman and Farrington 2007a, b) using portable diagnostic blood analyzers thermostatted to 37°C and typically utilized for the assay of mammalian blood. In this study, there was a need to assay whole-blood for blood gas profiles immediately following the capture of sharks in the field environment. However, there is evidence that the use of the analyzers in our study did not interfere with the accuracy of the values obtained. For example, although the raw pCO_2 (11–25 torr) values presented in our study expectedly fall well below normal mammalian ranges, they are well within confines of the readable ranges reported as accurate for the IRMA (4.4–149.4 torr) and i-STAT (5–130 torr), and as stated previously, when adjusted for temperature are in accord with the literature on fishes.

Interspecific differences in blood acid–base chemistry

Fish exhibit substantial depressions in blood pH following exhaustive activity and other forms of acute stress (e.g. Wood 1991). This parameter, coupled with other blood biochemistry, has been a reliable indicator of physiological status in previous studies on stressed sharks. Piiper et al. (1972) and Holeton and Heisler (1978) stimulated spotted dogfish (*Scyliorhinus stellaris*) with electric shocks until fatigued and observed a significant drop in pH coupled with a rise in blood carbon dioxide and blood lactate. Cliff and Thurman (1984) examined changes in blood biochemistry in dusky sharks caught on rod and reel and found that blood pH and bicarbonate declined, while carbon dioxide, metabolites (glucose, lactate), and electrolytes increased. Hoffmayer and Parsons (2001) found significant increases in blood lactate, while pH declined in serially sampled Atlantic sharpnose sharks after rod and reel capture. Spargo (2001) subjected sandbar sharks to 10 min of rod and reel angling and found significant changes in blood acid–base status, but recovery within 6–10 h. Similarly, in relation to presumed steady-state (baseline) levels, Skomal (2006) found significant disturbances in acid–base status in blue (*Prionace glauca*), shortfin mako (*Isurus oxyrhincus*), and spinner (*Carcharhinus brevipinna*) sharks subjected to rod and reel capture. Mandelman and Farrington (2007a, b) found that whole blood pH and gases were markedly disturbed in trawl-caught spiny dogfish.

The extent to which changes in blood pH in response to stress can be quantified in large fishes is hampered by the lack of baseline data. This is an inherent problem with this study and many of the aforementioned field studies during which the very act of handling and blood sampling the shark induces physiological stress. Given this methodological constraint, which is not likely to be rectified, we must acknowledge this potential limitation in studies of this nature. Nonetheless, it is generally accepted that baseline blood pH in elasmobranchs is not markedly different from that in teleosts, a range of roughly 7.7–8.0 dependent on species and temperature (Heisler 1988; Claiborne 1998). Presumed steady-state plasma pH values at 10–20°C from within this range have been reported across several of the aforementioned studies on elasmobranchs (e.g. Piiper et al. 1972; Heisler et al. 1980; Holeton and Heisler 1983). On the assumption that baseline blood pH of field-sampled sharks in the present study falls within this range, the temperature corrected pH (pH_{TC}) of 7.0–7.4 found in sharks exposed to longline capture indicates that these species were acidemic to varying degrees. This is further supported by the comparatively higher blood pH_{TC} ($\sim$7.6) exhibited by the minimally stressed spiny and smooth dogfish controls.

The extent to which the field-sampled sharks in this study were physiologically disturbed by the capture event varied significantly by species. Relative to the other species, the tiger shark and, to a lesser extent, the sandbar shark were clearly the least impaired by longline capture. Conversely, the blood acid–base chemistry of the dusky, Atlantic sharpnose, and blacktip sharks was highly disturbed, displaying the most depressed pH and bicarbonate values, the most elevated blood lactate levels, and high temperature corrected blood CO_2 tensions.

Animal size was not a significant predictor of blood acid–base status. Although this could have resulted from low intraspecific size variability, it is more likely a function of variability in hook-times. Without the ability to quantify the precise time of capture within each set, the potential effects of shark size may be obscured (e.g. a larger sandbar shark on a hook for 2.5 h may display a more pronounced stress response than a smaller sandbar shark on a hook for 0.5 h).

