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Hematological indicators of stress in longline-captured sharks ☆

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ABSTRACT

For many shark species, little information exists about the stress response to capture and release in commercial longline fisheries. Recent studies have used hematological profiling to assess the secondary stress response, but little is known about how, and to what degree, these indicators vary interspecifically. Moreover, there is little understanding of the extent to which the level of relative swimming activity (e.g., sluggish vs. active) or the general ecological classification (e.g., coastal vs. pelagic) correlates to the magnitude of the exercise-induced (capture-related) stress response. This study compared plasma electrolytes (Na^+, Cl^-, Mg^{2+}, Ca^{2+}, and K^+), metabolites (glucose and lactate), blood hematocrit, and heat shock protein (*Hsp*70) levels between 11 species of longline-captured sharks ($n = 164$). Statistical comparison of hematological parameters revealed species-specific differences in response to longline capture, as well as differences by ecological classification. Taken together, the blood properties of longline-captured sharks appear to be useful indicators of interspecific variation in the secondary stress response to capture, and may prove useful in the future for predicting survivorship of longline-captured sharks where new technologies (i.e., pop-up satellite tags) can verify post-release mortality.

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1. Introduction

In recent decades, the interaction between sharks and fishing gear has increased, and these fish now account for a large percentage of incidental bycatch in pelagic commercial fisheries worldwide (Beerkircher et al., 2002; Gilman et al., 2008; Skomal and Bernal, 2010). It is estimated that elasmobranchs, although rarely targeted, constitute 25% of the overall catch in U.S. commercial longline fisheries (Mandelman et al., 2008) and comprise as much as 94% of total bycatch in commercial longline operations worldwide (McKinnell and Seki, 1998). The commercial value of sharks is often low and certain species (e.g., dusky shark, *Carcharhinus obscurus*) are mandated to be released in an effort to reduce fishing mortality (NMFS, 2008). Therefore, the release of longline-captured sharks has become a common practice (Skomal, 2007; Mandelman et al., 2008; Skomal and Bernal, 2010).

Fish react to capture and handling with more exaggerated physiological responses than most other higher vertebrates (reviewed by Skomal and Bernal, 2010; Skomal and Mandelman, 2012) and the stress response has been classified into three levels: primary, secondary, and tertiary (Mazeaud et al., 1977). Like teleosts, sharks exhibit primary and secondary responses to stress that are manifested in their blood biochemistry (reviewed by Skomal and Bernal, 2010; Skomal and Mandelman, 2012). Recently, stress responses in fish have been shown to vary interspecifically and may be linked to: 1) a fish's metabolic scope and its cruise/burst swimming capacity, 2) the ability to physiologically respond to stress, and 3) the capacity to recover from the stressor (Mandelman and Skomal, 2009; Skomal and Bernal, 2010). Indeed, at-vessel mortality data from commercial fisheries and research surveys show that shark species respond differently to capture by the same fishing gear (e.g., Beerkircher et al., 2002; Morgan and Burgess, 2007; Mandelman and Skomal, 2009; Morgan and Carlson, 2010).

Given the increasing number of sharks released from commercial longline gear and the potential interspecific variation in response to longline capture, the objective of the current study was to determine if blood samples can be used to characterize and compare the secondary stress response in several species of sharks exposed to longline capture. Due to the challenges associated with keeping many of these large sharks in captivity, such stress parameters have not been published before and we hope these preliminary data will be a

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starting point for future stress physiology research on sharks having different levels of swimming activity (e.g., sluggish vs. active swimmers), and inhabiting ecologically distinct areas (e.g., coastal waters that are relatively shallow [<80 m] and well-mixed vs. the deep [>200 m] and well-stratified pelagic environment). In addition to commonly used blood parameters, we investigated the use of heat shock proteins (*Hsps*) as potential indicators of the stress response in sharks. We also examined the extent to which the published at-vessel mortality rates for these species (Beerkircher et al., 2002; Yokota et al., 2006; Morgan and Burgess, 2007; Morgan and Carlson, 2010) may be related to the magnitude of the stress response.

2. Materials and methods

2.1. Sampling

Fresh blood samples were collected from 2006 to 2009 during NOAA/NMFS longline research cruises assessing fish abundance [western Pacific ocean (WPO): R/V Oscar Elton Sette, 2008; eastern North Pacific ocean (ENP): R/V David Starr Jordan, 2007; western North Atlantic (WNA): R/V Delaware II, 2007, 2009 and F/V Eagle Eye II, 2006]. Two types of longline gear were used (demersal and pelagic) with different overall "soak times" (i.e. time period that the line is fishing) and hook types (circle vs. J hooks). For demersal sets, circle hooks were suspended at or near the bottom (30–60 m) and soaked for 2–6 h, and for pelagic sets, circle and J hooks were suspended within the upper 150 m of the water column and soaked for 4–12 h. Unfortunately, the inherent logistical difficulties of working on numerous vessels with varying scientific agendas did not allow for the deployment of hook timers to measure time on the line. Longlines, regardless of type, were set at sea surface temperatures ranging from 17 to 24 °C. Immediately after capture, sharks were brought on the boat and blood samples (2–10 mL) were collected via caudal puncture using an 18-gauge needle and heparinized syringe. Blood samples were kept at 4 °C until processing, which occurred mostly 2 to 4 min after capture, but in some cases were up to an hour after capture. Blood was also sampled from moribund sharks, but no blood was taken from dead specimens.

2.2. Hematocrit

Whole blood samples ($n = 3$–5 per shark) were transferred into microcapillary tubes and centrifuged (ZipOCRIT, LW Scientific, Inc.) for 5 min. Hematocrits were calculated as the proportion of red blood cell (RBC) volume.

