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Research article

Hematology and plasma biochemistry value differences between acclimated and recently captive female southern stingrays (*Dasyatis americana*)

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Abstract

Southern stingrays (*Dasyatis americana)* are used for interaction and education in captive and wild settings; therefore, it is important to monitor their health conditions. Diagnostic tools that are useful for assessing health in other animals include hematology and plasma biochemistry profiles. Certain reference intervals have been established in this species; however, interpretation of intervals in stingrays under different conditions is lacking. The primary aim was to compare hematological and plasma biochemical values between 17 female stingrays that were acclimated to captivity (n=8 adult) to those recently collected from the wild (n=9 immature). Examinations included measuring disc width, ultrasound evaluation of the coelomic cavity and blood collection. The examinations were performed on both test groups at two time points: prior to introduction of the recently captive rays to the aquarium exhibit and 8 months after cohabitation. Hematology analysis included manual WBC counts, leukocyte differential, PCV and plasma protein. Plasma chemistry profiles included aspartate aminotransferase, bicarbonate, urea, calcium, creatine kinase, cholesterol, chloride, globulin, glucose, phosphorus, potassium, sodium and total protein. The two groups of stingrays' results were compared using the Wilcoxon Rank Sum test. The following parameters were found to have statistically significant differences ($P<0.05$) prior to introduction: bicarbonate, urea, calcium, cholesterol, chloride, globulin, potassium, total protein and PCV. The recently captive rays had higher median values of urea, chloride and potassium. There were no significant differences after 8 months of cohabitation. Data interpretation for hematology and plasma chemistry values may be affected by the environmental changes for stingrays.

Introduction

Southern stingrays (*Dasyatis americana*) belong to the Dasyatidae family, subclass elasmobranchii, and naturally reside in the western Atlantic ocean and Gulf of Mexico (Grubbs et al. 2006). In the wild, they are used in nature-based tourism and, in captivity, they are one of the most represented marine stingray species in public aquaria (Firchau et al. 2004; 2008 AES Census; Semeniuk et al. 2009). In public aquaria they are often displayed in interactive exhibits, such as feeding or touch pools, which contributes to their popularity. Maintaining a healthy collection is important both for the animals and for public education. Diagnostic tools that may be useful in assessing the health of these animals are hematological and plasma biochemical profiles (Campbell 2015; Grant 2015). There are, however, a lack of reference intervals for many species and little information exists regarding interpretation of changes outside of those intervals. There are many factors that may influence cellular or physiologic changes in elasmobranchs, such as environment (water parameters and quality, temperature, season), nutrition, age, sex, species, stress and disease (Southgate 2001; Clauss et al. 2008). Intervals and medians for selected blood values for this species have been previously reported based on 28 individuals caught in trawls (Cain et al. 2004).

The facility used in this study maintains a southern stingray collection in a touch tank for public interaction. Wild southern stingrays were acquired to add to the collection. The objective of this study was to compare hematological and plasma biochemical values between female southern stingrays that were acclimated to a captive aquarium environment to those recently introduced to the facility from the wild. It was suspected that the recently captive stingrays were nutritionally deprived as well as stressed from capture and environmental changes at the time of examinations and therefore differences in analytes relating to those changes would be seen.

Materials and methods

Study design

This study was approved by the animal care and use committee at Colorado State University. This was an observational, prospective study using a collection of captive, female southern stingrays, divided into two groups: acclimated and recently captive. Each group was evaluated for biometric measurements (disc width or wingspan, follicle size, hematological parameters and plasma biochemical parameters) and compared between groups at two points in time: before and after cohabitation of 8 months.

