Journal of Wildlife Diseases, 48(2), 2012, pp. 295–306

BASELINE HEALTH PARAMETERS AND SPECIES COMPARISONS AMONG FREE-RANGING ATLANTIC SHARPNOSE (*RHIZOPRIONODON TERRAENOVAE*), BONNETHEAD (*SPHYRNA TIBURO*), AND SPINY DOGFISH (*SQUALUS ACANTHIAS*) SHARKS IN GEORGIA, FLORIDA, AND WASHINGTON, USA

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ABSTRACT: Sharks are of commercial, research, conservation, and exhibition importance but we know little regarding health parameters and population status for many species. Here we present health indicators and species comparisons for adults of three common wild-caught species: 30 Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*) and 31 bonnethead sharks (*Sphyrna tiburo*) from the western Atlantic, and 30 spiny dogfish sharks (*Squalus acanthias*) from the eastern Pacific. All animals were captured during June–July 2009 and 2010. Median values and preliminary reference intervals were calculated for hematology, plasma biochemistry, trace nutrients, and vitamin A, E, and D concentrations. Significant differences, attributable to physiologic differences among the species, were found in the basic hematologic and plasma biochemistry variables. Significant species differences in arsenic and selenium plasma concentrations were found and appear to coincide with diet and habitat variability among these three species. Vitamin E was significantly higher in the bonnethead shark, again related to the foraging ecology and ingestion of plant material by this species. The Atlantic sharpnose had significantly higher vitamin A concentrations, supported by the higher proportion of teleosts in the diet. Vitamin D was below the limit of quantification in all three species. These preliminary reference intervals for health variables can be used to assess and monitor the population health and serve as indicators of nutritional status in these populations of wild elasmobranchs.

Key words: Atlantic sharpnose, bonnethead, complete blood count, hematology reference intervals, plasma biochemistry, shark, spiny dogfish, trace nutrients, vitamins.

INTRODUCTION

Elasmobranchs are an integral component of the marine ecosystem, though many populations are in decline (Gallucci et al., 2006; Camhi et al., 2009). Commercial and recreational fisheries affect the populations of the Atlantic sharpnose (*Rhizoprionodon terraenovae*), bonnethead (*Sphyrna tiburo*), and spiny dogfish sharks (*Squalus acanthias*; Trent et al., 1997; Rulifson, 2007). Although the bonnethead and Atlantic sharpnose are species of Least Concern, the spiny dogfish is considered a Vulnerable species (IUCN, 2011). The bonnethead is subtropical to tropical (Hoese and Moore, 1958), whereas the spiny dogfish is found in cold temperate waters of the Atlantic and Pacific oceans (Verissimo et al., 2010). The Atlantic sharpnose is found only in the western North Atlantic along the eastern shore of the United States and Mexico (Bethea et al., 2006). Both the bonnethead and Atlantic sharpnose inhabit coastal and estuarine waters and do not undertake extensive migrations (Gurshin and Szedlmayer, 2004; Bethea et al., 2007), whereas the spiny dogfish migrates across ocean basins (Jensen, 1961; Hess, 1964).

In addition to having minimally overlapping habitats, these three species also

have different foraging ecologies. The bonnethead eats mostly crustaceans (crabs) and to lesser extents cephalopods, teleosts, and minor amounts of secondarily ingested plant matter (Cortes et al., 1996; Bethea et al., 2007). Sharpnose feed primarily on teleosts and elasmobranchs (Dasyatidae), with crustaceans comprising a minor portion of their diet (Bethea et al., 2004, 2006). Spiny dogfish primarily forage on teleosts and crustaceans but also cephalopods, jellyfish, and sea cucumbers (Hanchet, 1991; Beamish et al., 1992; Laptikhovsky et al., 2001).

Despite their ecological importance as top predators in the marine environment, few studies have provided reference ranges for health parameters of sharks in the wild (Harms et al., 2002; Cain et al., 2004; Stoskopf, 2010). Our objectives were to provide a comprehensive set of indices for health parameters in three wild-caught shark species of varying habitats and foraging ecologies and to compare these values among species.