Despite the presumed broad range of time spent on hooks (within a species), intraspecific variability in blood parameters was very low and clear interspecific differences were conserved. Assuming some sharks of a given species were hooked early in sets and some late in sets, this also suggests that hooked sharks of a given species experienced profound changes in acid–base status quickly and were unable to fully compensate during the remainder of the time on the line. Disturbances to acid–base equilibria are known to be manifested rapidly in fishes, but compensatory mechanisms are tightly regulated (Heisler 1988). Therefore, the inability of longline-caught sharks to fully resolve acid–base imbalances while on the line indicates that being hooked and restricted by a longline is particularly stressful. Despite this, however, the lower at-vessel mortality rates seen in tiger and sandbar sharks (Morgan and Burgess 2007) support a high threshold for coping with this type of stress.

Blood acid–base status and mortality

There is strong evidence in the current study that the level of physiological disturbance as manifested in the blood of sharks is indicative of the capacity of a species to recover from or succumb to the stress associated with longline capture. We found a strong correspondence between blood acid–base indicators and observed at-vessel mortality rates (Morgan and Burgess 2007; Table 3). Tiger and sandbar sharks were not as physiologically impaired as the dusky and blacktip sharks, and are also known to exhibit far lower at-vessel mortality rates (9 and 36 vs. 81 and 88%, respectively). Although mortality was rarely observed in the current study, it should be noted that our soak time of three h is much shorter than those typical of commercial demersal longline operations (~9–16 h). Hence, dusky and blacktip sharks are more sensitive to longline capture and the threshold for mortality appears to fall between 3 and 12 h of soak time. These findings support the conclusion that interspecific differences in the physiological threshold to acute stress exists among these closely related carcharhinids and, likely, all sharks.

Without observations of post-release behavior, the extent in which acid–base chemistry can be used to portend post-release (delayed) mortality remains unknown (Skomal 2007). However, there are indications from conventional tag-recapture rates that these physiological data also reflect the capacity of the shark to recover from acid to base disturbances associated with longline capture. When ranked from highest to lowest, the recapture rates of longline-caught sharks mirror results from our Meta analysis (Table 3): tiger (8.0%), sandbar (4.2%), dusky (1.7%), Atlantic sharpnose (1.4%), and blacktip (1.2%) (N Kohler, NMFS Cooperative Shark Tagging Program, personal communication). Therefore, while the set duration in this study may not have been sufficient to cause at-vessel mortality, acid–base disruptions may have been of sufficient magnitude to impede recovery and, thereby, cause delayed mortality. The ability to tightly regulate extracellular acid–base balance has been shown as a key factor in the ultimate survival of salmonids following exercise. For example, Atlantic salmon (*Salmo salar*) exhibiting no resolution to extracellular acid–base perturbations were also those that ultimately died following exhaustive activity in soft water (Kieffer et al. 2002).

Respiratory and metabolic acidosis

The acute stress associated with longline capture initiates a suite of physiological responses. In obligate ram ventilating sharks, immobility imposed by the line can restrict ventilation, thereby causing physiological disturbances typical of gillnet (Manire et al. 2001) or trawl net (Mandelman and Farrington 2007a, b) capture. The impedance of gas exchange results in the accumulation of blood carbon dioxide and results in respiratory acidosis. In addition, longline-hooked fish struggle to escape, typically mobilizing anaerobic muscle, which results in metabolic acidosis caused by significant lactate production, a dramatic decline in intracellular and extracellular buffers (e.g. blood bicarbonate), and large oxygen debt. The magnitude of the acidoses, either respiratory, metabolic, or both, is known to be species-specific and tightly linked to the aerobic and anaerobic capacity of the species. In this study, the specific physiological pathways contributing to overall blood acidemia differed between species. In the tiger, sandbar, and blacktip sharks, pCO_2 levels had the most influence on blood pH, thereby indicating that the acidemia was largely respiratory in origin. However, metabolic acidosis was also occurring in the tiger and sandbar sharks, but to a lesser degree. Regardless of the mixed acidoses, the magnitude of the acidemia was less in these two species and may not compromise survivorship. Brill et al. (2008) found that blood oxygen affinity was not compromised in sandbar sharks exposed to the acute stress of hook and line capture. This is further supported by the results of Spargo (2001), who found that sandbar shark blood pH returned to control levels in 6 h after exposure to 10 min of angling