2.3. Blood processing and plasma parameter quantification

Whole blood was transferred into 2 mL tube, centrifuged for 5 min at 2000*g*, and the plasma was separated and frozen immediately at −80 °C. In the laboratory, plasma samples were thawed and diluted 2–3× with HPLC-grade water, and approximately 55 μL of the diluted samples were injected into a Critical Care Xpress (CCX, Nova Biomedical, Waltham MA) benchtop analyzer to quantify (mmol/L) Na^+, Cl^-, Mg^{2+}, Ca^{2+}, K^+, lactate, and glucose (converted from mg/dL to mmol/L).

2.4. Heat shock proteins

The presence of a ~70 kDa heat shock protein (*Hsp*70) in the RBCs was determined using a modified western blot procedure from Currie and Tufts (1997) and Heberer et al. (2010). RBCs were hypotonically lysed in distilled water in the presence of a protease inhibitor cocktail (ROCHE Complete-mini) and the amount of protein was quantified using the BCA method. Approximately 100 μg of total protein per sample was loaded on an 8% polyacrylamide gel along with a Bio-Rad protein ladder to determine the proper separation of the ~70 kDa band and to verify the position of an *Hsp* standard (Sigma Aldrich, St. Louis MO, USA). Proteins were transferred onto a PVDF-fluorescent membrane (Millipore, Billerica, MA USA) and stained with a primary antibody against *Hsp*70 (Agrisera AS05-083) followed by a fluorescent secondary antibody (Li-Cor IRDye680) and detected by a Li-Core Odyssey Infrared Imaging System (version 3.0.16). The *Hsp70* levels in each species were compared by normalizing the signal of the blue shark (*Prionace glauca*), therefore providing relative levels of *Hsp*70 between species. Work on blue sharks has shown this species to have low at-vessel mortality rates (Yokota et al., 2006) and a relatively high post-release survival (Moyes et al., 2006). For this reason, we selected the blue sharks as the benchmark of a low-stress condition (verified by the values of other blood stress parameters in the present study).

2.5. Statistical analysis

Although our goal was to sample a large number of species, the sample sizes for each species were limited by our catch. For this reason, even species with a low sample size were included in the interspecific comparisons.

A general linear model univariate analysis of variance (ANOVA) was used to determine how blood parameters varied by species and family, and two-sample *T*-tests were used to detect differences by ecological classification (i.e., pelagic versus coastal association) and between moribund and non-moribund shortfin mako sharks (the only species with moribund specimens) (Sokal and Rohlf, 1995). Assumptions were satisfied using Levene's test of homogeneity of variances and a visual examination of the data for normality, and Tukey post-hoc tests were performed to compare values between groups.

Regression analyses (Sokal and Rohlf, 1995) were used to determine if at-vessel mortality rates were related to blood stress parameters. Because each species in this study was caught in only one body of water (e.g., the sandbar sharks were only caught in the WNA and mako sharks were only captured in the ENP), intraspecific differences due to geography were ruled out. Species-, gear- (i.e., pelagic vs. demersal longline) and location-specific at-vessel mortality rates were taken from published values (Beerkircher et al., 2002; Yokota et al., 2006; Morgan and Burgess, 2007; Morgan and Carlson, 2010). A Dixon test was used to detect and eliminate intraspecific outliers for each parameter (Sokal and Rohlf, 1995) prior to any statistical analysis. All analyses used $\alpha = 0.05$.

3. Results

A total of 164 sharks representing 11 species from 3 families (Alopiidae, Lamnidae, Carcharhinidae) and 2 ecological classifications (i.e., pelagic and coastal) were captured on longline gear and blood sampled (Table 1).

3.1. Electrolytes

Plasma electrolyte levels (Fig. 1, Table 1) were found to differ interspecifically (ANOVA, $p<0.05$). Mean (±SD) Na^+ levels for the blue shark were approximately 10% lower (260 ± 22 mmol/L) than those of blacktip (*Carcharhinus limbatus*) and sandbar (*Carcharhinus plumbeus*) sharks (298 ± 6 and 282 ± 14 mmol/L, respectively) (Fig. 1A). Mean Ca^{2+} levels were highest in blacktip sharks (3.3 ± 0.2 mmol/L), followed by the porbeagle (*Lamna nasus*) and pelagic thresher shark (*Alopias pelagicus*) (2.8 ± 0.1 and 2.8 ± 0.2 mmol/L, respectively), all being between 20 and 30% higher than those of other sharks (Fig. 1B). Mean K^+ levels were between 1.8 and 2-fold higher in the blacktip and pelagic thresher sharks (10.2 ± 3.9 and 9.5 ± 1.3 mmol/L, respectively) relative to all other species (Fig. 1C). No

Table 1
Hematological secondary stress parameters and at-vessel mortality rates for sharks captured using commercial longline gear. Values are mean ± SD.