MATERIALS AND METHODS

Capture and sampling

Atlantic sharpnose and bonnethead sharks were captured as by-catch in a trawl survey for sea-turtle abundance off the coasts of Georgia and northern Florida in June–July 2009 and 2010. The sharks were captured between St. Catherine's Sound, Georgia (31°42′N, 81°08′W) and St. Augustine, Florida (30°25″N, 81°23′W) within 40 km of shore. Stretch nets with 20-cm mesh were in water ranging from 3 to 13 m deep for 20 min. Only individuals that could be processed within 3 min of coming onboard were sampled.

Because of funding limitations, spiny dogfish were not captured in the Atlantic but were instead opportunistically captured by hook and line (rod and reel) in July 2010 in Admiralty Inlet (Puget Sound), Washington (47°56′N, 122°40′W). Sharks were brought to the surface at a rate of 1 m/s, pulled onboard, and the hook removed. No animal remained on deck longer than 7 min.

Physical examinations were conducted on each animal immediately upon removal from net or hook. Body condition score (BCS) was based on palpation of muscle mass along the vertebrae and ventral abdomen using an ordinal scale: 1=emaciated; 2=thin; 3=normal; 4=overweight; 5=obese. Sharks with a BSC <2 or evidence of trauma were excluded from this study. Morphometrics (straight length from tip of nose to precaudal pit and forked-straight length from tip of nose to tip of tail) were measured to the nearest centimeter using a measuring board (Wildco Wildlife Supply Co., Buffalo, New York, USA). Sharks were categorized as mature on the basis of total straight-line length (cm; Parsons, 1985; Saunders and McFarlane, 1993; Carlson and Parsons, 1997). Only adult sharks were used in this study. Sex was determined by the presence or absence of claspers. During the time on deck, all sharks were irrigated with flowing salt water from a deck hose (1.6-cm inside diameter) to deliver oxygenated salt water to the gills.

Blood collection, processing, and analysis

Sharks were placed in lateral recumbency and manually restrained during venipuncture. A maximum of 12 ml of blood was collected from the ventral tail artery (Mylniczenko et al., 2006) using the Vacutainer® system (Pulmo Medical Supplies, Porter Ranch, California, USA) with a 22-G by 2.5-cm needle. Whole blood was collected into one 10-ml sodium heparin tube and one 3-ml ethylenediaminetetra-acetic acid (EDTA) tube. Four blood smears were made within 10 min of collection from the EDTA blood. The smears were dried, fixed in methanol, and stained with Wright–Giemsa stain (JorVet, Dip-Quick, Jorgensen Laboratories, Loveland, California, USA). Blood smears were prepared and examined using light microscopy by the same individual (KHH) for calculation of a differential white blood cell count (WBC) based on 100 WBC counted at 1,000× magnification. Cells were identified on the basis of standard elasmobranch identification methods (Walsh and Luer, 2004; Arnold, 2005). The EDTA samples were stored at 4 C for up to 7 hr before complete blood count evaluation. Natt–Herrick stain was modified for elasmobranch osmolality (1,190 mOsm/l): 25 µl of Natt–Herrick stain (3050, ENG Scientific Inc., Clifton, New Jersey, USA) was mixed with 0.79 g of urea and 0.34 g of NaCl and filtered at 0.2 mm (Walsh and Luer, 2004; Arnold, 2005). The EDTA blood was diluted 1:100 with this modified Natt–Herrick solution, mixed for a minimum of 20 min, and the hemocytometer technique (Walsh and Luer, 2004) used by the same individual (KHH) to obtain total WBC counts. Hematocrit (Hct) was measured by

microhematocrit centrifugation and total protein by refractometer. Heparin plasma was separated within 10 min of collection and temporarily stored in liquid nitrogen (Atlantic sharpnose and bonnethead) or at −20 C (spiny dogfish) until transferred to −70 C. All plasma biochemistry analyses were conducted within 4 wk of sample collection.