stress. Although similar studies are lacking for the tiger shark, it is likely that this species has similar compensatory mechanisms. In contrast, the blacktip shark experienced a significantly higher blood acidemia driven by respiratory perturbation. In all likelihood, this species lacks mechanisms for maintaining or increasing oxygen delivery during strenuous exercise.

The acidemia measured in dusky and Atlantic sharpnose sharks was found to be exclusively or primarily metabolic in origin. While it has been suggested that increased blood carbon dioxide levels in the dusky shark are driving blood pH depressions following capture and confinement (Cliff and Thurman 1984), our study found that blood lactate (and indirectly bicarbonate) was the only significant correlate of pH in this species. This suggests that gas exchange was not severely compromised and changes in blood pH were driven by excessive anaerobic activity (Wood 1991), which resulted in markedly depressed bicarbonate levels, and an acidemia of the greatest magnitude measured in the current study.

Independent of whether respiratory or metabolic acidosis was more influential on blood pH in those species sampled this study, the overall magnitude of pH depression differed by species. As has been suggested for bull sharks (*Carcharhinus leucas*) captured by gillnet (Manire et al. 2001), it is possible that those species least impaired [e.g. highest pH_{TC} and lowest (lactate)] in the present study simply responded less vigorously on the longline. The tiger and sandbar shark, for example, are thought to respond more sluggishly to capture than other species, tempering ventilation and metabolic rates, and thus avoiding potentially deleterious physiological alterations (Morgan and Burgess 2007). Post hooking observations are necessary to further investigate the role of behavior.

Phylogenetic considerations

Comparative studies on metabolic enzyme activities of white (anaerobic) and red (aerobic) muscle in fishes have shown that sharks do not have lower aerobic or anaerobic capacities when compared to ecologically similar teleosts species (Dickson et al. 1993; Dickson 1996; Bernal et al. 2001, 2003a, b). Since muscle biochemistry is strongly reflected in the blood (Wells et al. 1986), the biochemical differences observed in the current study may very well be related to phylogeny. Although the sharks sampled in this study were all closely related carcharhinids, including three congeneric species (Carcharhinus), we found significant differences in how each reacted to acute capture stress. While post-hooking behavior may influence the magnitude of the stress response, we primarily attribute this variation to inherent physiological differences between these species. Specifically, the magnitude of the stress response in each species is likely linked to metabolic scope as it pertains to the potential for cruise and burst swimming, the ability to physiologically respond to stress (e.g. enhanced oxygen delivery), and the capacity to recover from physiological perturbation (e.g. acid–base disturbances). It is beyond the scope of this current study to define the physiological mechanisms underlying the interspecific differences reported herein; additional studies are much needed.

Nonetheless, we found that tiger, sandbar, dusky, Atlantic sharpnose, and blacktip sharks are, in increasing order, more sensitive to the acute stress of longline capture, with the first two species markedly more physiologically resilient. The extent to which this stress may result in at-vessel or post-release mortality appears to be linked to species as well set duration. Dusky, Atlantic sharpnose, and blacktip sharks physiologically respond with more exaggerated acid–base disturbances and greater rates of both at-vessel and post-release mortality, which appears to increase with longline sets in excess of 3 h. Future studies of this realm should employ hook timers to elucidate the relative impacts of longline hook-time as well as ontogeny on shark physiology across taxa.

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