Ecological classification	Family	Species	*n*	Electrolytes					Metabolites		Hct	At-vessel mortality
				Na^+	Ca^{2+}	Cl^-	K^+	Mg^{2+}	Glucose	Lactate		
				(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(mmol/L)	(%, *n*)	(%)
Pelagic	Lamnidae	*Lamna nasus* (porbeagle shark)	11	272 ± 8	2.8 ± 0.1	250 ± 7	5.4 ± 1.5	1.2 ± 0.1	6.9 ± 1.2	22.6 ± 6	36 ± 7, 10	24%[a] WNA
		Isurus oxyrinchus (shortfin mako shark)	46	272 ± 16	2.4 ± 0.2	265 ± 16	4.5 ± 0.8	1.1 ± 0.3	6.7 ± 0.9	16.7 ± 12[*] 34.3 ± 5[**]	27 ± 9, 41	24%[c] ENP
	Alopiidae	*Alopias pelagicus* (pelagic thresher)	4	280 ± 8	2.8 ± 0.2	267 ± 3	9.5 ± 1.3	2.1 ± 0.6	10.6 ± 1.5	32.1 ± 6	32 ± 4, 3	? WPO
	Carcharhinidae	*Prionace glauca* (blue shark)	40	260 ± 22	2.3 ± 0.3	265 ± 18	4.5 ± 0.7	1.0 ± 0.7	5.9 ± 1.1	4.8 ± 4	19 ± 5, 38	6.8%[c] ENP
		Carcharhinus longimanus (oceanic whitetip shark)	3	276 ± 22	2.6 ± 0.2	266 ± 24	4.8 ± 0.6	1.0 ± 0.1	4.6 ± 1.4	0.3 ± 0		? WPO
		Carcharhinus falciformis (silky shark)	4	277 ± 35	2.4 ± 0.5	264 ± 25	5.1 ± 0.8	1.0 ± 0.2	7.0 ± 0.4	16.5 ± 14	4, 1	66.3%[b] WNA
Coastal		*Galeocerdo cuvier* (tiger shark)	9	267 ± 20	2.4 ± 0.5	263 ± 12	5.3 ± 1.2	1.0 ± 0.2	6.4 ± 1.1	4.9 ± 6	21 ± 8, 9	8.5%[d] WNA
		Carcharhinus plumbeus (sandbar shark)	25	282 ± 14	2.3 ± 0.2	269 ± 20	4.9 ± 0.8	1.1 ± 0.1	4.3 ± 0.7	11.5 ± 7	20 ± 8, 26	36.1%[d] WNA
		Carcharhinus obscurus (dusky shark)	14	276 ± 20	2.4 ± 0.4	266 ± 19	5.7 ± 1.2	1.1 ± 0.2	6.0 ± 1.4	13.0 ± 7	14 ± 8, 15	81.1%[d] WNA
		Rhizoprionodon terraenovae (Atlantic sharpnose)	3	271 ± 36	2.3 ± 0.4	259 ± 34	5.6 ± 1.5	1.4 ± 0.4	7.9 ± 3.4	17.0 ± 14	19 ± 7, 3	91%[e] WNA
		Carcharhinus limbatus (blacktip shark)	5	298 ± 6	3.3 ± 0.2	276 ± 6	10.2 ± 3.9	1.8 ± 0.3	5.7 ± 1.5	36.8 ± 13	28 ± 4, 5	88%[d] WNA

Ref: [a]G. Skomal, L. Natanson and D. Bernal (unpublished data); [b]Beerkircher et al. (2002); [c]Yokota et al. (2006); [d]Morgan and Burgess (2007); [e] Morgan and Carlson (2009).
Lactate values for healthy ([*], $n = 43$) and moribund ([**], $n = 3$) shortfin mako sharks, which are significantly different ($p < 0.05$). Locations caught: ENP, eastern North Pacific; WNA, western North Atlantic; WPO western Pacific ocean. Question marks (?) refer to unknown at-vessel mortality rates.

significant interspecific differences were detected for plasma Cl^- and Mg^{2+} (Table 1).

3.2. Metabolites

Blood and plasma metabolite levels also showed significant interspecific variation (ANOVA, $p < 0.05$). Mean glucose levels in the pelagic thresher (10.6 ± 1.5 mmol/L) were from ~1.3 to 2.5-fold higher than those of all other species, and the sandbar shark (4.3 ± 0.7 mmol/L) was significantly lower than all species with the exception of the blacktip and oceanic whitetip sharks (*Carcharhinus longimanus*) (5.7 ± 1.5 and 4.6 ± 1.4 mmol/L, respectively) (Fig. 2A). Mean lactate values were highest in the blacktip shark (36.8 ± 13 mmol/L), followed by the pelagic thresher and porbeagle sharks (32.1 ± 6 and 22.6 ± 6 mmol/L, respectively), and lowest in the tiger (*Galeocerdo cuvier*), blue and oceanic whitetip sharks (4.9 ± 6, 4.8 ± 4, and 0.3 mmol/L, respectively) (Fig. 2B). Three moribund shortfin mako sharks (*Isurus oxyrinchus*; excluded from all other analyses involving mako lactate values) had significantly higher lactate values (34.3 ± 5 mmol/L) than non-moribund mako sharks (16.7 ± 12 mmol/L); there were no significant differences between the other blood parameters of the moribund and non-moribund mako sharks.

3.3. Hematocrit

Mean hematocrit values for porbeagle (36 ± 7%), pelagic thresher (32 ± 4%), blacktip (28 ± 4%), and shortfin mako (27 ± 9%) sharks were between ~1.3 and 9-fold higher than those of other sharks (Fig. 3).

3.4. Hsp70

The levels of RBC *Hsp70* were quantified in 32 sharks (7 species, 2 families) (Fig. 4). Although *Hsp70* had high intraspecific variability and significant differences could not be established (by species, family, or ecological classification), the highest values were documented for the dusky and shortfin mako sharks, which were from 4 to 7-fold higher than those of the blue shark (Fig. 4).

3.5. Group comparisons

Hematological blood parameters pooled by family (Table 2) showed several family-level differences. Specifically, mean K^+ levels for the Alopiidae (in this study, only the pelagic thresher represents the alopiids) (9.5 ± 1 mmol/L) were more than 1.9-fold higher when compared to both the Lamnidae and Carcharhinidae (Table 2, Fig. 1). Similarly, the alopid had the highest level of glucose (10.6 ± 1.5 mmol/L), which was between 1.6 and 1.9-fold higher than that of lamnids and carcharhinids. Lactate values were also highest in the alopid (32.1 ± 6 mmol/L), followed by the lamnids (18.1 ± 11 mmol/L), which were about 1.8 fold higher than those in the carcharhinids (10.2 ± 11 mmol/L) (Table 2, Fig. 2). Mean hematocrit values were highest in alopid (32 ± 4%) and lamnids (29 ± 9%), which were significantly higher than those in the carcharhinids (19 ± 7%) (Table 2, Fig. 3). Family-level comparisons revealed no significant differences in the mean levels of Na^+, Ca^{2+}, Cl^-, or Mg^{2+} (Table 2).