Animals

Two groups of female stingrays were used in this study: the first group had been in captivity for at least 2 years (acclimated rays) and the second group were newly acquired from the wild and transported approximately 2,000 miles to the aquarium (recently captive rays). There were 25 stingrays in total (13 acclimated rays and 12 recently captive rays) and the sample size for each group depended on the stingrays caught during a given examination session and successful data or blood collection from individual animals. For this study, eight acclimated and nine recently captive rays were used. This population was used in another study (Grant et al. 2013); therefore, some descriptive statistics, like disc width, may vary slightly due to the different combinations of animals used within each group. It was previously established that the acclimated group had a significantly larger wingspan compared to the recently captive group, implying that the newly added stingrays were younger (Grant et al. 2013).

All stingrays were uniquely identified with a passive integrated transponder (PIT) tag (Avid Identification Systems, Inc., Norco CA). Physical examinations were performed and blood was collected, prior to the arrival of the new stingrays, on the acclimated rays and within one month of arrival, during two sessions, on the recently-captive rays. Information, such as capture process, the duration from capture to arrival, the water quality during transport and other tranport conditions, was unknown.

Husbandry

Upon arrival to the facility, the recently captive rays were quarantined for 2 weeks and treated with 2 parts per million (ppm) of praziquantel (Fishman Chemical, LLC, Ft. Pierce, FL) for 5 days for potential parasites. Both the exhibit and quarantine systems were maintained under similar parameters. The acclimated and recently captive rays were housed in a 45,000-l exhibit and 11,400-l quarantine tank, respectively. Each tank contained artificial saltwater with average water quality parameters maintained at 75°F (24°C), 7.5–8.0 pH, 33% salinity, zero ammonia, less than 0.05 ppm nitrite, and less than 150 ppm nitrate. Their diet consisted of smelt, pollock, capelin, mackerel, squid, or shrimp daily along with an elasmobranch vitamin supplement (Vita-Zu®, Mazuri®, St. Louis, MO) provided once weekly. The acclimated rays were also presented with feed purchased from the public of varied amounts.

Biometric measurements and blood sampling

Physical examinations, blood sampling and processing were performed prior to introduction (acclimated rays within 3 months of new ray introduction; recently captive rays within 1 month after arrival) and 8 months after cohabitation of the two groups within the same population of stingrays exhibited in the touchpool. The stingrays were handled by manual restraint and placed in dorsal recumbency to induce tonic immobility (Henningsen 1994; Stamper 2007). Physical examinations were conducted after blood collection and included measuring the disc width (DW) of each stingray and a coelomic ultrasound examination. During the ultrasound examination, liver lengths were measured (Grant et al. 2013) as well as follicle diameters when possible. Although follicle size was not initially recorded in medical records, review of the saved ultrasound images allowed for follicle diameter measurement to help ascertain life stage.

Blood was collected from the caudal tail vein by a ventral approach using a 3-mL syringe and 23-gauge needle (Noga 2010; Campbell 2015). Blood was immediately transferred into 500uL lithium heparin containers (Microtainers® BD, Franklin Lakes, NJ) and fresh blood smears were made and allowed to air dry. The whole blood samples were maintained in a cooler and submitted to the Colorado State University Diagnostic Laboratory (Clinical Pathology Laboratory, Fort Collins, CO) within 4 hours of collection. The whole blood samples were processed at the laboratory which included plasma separation. Hematological and plasma biochemical diagnostic profiles were performed. Hematological profiles included manual WBC counts using the Natt-Herrick method (Natt-Pette™, Exotic Animal Solutions, Inc., Hueytown, AL), leukocyte differentials, plasma protein and packed cell volume (PCV). Leukocyte differentials were determined using Wright's-giemsa stained blood smears and the following cell nomenclature: G1 (granulocyte type I or heterophil-like cells), G2 (granulocyte type II or neutrophil-like cells), G3 (granulocyte type III or eosinophil-like cells), basophils, lymphocytes and monocytes (Campbell 2015; Grant 2015). Leukocyte differentials were performed by trained laboratory technicians, who were blinded to the stingray groups. Plasma protein was measured by refractometer and PCV by microhematocrit centrifugation. The following plasma chemistry tests were analysed using the Roche Hitachi 917 (Block Scientific, Nutley, NJ): aspartate aminotransferase (AST), bicarbonate, blood urea nitrogen (urea), calcium, creatine kinase (CK), cholesterol, chloride, globulins, glucose, phosphorus, potassium, sodium and total protein (biuret method).