Plasma biochemical profiles were performed at the University of Miami Avian and Wildlife Laboratory using standard dry slide determinations with an Ortho Vitros 250 (Ortho Vitros, Rochester, New York, USA) chemical analyzer. The following plasma values were measured: Alanine aminotransferase, amylase, aspartate aminotransferase, blood urea nitrogen (BUN), calcium, carbon dioxide, chloride, cholesterol, creatinine, creatine phosphokinase (CPK), gamma-glutamyl transferase (GGT), glucose, lactate dehydrogenase (LDH), lipase, phosphorus, potassium, sodium, triglycerides, and uric acid. Plasma was not diluted to obtain BUN, chloride, or sodium values. Lipid and protein fractions were evaluated by plasma electrophoresis (RIA; University of Miami, Miami, Florida, USA). Plasma vitamin D was measured at Boston University School of Medicine by competitive protein binding as described by Chen et al. (1990) and Singh (2010). Plasma vitamin A and E profiles were performed at Arizona State University School of Life Sciences using a Waters Alliance 2695 high-performance liquid chromatography system (Waters Corporation, Milford, Massachusetts, USA) that had been fitted with a Waters YMC carotenoid 5.0 micrometer (4.6 mm × 250 mm) and a built-in column that was set at 30 C (Dierenfeld et al., 2009). Plasma osmolality was measured at the Georgia Aquarium using an Advanced Instruments 3250 freezing-point osmometer with a precision of ±mOsm. Nutritional trace mineral analysis was performed at the University of Florida, College of Veterinary Medicine, on a Thermo Electron X Series inductively coupled plasma mass spectrometer (Waltham, Massachusetts, USA). The following minerals were measured: aluminum, arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, molybdenum, nickel, selenium, silver, and zinc. Iridium was used as an internal standard and quantified against a standard seven-point curve for each metal (Barber et al., 2007).

Data analysis

Any variables having >20% of reference individuals (per species) with results below or above the analyzer detection limit were omitted from the reference interval determination for that species. Normality was assessed for all remaining blood parameters using the Shapiro–Wilk test (Kleinbaum et al., 1998). Since most variables (95%) did not meet the assumption of normality, nonparametric statistics were used to determine the reference interval for each variable (Carlson-Bremer et al., 2010). The reference intervals therefore represent the central 95% interval bounded by the 2.5% and 97.5% percentiles (Harr et al., 2001). Nonparametric methods were also used to assess statistical differences between species. Kruskal–Wallis tests were used to evaluate differences in blood parameters by species (Chaffin et al., 2008). Significant values were noted as those with $\alpha<0.05$. Family-wise error was corrected for using the Dunn's post hoc test in comparisons between species, but only within each variable. The between-variable family-wise error rate as a result of the many Kruskal–Wallis tests was not corrected for using the standard Bonferroni correction because of its tendency to increase the type II error rate, particularly when sample sizes are small (Chandler, 1995; Garamszegi, 2006). We therefore chose to leave the question of biological significance in the hands of the readers, and limited our discussion to those variables that were highly significantly different between species ($P<0.001$). The pgirmess package in R (http://cran.r-project.org/web/packages/pgirmess/index.html) was used for the Kruskal–Wallis and Dunn's post hoc tests. All data analyses were conducted using the software package R Project for Statistical Computing (http://www.r-project.org).

RESULTS

Blood samples were collected from 30 Atlantic sharpnose (22 males, 8 females), 31 bonnethead (10 males, 21 females), and 30 spiny dogfish (8 males, 22 females). Slight hemolysis was noted in one bonnethead. The lipemia index for all samples from all species was negligible. The mean total length of captured Atlantic sharpnose was 84.6 cm (range: 55–110 cm); mean total length of bonnethead was 98.3 cm (range: 72–117 cm); and mean total length of spiny dogfish was 80 cm (range: 60–86 cm). There were no statistically significant differences between the total length of males and females for any species

sampled. All sharks evaluated for body condition ($n=91$) had thin to normal body condition scores (BCS 2–3). There were no external parasites or other abnormalities noted on physical exam and all reference individuals were presumed healthy.