When grouped by ecological classification, mean glucose and hematocrit levels in pelagic sharks (6.4 ± 1.3 mmol/L and 25 ± 9%, respectively) were significantly higher (by ~1.25 fold) than those for coastal sharks (5.4 ± 1.6 mmol/L and 19 ± 8%, respectively) (Figs. 2, 3, Table 2). In addition, mean Na^+ and K^+ levels were significantly higher in coastal sharks (279 ± 19 and 6.0 ± 3 mmol/L, respectively) than in pelagic sharks (268 ± 19 and 4.7 ± 1 mmol/L, respectively). Pelagic and coastal sharks showed no significant differences in the mean plasma levels of Ca^{2+}, Cl^-, Mg^{2+}, and lactate (Table 3).

3.6. At-vessel mortality

For several species, the relative levels of some blood-stress parameters (e.g., Na^+, Ca^{2+}, K^+, Mg^+ and lactate) were correlated with published at-vessel mortality rates (Beerkircher et al., 2002; Yokota et al., 2006; Morgan and Burgess, 2007; Morgan and Carlson, 2010).

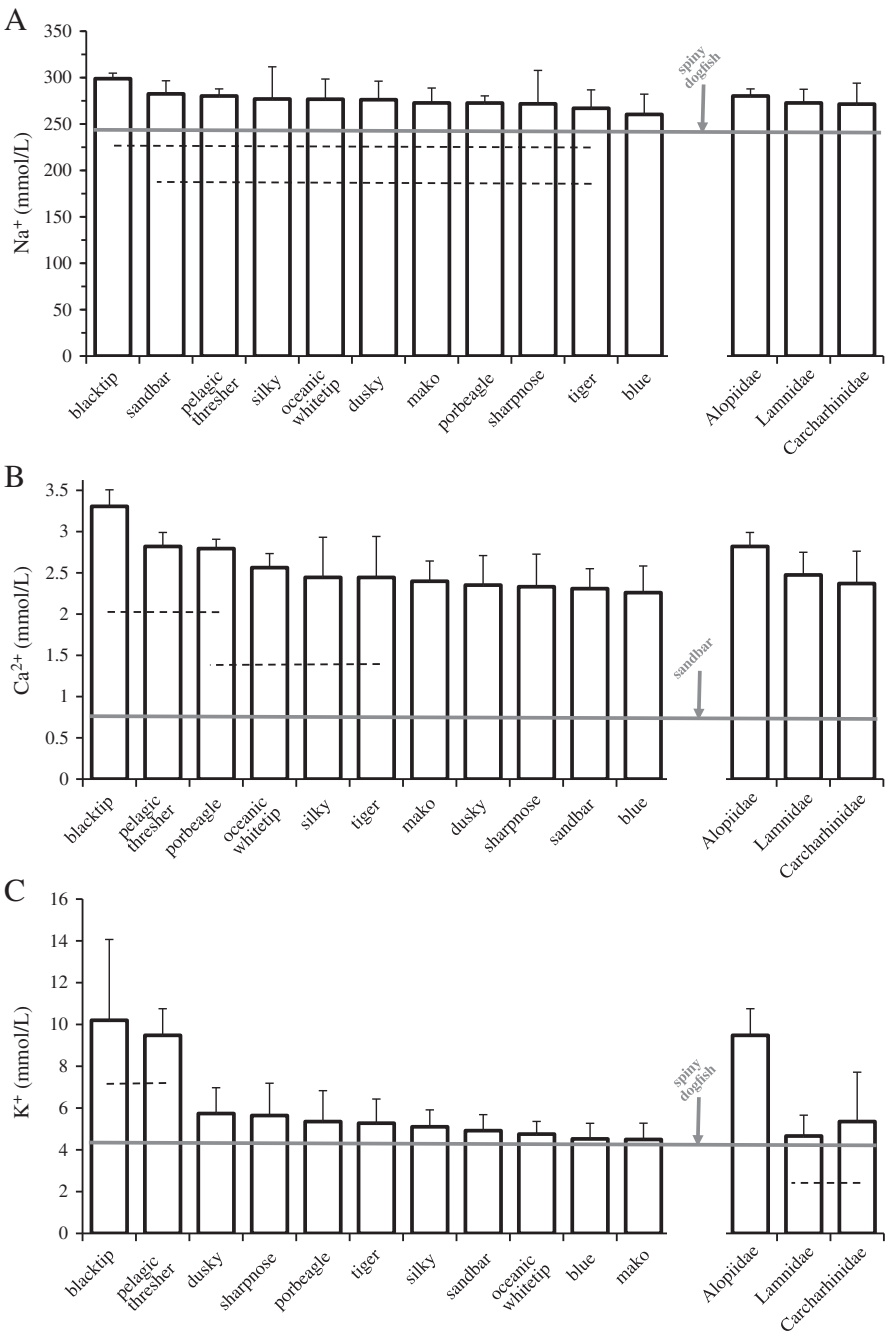

Fig. 1. Plasma electrolytes in sharks captured by longline gear. Values are mean ± SD, sample sizes given in Tables 1 and 2. Hatched lines represent significantly similar groups ($p<0.05$; obtained through Tukey post-hoc comparisons) and gray horizontal line represents baseline values from unstressed spiny dogfish (A and C) (Mandelman and Farrington, 2007) or sandbar sharks (B) (Spargo, 2001).

However, the Pearson correlation coefficient (r^2) values for the regression between Na^+, Ca^{2+}, and Mg^{2+} and at-vessel mortality rates were low (0.03–0.09). By comparison, the regression analyses for both lactate and K^+ (Fig. 5) had a slightly higher r^2 value (0.16, $p=5\times10^{-7}$, and 0.20, $p=1.1\times10^{-8}$, respectively) indicating that between 16 and 20% of these values may be explained by the at-vessel mortality rates.