Statistical analysis

The data were analysed using a commercial statistical software package (IBM® SPSS® Statistics Subscription Build 1.0.0.1275, IBM Corporation, Armonk, NY). Histograms of the data were used to evaluate distribution. Due to the small sample size and violations of assumptions for parametric testing, a nonparametric test, the Mann-Whitney U Test, was used to compare the values for the hematological profiles, plasma biochemistry profiles, disc widths and follicle size between the two groups. Statistical significance was considered with a probability value of less than 0.05.

The entire process including physical examinations, blood sampling and collection, sample processing and analysis was repeated in the same population 8 months after the recently captive rays were introduced into the touch tank. The examinations were conducted over two consecutive days.

In addition to the comparative analysis, the correlation between protein values from the hematological profile reports (refractometer) and the biochemistry profile reports (biuret) from both sessions was evaluated using the Spearman's rho correlation coefficient (IBM® SPSS® Statistics version 23 release 23.0.0.0, IBM Corporation, Armonk, NY).

Results

All of the female stingrays were apparently healthy upon physical examination. Prior to introduction, the DW of the acclimated rays (median=60 cm) was significantly larger (P=0.001) compared to the recently-captive rays (median=40 cm). The diameter of the follicles in the acclimated rays (median=1.41 cm) were significantly larger (P=0.001) compared to the diameter of the follicles of the recently captive rays (median=0.60 cm). Eight months after cohabitation, the DW of the acclimated rays (median=64 cm) was

still significantly larger (P<0.001) compared to the recently captive rays (median=51 cm). After 8 months, there was no difference in follicle diameter (acclimated ray median=1.7 cm, recently captive ray median=1.52 cm, P=0.277).

The descriptive and comparative results from the hematological and plasma biochemistry profiles are shown (Tables 1–4). Globulin was not reported in one plasma sample from the acclimated group and glucose was not reported in one plasma sample from the recently captive group. Significant differences were found between the two test groups in the first sample session for PCV and protein by refractometer, both of which were higher in the acclimated group (Table 1). There were no significant differences in WBC counts between the two test groups at either of the two sampling sessions. There were significant differences in bicarbonate, urea, calcium, cholesterol, chloride, globulin, potassium and total protein between stingray groups prior to introduction (Table 3). The acclimated rays had higher bicarbonate, calcium, cholesterol, globulin and total protein compared to the recently captive rays which had higher urea, chloride and potassium at introduction. These results, along with the results from two other studies (Cain et al. 2004; Phillips et al. 2016) that established reference intervals of wild and captive stingrays are summarised in Table 5. After 8 months of cohabitation in the touch tank exhibit, all hematological and plasma biochemical values showed no statistically significant difference therefore the data were combined and summarised in Tables 2 and 4, respectively.

There was a significant positive correlation (n=33, r=0.954, P<0.0001) between protein values when computing the results from the refractometer and biuret methods on all stingrays from both time periods (Figure 1).

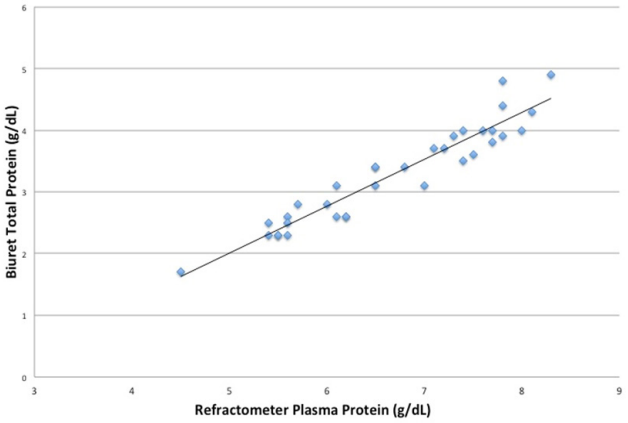

Figure 1. The total protein Spearman's rho correlation (n=33, r=0.954, P<0.0001) between the results from the refractometer (RP) and biuret (BP) methods in all the southern stingrays from both time periods.