Descriptive statistics for hematologic variables are shown in Table 1. There were significant differences ($P<0.05$) among the three species for the WBC, Hct, total protein by electrophoresis, and total dissolved solids. Significant differences ($P<0.05$) were also observed in the leukocyte differential for both percent and absolute value of neutrophils, heterophils (or fine eosinophilic granulocyte), eosinophils (or coarse eosinophilic granulocyte), and granulated thrombocytes (GT cells) as well as for the absolute value of lymphocytes. Heterophils, lymphocytes, and GTs were the most commonly identified WBC in the Atlantic sharpnose and bonnethead. Lymphocytes, neutrophils, and GTs were the most commonly identified WBC in the spiny dogfish. Monocytes were uncommon and basophils rarely observed in all three species (Table 1).

Descriptive statistics for plasma chemistry variables are shown in Table 2. There were several plasma chemistry variables assayed but not analyzed because of measured values consistently above the maximum detectable levels in all three species: sodium (>250 mmol/l) and BUN (>120 mg/dl). Because of the high BUN, the BUN/creatinine ratio could not be determined. Conversely, the measured values LDH (<100 U/l) and GGT (<5 U/l) in all three species were consistently below minimum detectable levels. Carbon dioxide was consistently lower than the minimum measurable level (<5 mmol/l) for both the Atlantic sharpnose and bonnetheads. Triglycerides (<10 mg/dl), and thus very-low-density lipoprotein (VLDL) and LDL were below minimum measurable values in the Atlantic sharpnose. Alanine aminotransferase (<3 U/l) was below the minimum measurable value in the bonnethead shark. Amylase (<30 U/l), CPK (<20 U/l), and uric acid (<0.2 mg/dl) were below the minimum measurable values in the spiny dogfish.

Significant interspecies differences ($P<0.05$) were observed in all plasma chemistry variables except CPK, amylase, triglycerides, VLDL, and osmolality (Table 2). Descriptive statistics for plasma proteins are shown in Table 3. There were significant interspecies differences ($P<0.05$) in total protein, albumin/globulin ratio, prealbumin, albumin, alpha-1 globulin, and alpha-2 globulins.

Descriptive statistics for plasma trace nutrients and vitamins are shown in Table 4. Statistically significant ($P<0.05$) differences between species were present for vitamin E, vitamin A, and zeaxanthine concentrations. All plasma trace nutrients have significant species differences ($P<0.05$) except iron. Plasma values for vitamin D were below the measurable minimum (<5.0 ng/ml) for all three species.

There were apparent differences between sexes of each species for plasma biochemistry variables (AST, GGT, triglycerides, VLDL) and trace nutrients (arsenic, cobalt, copper, and zinc). However, because of the small sample size there was insufficient statistical power to perform analyses of these differences.

DISCUSSION

Hematology and plasma chemistry

The Hct, total solids, and total proteins found in the three species of this study are within ranges reported for other wild elasmobranchs (Hoffmayer and Parsons, 2001; Harms et al., 2002; Cain et al., 2004; Stoskopf, 2010). The total WBC counts determined in the Atlantic sharpnose, bonnethead, and spiny dogfish are higher than those in other species of elasmobranch (Stoskopf, 2010). It should be noted that GTs were included in the complete WBC counts in this study, thus explaining the higher overall counts.

TABLE 1. Preliminary hematologic reference intervals for three US coastal shark species collected June–July 2009 and 2010.