4. Discussion

Although recent works have focused on the stress response in fishes, few studies have had the opportunity to assess and to compare this response among a diverse assemblage of sharks. Studies that have assessed stress parameters in more than one species of shark have produced varying results. For example, a study by Frick et al. (2009) on two captive species, the Port Jackson shark (*Heterodontus portusjacksoni*) and Australian swellshark (*Cephaloscyllium laticeps*), found little variation in the blood-chemistry between the two species. Conversely, Mandelman and Skomal (2009) compared the levels of blood gases, pH, and lactate in sandbar, tiger, Atlantic sharpnose (*Rhizoprionodon terraenovae*), dusky, and blacktip sharks exposed to demersal longline capture and found clear species-specific differences. Considering the relatively small amount of published literature on the stress-response in sharks, there does appear to be species-specific variation in stress-induced blood parameters (e.g., lactate, glucose) (e.g., Wells and

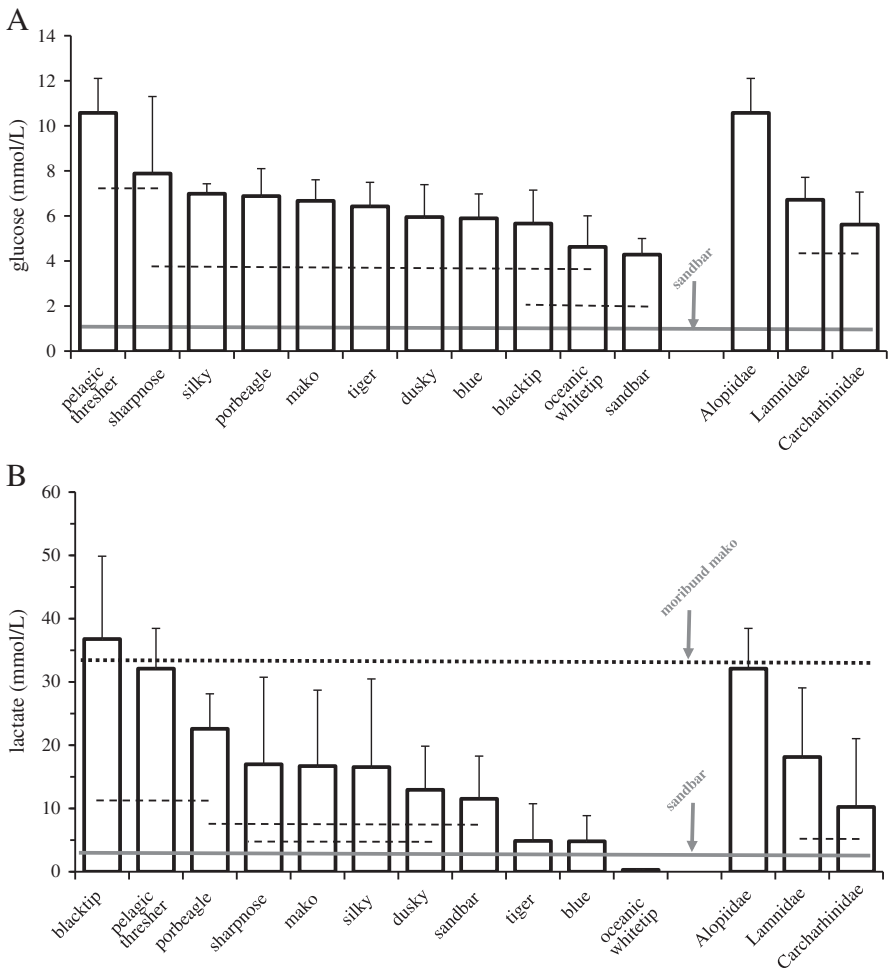

Fig. 2. Plasma metabolites in sharks captured by longline gear. Values are mean ± SD, sample sizes given in Tables 1 and 2. Hatched lines represent significantly similar groups ($p<0.05$; obtained through Tukey post-hoc comparisons), gray horizontal lines represent baseline values from unstressed sandbar sharks (Spargo, 2001), and in 2B, the black dotted line represents the lactate value of the moribund mako sharks seen in this study.

Davie, 1985; Hoffmayer and Parsons, 2001; Moyes et al., 2006; Mandelman and Farrington, 2007; Brill et al., 2008; Frick et al., 2010). Additional evidence comes from published at-vessel mortality rates for various species of sharks, which show that the lethal effects of longline gear can range from minimal (e.g., less than 9% in tiger sharks; Morgan and Burgess, 2007) to very high (e.g., in excess of 88% in blacktip sharks and up to 91% in Atlantic sharpnose; Morgan and Burgess, 2007; Morgan and Carlson, 2010) and thus

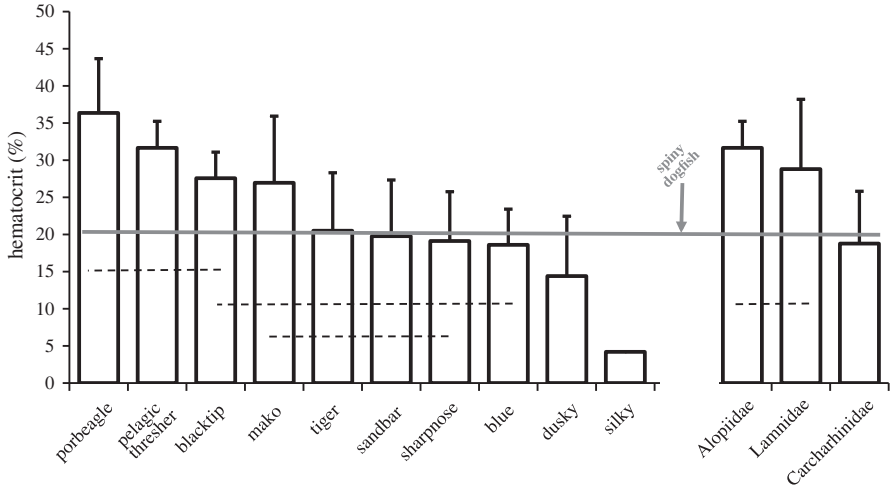

Fig. 3. Blood hematocrit concentration in sharks captured by longline gear. Values are mean ± SD, sample sizes given in Tables 1 and 2. Hatched lines represent significantly similar groups ($p<0.05$; obtained through Tukey post-hoc comparisons) and gray horizontal line represents baseline values from unstressed spiny dogfish (Mandelman and Farrington, 2007).