Discussion

Biometric measurements with a statistically significant difference, in two groups of female stingrays, included DW, follicle size, plasma protein, PCV, bicarbonate, urea, calcium, cholesterol, chloride, globulin, potassium and total protein at introduction; and only DW remained significantly different after 8 months of cohabitation. These results show how extrinsic factors likely contribute to the change, and direction of change, and should always be considered when evaluating physiologic parameters in these animals.

The exact ages of the rays here are unknown, but life stage and age comparisons can be made based on the DW, follicle size and PCV. It is suspected that the recently captive rays were younger and likely not yet reproductively mature. Younger fish of the same species tend to have lower PCVs compared to older fish, which was consistent in this study (Clauss et al. 2008; Roberts et al. 2010). The follicle size in the acclimated group (1.41 cm) compared to the recently captive group (0.6 cm) was larger indicating reproductive activity in the acclimated group and being at a more advanced life stage (Grant 2016).

The difference in size and reproductive maturity may also serve as an explanation of the difference in protein results between the two groups. Pregnant southern stingrays typically have a minimum DW of 70 cm (Grant 2016; Henningsen and Leaf 2010; Ramirez-Mosqueda et al. 2010). With many of the rays in this study having a DW of at or near 70 cm, and presumably being reproductively

Table 1. Comparative results of hematological values between acclimated and recently captive southern stingrays prior to introduction. P-values < 0.05 are considered statistically significant. *The majority of results were zero.

	Acclimated stingrays		Recently captive stingrays		
Plasma Biochemistry Value	n	Median (min-max)	n	Median (min-max)	P-value
WBC (x103/uL)	8	15.2 (6.3-27.9)	9	18 (3.8-40.2)	1.000
G1 Heterophil (x103/uL)	8	5.8 (3.7-8.9)	9	3.9 (1.0-8.8)	0.321
G3 Eosinophil (x103/uL)	8	1.0 (0.1-3.1)	9	0.7 (0.1-2.1)	0.236
Basophils (x103/uL)*	8	0.1 (0.0-0.3)	9	0.0 (0.0-0.1)	0.167
Lymphocytes (x103/uL)	8	8.7 (1.5-15.1)	9	8.9 (2.5-30.1)	0.888
Monocytes (x103/uL)	8	0.2 (0.0-6.3)	9	0.2 (0.1-0.8)	0.328
Plasma protein (g/dL)	8	7.9 (7.1-8.6)	9	5.6 (5.4-6.2)	<0.001
PCV (%)	8	29 (24-36)	9	24 (21-31)	0.015

Table 2. Descriptive results of hematological values combined from the two stingray groups after 8 months of cohabitation.

Hematological Value	n	Median (min-max)
WBC (x103/uL)	17	28.9 (6.2-55.5)
G1 (x103/uL)	17	5.4 (2.2-13.8)
G2 (x103/uL)	17	0.0 (0.0-0.3)
G3 (x103/uL)	17	1.2 (0.2-5.0)
Basophils (x103/uL)	17	0.0 (0.0-0.1)
Lymphocytes (x103/uL)	17	21.7 (1.4-46.2)
Monocytes (x103/uL)	17	0.0 (0.0-2.0)
Plasma protein (g/dL)	17	7.0 (4.5-7.8)
PCV (%)	17	31 (20-48)

mature, protein would be required for folliculogenesis and the mobilisation of vitellogenin would be initiated. The increased follicle size in the recently captive group between the two sampling sessions, from a median of 0.6 cm to 1.52 cm, indicates a potential shift to active folliculogenesis. Dietary changes (having food readily and routinely available) may have initiated or perpetuated folliculogenesis as well as assisted with the acclimation of the plasma biochemical parameters in this group. A study done on wild stingrays exposed to public interaction showed a possible association between increased DW and elevated serum protein levels; however, the stingrays analysed in that study were all larger than the largest ray in this study (Semeniuk et al. 2009). The suspected difference in reproductive status may also account for the difference in plasma calcium levels with the acclimated rays mobilising more calcium (Palmeiro et al. 2007). Size alone is not the contributing factor to the differences in these analytes; size representing life stage, environment, diet and stress are more likely contributing to the differences as size alone was the only significant difference after 8 months.