Variable[a]	Atlantic sharpnose (n=30)		Bonnethead (n=31)		Spiny dogfish (n=30)		P-value[b]
	Median	Reference interval	Median	Reference interval	Median	Reference interval	
WBC count ($\times 10^3/\mu l$)	57.2[c]	34.6–119.6	50.7[c]	35.3–83.1	40.2	21.4–55.9	<0.001
Hematocrit (%)	22	18.9–30.8	25	22–35	20	15–22.8	<0.001
Total solids (mg/dl)	5.5	2.3–7.1	6.2	5.35–7.25	4.8	3.95–6.36	<0.001
% Neutrophils	0	0–2.3	7	1.5–16	17.5	5.17–39.2	<0.001
Neutrophils ($\times 10^3/\mu l$)	0	0–2.6	3.8[c]	6.7–8.5	6.6[c]	1.3–18.2	<0.001
% Heterophils (FEG)	25[c]	13.5–38.3	20[c]	9.75–40.5	11.5	5.45–26.8	<0.001
Heterophils ($\times 10^3/\mu l$)	16.3[c]	5.7–26.8	10.6[c]	4.7–19.1	4.4	2.0–11.2	<0.001
% Lymphocytes	36	20.2–57.1	36	22.7–55	35	20–49	0.55
Lymphocyte ($\times 10^3/\mu l$)	19.8[c]	10.4–47.4	20.5[c]	10.4–37.5	13.2	7.6–23.6	<0.001
% Monocytes	4	0–8.3	3	1–7	5	1–8.3	0.13
Monocytes ($\times 10^3/\mu l$)	2.2	0–6.5	1.8	0.47–4.6	1.7	0.41–3.3	0.37
% Eosinophils (CEG)	11[c]	4–26.2	5	0.75–17	11[c]	4–24.3	<0.001
Eosinophils ($\times 10^3/\mu l$)	6.2[c]	2.2–22.7	2.2[d]	0.34–12.1	4.3[c,d]	1.1–11.4	<0.001
% Basophils	0	0–5.3	0	0–2	0	0–3.5	0.05
Basophils ($\times 10^3/\mu l$)	0	0–4.7	0	0–1.6	0	0–1.3	0.04
% GT	20.5[c,d]	6.5–36	27[c]	7–39	18[d]	10.7–26	0.002
GT ($\times 10^3/\mu l$)	12.6[c]	3.7–29.0	13.9[c]	3.4–27.5	6.5	3.5–12.6	<0.001

[a] WBC = white blood cell; FEG = fine eosinophilic granulocytes; CEG = coarse eosinophilic granulocytes; GT = granulated thrombocytes.

[b] Statistical significance (P<0.05) in species differences as determined by Kruskal–Wallis and Dunn's post hoc tests.

[c,d] Medians sharing this superscript in common are not significantly different among species.

TABLE 2. Preliminary plasma biochemistry reference intervals for three US coastal shark species.

	Atlantic sharpnose			Bonnethead			Spiny dogfish			
Plasma variable[a]	*n*	Median[b]	Reference interval	*N*	Median	Reference interval	*n*	Median	Reference interval	*P*-value[c]
Glucose (mg/dl)	30	167[d]	129–222	31	175[d]	134–225	30	42	28.2–58.0	<0.001
Creatinine (mg/dl)	30	0.45[d]	0.2–1.1	31	0.3[d]	0.1–0.7	29	0.1	0.1–0.13	<0.001
Potassium (mmol/l)	30	5.9[d]	4.9–7.6	31	6[d]	5.0–7.8	29	4	3.2–4.8	<0.001
CO_2 (mmol/l)		BDL			BDL		29	7	5.0–9.0	NA
Calcium (mg/dl)	30	18.3[d]	15.9–21.7	31	18.9[d]	16.2–22.6	30	13.6	9.3–15.6	<0.001
Phosphorus (mg/dl)	30	7.15[d]	5.5–9.6	31	7.3[d]	5.1–9.7	30	4.4	2.8–5.7	<0.001
Uric acid (mg/dl)	28	0.6	0.3–1.5	31	0.8	0.3–1.8		BDL		0.002
AST (U/l)	30	25.5	8.2–51.6	31	39	15.7–146	28	7	1.7–19	<0.001
ALT (U/l)	24	6.5	3.5–16.0		BDL		30	13	5.9–21.6	<0.001
CPK (U/l)	30	258	107–626	31	172	53.5–2,794		BDL		0.207
Amylase (U/l)	30	1,183	584–2,030	31	1,135	812–1,800		BDL		0.77
Lipase (U/l)	24	11.5[d]	1.5–19.7	31	20[d]	8.0–68.3	29	11	2.7–152	<0.001
Cholesterol (mg/dl)	30	86[d]	6.8–165.2	31	109	75–149	27	98[d]	56.5–145	0.007
Triglycerides (mg/dl)		BDL		31	29	20.2–45.3	28	30.5	20–82.3	0.233
HDL (mg/dl)	30	8	5–12	31	10[d]	6–16.5	30	12[d]	5.2–34.6	<0.001
VLDL (mg/dl)		BDL		31	6	4–9	28	6	4–17	0.191
LDL (mg/dl)		BDL		31	94	60.5–128	26	74	45.1–101.3	<0.001
Osmolality (mOsm)	30	1,091	1,013–1,300	31	1,088	997–1,329	30	1,064	699–1,210	0.160