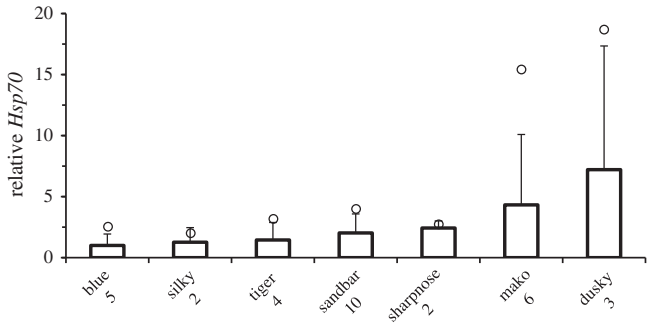

Fig. 4. Relative heat shock protein 70 (*Hsp70*) in the red blood cells of sharks captured by longline gear. Values are expressed in reference to blue shark = 1 and are mean ± SD (except for silky and sharpnose, where values are mean ± range). Circles are maximum values for each species. Sample size is shown below common names.

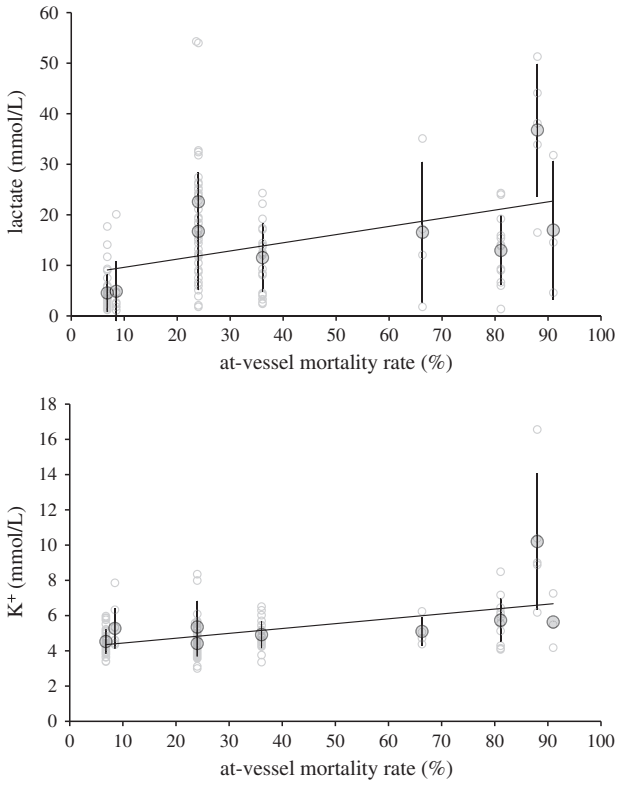

Fig. 5. Lactate and potassium concentrations in the blood plasma of sharks as a function of published at-vessel mortality rates (Beerkircher et al., 2002; Yokota et al., 2006; Morgan and Burgess, 2007; Morgan and Carlson, 2010). Lines represent the significant relationship (for all data) between lactate and potassium values and at-vessel mortality rate, where lactate (mmol/L) = 7.9 + 0.2(% at-vessel mortality rate), $p = 5 \times 10^{-7}$, $r^2 = 0.16$ and potassium (mmol/L) = 4.2 + 0.03(% at-vessel mortality rate), $p = 1.1 \times 10^{-8}$, $r^2 = 0.20$. Filled circles show the mean ± SD (bars) for each mortality rate in 9 species with known published values (see Table 1).

suggest that the tolerance to longline-induced stress is species-specific (Table 1).

4.1. Electrolytes

The efflux of K^+ (a largely intracellular ion) from the muscle cells and its accumulation in the blood (i.e.,. hyperkalemia) may result from intracellular acidosis, which can ultimately induce cellular damage, alter electrochemical gradients, and affect locomotor and heart muscle function (Cliff and Thurman, 1984; Moyes et al., 2006). Hyperkalemia has been reported in several studies, in which K^+ levels rise significantly in response to the stress event (e.g., Wells et al., 1986; Cliff and Thurman, 1984; Manire et al., 2001; Mandelman and Farrington, 2007; Frick et al., 2010). In addition, significantly higher K^+ levels have also been detected in moribund sharks relative to conspecifics that were exposed to and survived a fishing event (Moyes et al., 2006). In the current study, we found that the blacktip and pelagic thresher sharks had higher potassium levels relative to other species, indicating potential hyperkalemia. When comparing the reported potassium baseline concentration (of approximately 4 mmol/L) for captive spiny dogfish (*Squalus acanthias*), Port Jackson sharks, and gummy sharks (Mandelman and Farrington, 2007; Frick et al., 2010), this value is similar to the results in this study for all species, except those measured in the pelagic thresher and blacktip sharks. This suggests that most sharks in this study were not experiencing hyperkalemia after longline capture. It is interesting to note that the blacktip and pelagic thresher sharks had potassium levels higher than 7 mmol/L, a value known to be the threshold above which myocardial disturbance occurs in mammals (Cliff and Thurman, 1984; Mandelman and Farrington, 2007). Potassium was one of two ions that showed a family and ecological classification effect, with the alopid having a higher value than lamnids and carcharhinids, and coastal sharks showing higher values than pelagic sharks. However, these differences may be driven by the unusually high values recorded for the *A. pelagicus* (the only thresher species studied, driving up values for Alopiidae) and the blacktip shark (driving up values for coastal species).