Electrolytes and urea can be affected by blood osmolarity thereby changes in water quality and salinity may contribute. The wild-caught stingrays introduced into captivity were captured off the southern coast of Florida and transported over 2000 miles to the aquarium. Information regarding the capture technique, the duration from capture to arrival, the life support system during transport, water quality during transport and feedings during capture and transport were unknown. Although the examinations of the recently captive rays were completed after they were introduced into a similar environment as the acclimated rays, their previous ocean and transport environments may have played a role in the differences in the electrolytes and urea. Marine elasmobranchs readily move water and salt across the gill epithelium and osmoregulation is achieved by balancing water through renal excretion and balancing sodium and chloride levels with various organs (Evans et al. 2004). They normally maintain their blood osmolarity slightly higher than their environment. This is accomplished by retaining high levels of solutes, such as sodium, chloride, urea and trimethylamine oxide (TMAO) (Evans et al. 2004; Hammerschlag 2006; Anderson et al. 2007). By remaining hyperosmotic compared to the environment, they have less water loss and thus avoid dehydration (Hammerschlag 2006). Because they have the ability of regulating their electrolyte and urea plasma concentrations based on their environment,

Table 3. Comparative results of plasma biochemistry values between acclimated and recently captive southern stingrays prior to introduction. P-values < 0.05 are considered statistically significant.

	Acclimated stingrays		Recently captive stingrays		
Plasma Biochemistry Values	n	Median (min-max)	n	Median (min-max)	P-value
AST (U/L)	8	12.0 (4.0-36.0)	9	11.0 (6.0-16.0)	0.606
Bicarbonate (mEq/L)	8	5.3 (3.9-5.7)	9	4.1 (3.1-5.4)	0.028
Urea (mg/dL)	8	1050 (880-1075)	9	1110 (780-1330)	0.036
Calcium (mg/dL)	8	17.2 (16.3-18.3)	9	15.5 (14.6-17.2)	0.002
CK (IU/L)	8	225 (69-943)	9	371 (114-784)	0.321
Cholesterol (mg/dL)	8	205 (139-291)	9	122 (23-176)	0.004
Chloride (mEq/L)	8	247 (168-269)	9	285 (259-313)	0.002
Globulin (g/dL)	7	3.4 (3.0-3.9)	9	1.5 (1.3-1.8)	0.008
Glucose (mg/dL)	8	34.5 (25-58)	9	38 (27-45)	0.673
Phosphorus (mg/dL)	8	4.7 (3.9-7.0)	9	4.0 (3/1-7.1)	0.277
Potassium (mEq/L)	8	3.1 (1.7-3.5)	9	3.7 (2.1-5.8)	0.006
Sodium (mEq/L)	8	274 (214-276)	9	277 (258-292)	0.236
Total protein (g/dL)	8	4.4 (3.7-4.9)	9	2.5 (2.3-2.8)	<0.001

Table 4. Descriptive results of plasma biochemistry values combined from the two groups of stingrays after 8 months of cohabitation.