[a] AST = aspartate aminotransferase; ALT = alanine aminotransferase; CPK = creatinine phosphokinase; HDL = high-density lipoprotein; VLDL = very-low-density lipoprotein; LDL = low-density lipoprotein; NA, not applicable.

[b] BDL = below detectable limits.

[c] Statistical significance ($P<0.05$) in species differences as determined by Kruskal–Wallis and Dunn's post hoc tests.

[d] Medians with this superscript in common are not significantly different among species.

TABLE 3. Preliminary reference intervals for plasma proteins by electrophoresis for three US coastal shark species.

	Atlantic sharpnose (n=30)		Bonnethead (n=31)		Spiny dogfish (n=30)		
Plasma proteins	Median	Reference interval	Median	Reference interval	Median	Reference interval	P-value[a]
Total protein (g/dl)	2.3[b]	1.8–3.4	2.8	2.3–3.6	2.1[b]	1.4–3.2	<0.001
A/G ratio[c]	0.14[b]	0.1–0.3	0.05	0.03–0.27	0.2[b]	0.09–0.5	<0.001
Prealbumin (g/dl)	0.03[b]	0–0.14	0.02[b]	0–0.14	0.14	0.02–0.2	<0.001
Albumin (g/dl)	0.25[b]	0.1–0.3	0.1	0.06–0.14	0.2[b]	0.1–0.4	<0.001
Globulins							
Alpha 1 (g/dl)	0.09	0.04–0.2	0.07	0.03–0.13	0.05	0.03–0.08	<0.001
Alpha 2 (g/dl)	0.69	0.3–1.1	1.08	0.83–1.4	0.20	0.1–0.3	<0.001
Beta (g/dl)	1.2	0.8–1.9	1.31	1.09–2.1	1.44	0.8–2.1	0.05
Gamma (g/dl)	0.1	0.07–0.16	0.1	0.06–0.15	0.1	0.05–0.2	0.640

[a] Statistical significance (P<0.05) in species differences as determined by Kruskal–Wallis and Dunn's post hoc tests.

[b] Medians sharing this superscript are not significantly different among species.

[c] A/G = albumin/globulin.

However, the leukocyte differentials determined in these three species are within range of those found in other species of both captive and wild elasmobranchs (Stoskopf, 2010).

Several key parameters in a plasma biochemistry panel were excluded from this study because of levels exceeding the upper limits of the analyzer. For example, BUN and nitrogen, both important indicators in elasmobranch health, were beyond the upper limits of our biochemical analyzer. To avoid this error in the future, analyzer limits should be determined before processing all samples so that dilutions can be completed, if necessary.

Overall, the plasma biochemistry values reported here show no extreme differences when compared with those obtained in previous studies of elasmobranchs (Torres et al., 1986; Tort et al., 1987; Harms et al., 2002; Cain et al., 2004; Ferreira et al., 2010). However, we found numerous statistically significant interspecies differences in plasma chemistry values that we think are attributable to basic physiologic differences between the Atlantic sharpnose, bonnethead, and spiny dogfish. These differences highlight the need to establish reference intervals for individual species.