Previous works have found increases in Ca^{2+}, Mg^{2+}, Na^+ and Cl^- in response to capture events (e.g., Cliff and Thurman, 1984; Wells et al., 1986; Moyes et al., 2006; Mandelman and Farrington, 2007; Brill et al., 2008; Frick et al., 2009; Frick et al., 2010). Similar to potassium, such increases in other ions may be the result of intracellular acidosis driving these electrolytes into the blood, a reflection of myotomal or myocardial cell damage, or due to loss of osmoregulatory ability during the capture event (Cliff and Thurman, 1984; Wells et al., 1986; Moyes et al., 2006). Our study found interspecific differences in Ca^{2+} and Na^+ levels, with the porbeagle, pelagic thresher, and blacktip sharks having higher Ca^{2+} values than the other sharks, and the

Table 2
Hematological secondary stress parameters for each family and ecological classification of sharks. Values are mean ± SD.

	Classification	*n*	Electrolytes					Metabolites		Hct
			Na^+ (mmol/L)	Ca^{2+} (mmol/L)	Cl^- (mmol/L)	K^+ (mmol/L)	Mg^{2+} (mmol/L)	Glucose (mmol/L)	Lactate (mmol/L)	(%)
Family	Lamnidae	57	272 ± 15	2.5 ± 0.3	262 ± 16	4.6 ± 1	1.2 ± 0.3	6.7 ± 1.0	18.1 ± 11 (n = 54)[a]	29 ± 9
	Carcharhinidae	103	271 ± 23	2.4 ± 0.4	266 ± 19	5.4 ± 2	1.1 ± 0.5	5.6 ± 1.4	10.2 ± 11	19 ± 7
	Alopiidae	4	280 ± 8	2.8 ± 0.2	267 ± 3	9.5 ± 1	2.1 ± 0.6	10.6 ± 1.5	32.1 ± 6	32 ± 4
Ecological classification	Pelagic	108	268 ± 19	2.4 ± 0.3	263 ± 17	4.7 ± 1	1.1 ± 0.5	6.4 ± 1.3	12.7 ± 11 (n = 105)[a]	25 ± 9
	Coastal	56	279 ± 19	2.4 ± 0.4	268 ± 20	6.0 ± 3	1.2 ± 0.3	5.4 ± 1.6	14.2 ± 12	19 ± 8

[a] Excluding moribund shortfin makos.

blacktip and sandbar sharks having higher Na^+ than the other sharks. The repeated presence of high ion concentrations in the blacktip shark suggests that the blacktip exhibits the largest physiological disruption when compared to the other species studied, a conclusion in concert with the findings of Mandelman and Skomal (2009) and the high at-vessel mortality rate reported for this species (~88%; Morgan and Burgess, 2007).

For the most part, this study was not able to determine neither a family-level nor an ecological-level effect in the relative levels of plasma electrolytes (with the exception of potassium). However, coastal species did show a slightly higher Na^+ value relative to the pelagic species. Although the difference was quite small (~10 mmol/L), future investigation is warranted to determine if the capture-related ionic disruption is greater within the coastal species. Overall, interspecific variations in ion concentrations were present, with the blacktip, pelagic thresher, sandbar, and porbeagle sharks showing some ionic disruption after longline capture.

4.2. Metabolites

In general, a stress event results in rising catecholamine levels that, in turn, trigger glycogenolysis leading to hyperglycemia (Wells et al., 1986; reviewed by Skomal and Bernal, 2010; Skomal and Mandelman, 2012). The onset of glucose mobilization appears to occur prior to the accumulation of lactate in the blood (Wells et al., 1986) and suggests that glucose mobilization may be integral to survival. Glucose levels are known to increase with longer stress events (reviewed by Skomal and Bernal, 2010; Skomal and Mandelman, 2012) and it is likely that this is the case for many of the sharks sampled in this study. Conversely, Cliff and Thurman (1984) reported that, upon capture, moribund or dead dusky sharks exhibited lower glucose levels relative to those that were alive, suggesting that a potential inability to mobilize glycogen stores may result in metabolic failure and death. Our study found that the pelagic thresher shark had the highest glucose levels among the sharks we sampled (Table 1). In general, threshers are known for being very aggressive when captured on fishing gear (i.e., experience strong fights on the line) and are thought to possess high metabolic scope to support such swimming activities (Dickson et al., 1993; Bernal et al., 2003; Heberer et al., 2010). The high glucose levels in the pelagic thresher may be due to higher overall glucose demand resulting from longer time on the line and/or a higher degree of struggling while on the line, but alternatively it may also simply reflect a higher metabolic scope. Glucose levels (Table 1) are lower in the blacktip, sandbar, and oceanic whitetip sharks. However, the lack of data on the actual time spent on the longline makes it difficult to differentiate between high glucose levels resulting from greater metabolic capacity versus those associated with longer stress events, or to understand exactly what the low glucose levels signify. Although interspecific trends in glucose levels were present for longline-captured sharks, more research is warranted to clearly understand how stress events affect these shark species, especially species with a wide array of activity levels.

In fishes, exhaustive exercise associated with struggling on the line during capture is powered by anaerobic glycolysis, resulting in the acute intramuscular accumulation of metabolic end-products and an efflux of lactate and protons from the muscle cells into the surrounding blood, leading to metabolic acidosis and possible irreversible cell damage (Abelow, 1998; Skomal, 2007; reviewed by Skomal and Bernal, 2010; Skomal and Mandelman, 2012). Moyes et al. (2006) found that lactate levels in blue sharks that had been exposed to capture stress and survived were 4.8-fold lower than those levels detected in moribund sharks, a similar finding for moribund shortfin mako sharks in this study, which had ~2-fold higher lactate levels than makos considered to be in good condition (Fig. 2B). This evidence suggests that exceptionally high levels of lactate may be indicative (although not necessarily causative) of severe, and potentially irreversible, physiological disruptions that in extreme cases can influence survivability after a stress event. However, the association of lactate accumulation and a stressed physiological state appear to be species-specific as, for example, moribund gummy sharks showed lower lactate values relative to survivors at the time of capture (Frick et al., 2010), and in this study, oceanic whitetip sharks showed extremely low lactate values after capture (Table 1).