Plasma trace nutrients

There is increasing concern about contaminants in the marine environment and although many of these are also nutritional trace elements, we do not know normal concentrations in most healthy animal populations. We found multiple differences in the plasma trace nutrient concentrations between Atlantic sharpnose, bonnethead, and spiny dogfish. For brevity we will focus our discussion only on the elements having a percentage difference between the calculated medians greater than 100: arsenic (As), selenium (Se), and nickel (Ni). Only As and Se will be discussed; Ni will not be included in the discussion since it was at the limits of detection (Barber et al., 2007), which inherently results in increased variability within the data. Atlantic sharpnose and bonnethead sharks captured off the coast of Georgia and Florida had significantly higher levels of As and Se than did the spiny dogfish captured in Puget Sound, Washington (Table 4). These differences are related to species differences in foraging ecology and environmental differences between the eastern North Pacific and the western North Atlantic oceans.

TABLE 4. Preliminary reference intervals for plasma trace nutrients and vitamins for three US coastal shark species.

Plasma trace nutrients and vitamins	Atlantic sharpnose (n=30)		Bonnethead (n=31)		Spiny dogfish (n=29)		
	Median[a]	Reference interval	Median	Reference interval	Median	Reference interval	P-value[b]
Aluminum (μg/g)	NT		NT		0.05	0.01–0.12	
Arsenic (μg/g)	3.1[c]	1.7–4.2	3.5[c]	1.29–5.58	0.20	0.04–0.32	<0.001
Cadmium (ng/g)	NT		NT		0.2	0.03–0.4	
Chromium (ng/g)	10[c]	7–2	20	9–30	10[c]	3–20	<0.001
Cobalt (ng/g)	10[c]	6–60	40	20–60	20[c]	3–30	<0.001
Copper (μg/g)	0.34[c]	0.26–0.62	0.34[c,d]	0.29–0.75	0.23[d]	0.07–0.32	<0.001
Iron (μg/g)	0.88	0.14–1.79	0.82	0.15–2.59	0.44	0.03–1.18	0.05
Lead (ng/g)	NT		NT		0.9	0.1–9	
Manganese (ng/g)	NT		NT		2	1–8	
Mercury (ng/g)	NT		NT		0.3	0.02–4	
Molybdenum (ng/g)	3[c]	1–8	3[c]	1–10	1	0.4–3	<0.001
Nickel (ng/g)	10[c]	0.2–30	12[c]	3–40	3	1–4	<0.001
Selenium (μg/g)	0.84[c]	0.63–1.54	1.03[c]	0.69–1.92	0.22	0.05–0.28	<0.001
Silver (ng/g)	0.4[c]	0–2	1	0.6–2	0.3[c]	0.09–0.7	<0.001
Zinc (μg/g)	0.79[c]	0.43–1.5	0.94[c]	0.47–4.2	0.37	0.14–0.64	<0.001
Vitamin E (μg/ml)	2.05[c]	1.30–2.78	2.69	1.16–4.57	1.64[c]	1.06–2.82	<0.001
Vitamin A (μg/ml)	0.28	0.1–0.61	0.11	0.01–0.27	0.02	0.01–0.04	<0.001
Zeaxanthine (μg/ml)	0.24	0.01–0.83	0.02	0–0.39	0	0	<0.001
Vitamin D (ng/ml)	<5.0		<5.0		<5.0		

[a] NT = not tested.

[b] Statistical significance (P<0.05) in species differences as determined by Kruskal–Wallis and Dunn's post hoc tests.

[c,d] Medians sharing this superscript in common are not significantly different.

Arsenic: Arsenic concentrates in hepatic and renal tissue (Burger et al., 2007), and since we used nonlethal sampling techniques, plasma concentrations presented here are lower than those reported in most marine fishes (Meador et al., 2004; Hayase et al., 2009). The spiny dogfish in this study had significantly lower concentrations of As than did the Atlantic sharpnose or bonnethead. This difference may be related to environmental exposure to As, which has been reported to accumulate in fish more rapidly in warmer waters than in colder waters due to enhanced uptake across the gills and intestines (McGeachy and Dixon, 1990). The waters off the coast of Georgia and Florida (29 C) are warmer than the water in the Puget Sound, Washington (13 C) in the summer months. In addition to warmer waters affecting the concentrations of As in the Atlantic sharpnose and bonnethead sharks, the sediment concentration of As in Georgia and Florida is much higher than that in Washington (Valette-Silver et al., 1999). Atlantic sharpnose and bonnethead sharks may be exposed to higher concentrations of As in their prey and environment than is the spiny dogfish of Puget Sound.

Selenium: Selenium in fish is typically measured from muscle or liver tissue (Branco et al., 2007); our reference intervals were measured from plasma because of our nonlethal sampling protocol. However, the concentrations of Se in the plasma of Atlantic sharpnose and bonnethead are similar to concentrations of Se found in the muscle and liver of blue sharks and swordfish from the northern Atlantic (Branco et al., 2007). This suggests that plasma Se may be representative of muscle and liver Se concentrations in marine fishes.

Spiny dogfish had an overall lower concentration of Se than the values reported

in the muscle of other marine carnivores (Branco et al., 2007), and compared with both the Atlantic sharpnose and bonnetheads in this study. Diet is related to the concentration of Se in marine carnivores (Maher et al., 1992), but the environment also plays a significant role (Branco et al., 2007). Similar to As (McGeachy and Dixon, 1990), Se is more rapidly absorbed across gill tissue in warmer waters compared with cooler waters (Fowler and Benayoun, 1976). Given the previously discussed temperature differences between the waters off the coast of Georgia and Florida compared with Puget Sound, the Atlantic sharpnose and bonnethead may be exposed to higher concentrations of bioavailable Se than the spiny dogfish of Puget Sound.

Plasma vitamins

Vitamin E: Little is known about the role of vitamin E in elasmobranchs or the critical value at which deficiency occurs. We found significant differences in plasma vitamin E between bonnethead and both sharpnose and spiny dogfish in this study. This difference may be directly related to the foraging ecology and diet of these sharks as plasma vitamin E has been found to increase linearly with increased concentrations in the diet (Peng and Gatlin, 2009; Hamre, 2011). Bonnethead sharks feed primarily on crustaceans (shrimp and crabs) and mollusks, both of which have been found to have very high concentrations of vitamin E (Villanueva et al., 2009). In addition, bonnetheads frequently ingest plant material (Bethea et al., 2007); plants are the only known synthesizers of vitamin E (Wedekind et al., 2010). The higher concentration of vitamin E in bonnetheads compared with the Atlantic sharpnose and spiny dogfish may reflect variation in foraging ecology among these three species.

Vitamin A: Hypervitaminosis A has been related to skeletal deformities and poor development in finfish (Hilton, 1983; Mazurais et al., 2009), though the role of vitamin A in elasmobranchs has yet to be fully elucidated. We found significant interspecies differences in the plasma concentrations of vitamin A, with the Atlantic sharpnose having the highest concentration. Similar to vitamin E, vitamin A concentration in plasma directly correlates with the concentration in the diet (Mazurais et al., 2009), so once again we conclude that differences between the species can best be explained by differences in foraging ecology. Fish liver is an extremely high source of vitamin A and because Atlantic sharpnose feed primarily on forage fish (clupeids) and other teleosts (Hoffmayer and Parsons, 2003; Bethea et al., 2006), they are exposed to higher dietary concentrations of vitamin A than either the bonnethead or spiny dogfish.

The health parameters presented here provide the baseline data necessary to monitor the population health of Atlantic sharpnose, bonnethead, and spiny dogfish sharks within the geographical ranges sampled and to further utilize these species as environmental indicators for the estuarine and nearshore ecosystems of Georgia, Florida, and Puget Sound, Washington.

ACKNOWLEDGMENTS

This project was funded in part by the US Army Medical Research and Material Command Training Grant (W81XWH-06-1-640) and by a fellowship award from Tufts Institute of the Environment. We thank the Georgia Aquarium and the Georgia Sea Turtle Center for funding. Joe Gaydos and the SeaDoc Society provided field supplies. Field assistance was provided by Amy Hupp, Lindsey Parker, Marty Dickens, Mike Arendt, and Julia Byrd. Marcie Oliva provided hematologic training. Further support was provided by Al Segars, Sharon Deem, Tristan Merriman, Michelle Kaylor, and Steven Nelson. Thank you to all else, not mentioned here, for providing support.

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Submitted for publication 1 May 2011.
Accepted 7 October 2011.