Several studies have found increases in lactate after a capture event (e.g., Cliff and Thurman, 1984; Wells et al., 1986; Mandelman and Farrington, 2007). Similarly, this study found significant species-specific differences in lactate values (Table 1, Fig. 2). In general, it appears that the pelagic thresher, shortfin mako, and porbeagle sharks generate higher lactate values than other species, except the blacktip shark (see below). In a broader context, when grouped by family, lamnids appear to have higher lactate values relative to carcharhinids, a result that agrees with previous findings where lamnids (e.g., mako sharks) exhibit a stronger stress response relative to carcharhinids (e.g., blue sharks) (Skomal and Bernal, 2010). This may be due to the relatively higher anaerobic capacities for burst swimming in lamnid sharks (as well as the common thresher), but at the same time may be offset by a higher aerobic scope, which may allow for increased rates of recovery after a certain amount of time (Dickson et al., 1993; Brill, 1996; Bernal et al., 2003). Little is known about the total lactate loads that sharks may experience as a result of the different degrees (duration and intensity) of burst swimming, how the absolute lactate load may vary by species, the rates of lactate and proton efflux from the white muscle (signaling muscular stress), or how the capacity to recover from anaerobically powered bouts of burst swimming (i.e., lactate oxidation or the use of lactate as a substrate for gluconeogenesis) may affect lactate accumulation in the myotomal muscles and blood.

4.3. Hematocrit

This study found that the porbeagle, shortfin mako, pelagic thresher, and blacktip sharks have higher hematocrit values than the other sharks sampled. All of these species, including the blacktip, are considered to be active sharks (Bernal et al., 2001, 2003, 2010; Brunnschweiler, 2005), suggesting that more aerobically active species have higher hematocrit values after capture relative to less active species. The hematocrit values of this study also correlated to family (with lamnids and alopiids having higher values) and ecological classification, with pelagic species having higher values. Taken together, species that inhabit the pelagic zone, and are highly active swimmers, may have higher baseline hematocrit values to sustain their high aerobic demands. On the other hand, it is possible that an increase in hematocrit may be induced by the stress event as a result of RBC swelling due to osmotic upset (Brill et al., 2008). There are no data documenting the baseline (i.e., unstressed) hematocrit values in large sharks and data of this nature are needed to determine the overall degree and effects of capture stress and to examine how aerobic capacity may correlate with hematocrit stress response. It is interesting to note, however, that species having elevated lactate levels also exhibit high hematocrit values, suggesting higher levels of stress on the longline relative to other species.

4.4. Hsp70

Previous work on fish indicates that *Hsp* concentrations increase with the stress response (Currie and Tufts, 1997; Moyes et al., 2006), that induction is rapid (Skomal and Bernal, 2010), and that the use of *Hsps* for identifying intracellular changes due to stress is becoming a reliable method (e.g., Currie and Tufts, 1997; Currie et al., 2000; Moyes et al., 2006; Mladineo and Block, 2009; Heberer et al., 2010; Skomal and Bernal, 2010). This study did not find any

evidence of species-specific differences in longline capture induction of heat shock protein (i.e., 70 kDa) (Fig. 4), suggesting that longline capture affected all of our sampled sharks, at a cellular level, in a similar manner. In addition, there was high intraspecific variation in the data, which precluded making a conclusive statement on the cellular stress response in this study. Nonetheless, within these preliminary data, if only the maximum *Hsp70* values are considered, mako and dusky sharks exhibited more than 4-fold higher relative amounts of *Hsp70* in the blood (Fig. 4). Clearly, future work in this area is warranted, as the assessment of *Hsp* concentration and expression in additional species where the degree of the stress event is known (i.e. time on the line) will allow our understanding of stress response to be addressed at the molecular level.

5. Conclusions

This study has documented species-, family-, and ecology-specific responses to longline capture for several blood parameters, corroborating previous research (e.g., Mandelman and Skomal, 2009; Skomal and Bernal, 2010; Skomal and Mandelman, 2012), and providing preliminary data concerning the stress response to longline gear in 11 species of sharks. As evidence builds that different species show variability in their physiological response and potential tolerance to longlining, it becomes important to consider the modification of fishing practices (e.g., shorter handling time and soak times) to minimize post-release mortality for those species that are more sensitive. When comparing the blood chemistries of the species sampled in the current study to published baseline samples available for sharks, the effects of longlining are quite evident as all the former had higher stress-related values (Figs. 1–3). Clearly, the physiological impact of longline capture on all sharks is significant and, depending on the species, may pass the physiological threshold for recovery. For example, in this study the elevated lactate levels in mako sharks considered to be moribund may have crossed a physiological threshold for recovery. An attempt to determine if any of the measured blood parameters could be correlated with the rate of at-vessel mortality in all species studied showed that lactate and potassium could serve as a potential indicator of mortality 16 and 20% of the time (Fig. 5). However, the relationship clearly is not strong for these two parameters and this warrants more investigation where capture variables can be controlled. As this study did not include the use of current technologies that can verify post-release mortality (i.e., PSAT tags), our goals did not include and allow for finding parameters to predict mortality rates. Recent studies have started using such practices (e.g., Moyes et al., 2006; Musyl et al., 2011), and pairing known survival events with blood parameters will hopefully help to clarify predicting variables of mortality in the future.

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