8 months of cohabitation	n	Median (min-max)
AST (U/L)	17	11 (5-27)
Bicarbonate (mEq/L)	17	2.7 (2.1-4.0)
Urea (mg/dL)	17	1050 (870-1130)
Calcium (mg/dL)	17	16.7 (12.6-18.5)
CK (U/L)	17	218 (94-653)
Cholesterol (mg/dL)	17	263 (78-335)
Chloride (mEq/L)	17	265 (254-280)
Globulin (g/dL)	17	2.4 (1.5-3.0)
Glucose (mg/dL)	16	45 (22-66)
Phosphorus (mg/dL)	17	4.8 (3.9-6.7)
Potassium (mEq/L)	17	3.3 (2.6-6.1)
Sodium (mEq/L)	17	261 (250-274)
Total protein (g/dL)	17	3.4 (1.7-4.0)

one explanation of the differences between chloride, potassium and urea may be that the recently captive rays were previously exposed to an environment that was higher in salinity. Although there was not a significant difference between groups when comparing sodium, the median values of sodium were higher in the recently captive group which would be expected if exposed to higher salinities. The primary organs involved with regulation of these solutes (sodium, chloride, potassium and urea) are the rectal gland and kidney. The rectal gland of elasmobranchs controls the majority of salt excretion, with secretory fluid having higher concentrations of NaCl compared to the surrounding seawater, but also contains ion pumps and channels that transport potassium across the basolateral cell membranes (Evans et al. 2004). A cotransport protein (NKCC), a Na-K activated ATPase, a K+ channel, and a Cl- channel on the basolateral cell membrane have been shown to osmoregulate *Squalus acanthias* (Evans et al. 2004). Initially being in an environment with a higher salinity may have resulted in higher concentrations of these ions until the rectal gland could excrete adequate amounts to regulate to the new environment. The kidney is involved with sodium and chloride movement, although to a lesser extent, as well as urea reabsorption and clearance (Evans et al. 2004; Hammerschlag 2006). Some marine elasmobranchs seem to acclimate to lower salinities, not only by increasing urine flow (thus eliminating urea, sodium and chloride), but also possibly by decreasing urea synthesis in the liver (Hazon et al. 2003; Tam et al. 2003; Anderson et al. 2005). Table 5 shows a summary of the significantly different values in this study and results from Cain's study and Phillips' study. Although a different analyser was used in the study by Cain et al., the results complement the trend in this study. Many of the parameter results in the study by Phillips et al., which was conducted on captive rays, fall between the results in this study. For example, the parameters that were elevated in the recently captive rays compared to the acclimated rays (urea, chloride and potassium) were also shown to be higher in the wild-caught rays and bookended the captive rays.

Another contributing factor explaining changes in electrolytes is stress. There are a number of factors that may influence stress in fish including water quality, environmental conditions, social environment, handling, transport, nutrition, therapeutics and pathogens (Clauss et al 2008; Pasnik et al. 2010). The recently captive rays were possibly experiencing chronic stress from

Table 5. A summary of the plasma biochemical parameter medians in this study as well as those from a study that established reference intervals for wild-caught southern stingrays (Cain et al. 2004) and captive southern stingrays (Phillips et al. 2016). a Cain et al. 2004; b Phillips et al. 2016 *Na was not significantly different in this study but included here due to its association with Cl. NR=not reported

Parameter	Acclimated rays	Recently captive rays	Wild-caught rays[a]	Captive rays[b]
Bicarbonate (mEq/L)	5.3	4.1	<5	NR
Urea (mg/dL)	1050	1110	1243	1014
Calcium (mg/dL)	17.2	15.5	16.5	14.6
Cholesterol (mg/dL)	205	122	NR	112
Chloride (mEq/L)	247	285	342	268
Globulin (g/dL)	3.4	1.5	NR	3.2
Glucose (mg/dL)	34.5	38	30.5	35
Phosphorus (mg/dL)	4.7	4.0	4.7	4.3
Potassium (mEq/L)	3.1	3.7	5.0	3.6
Total protein (g/dL)	4.4	2.5	2.6	3.7
PCV (%)	29	24	22	25
Sodium (mEq/L)*	274	277	315	259

capture, confinement, overcrowding, transport, or environmental (poor water quality) and dietary change (Skomal and Bernal 2010). The transition of wild animals into captivity is classified as chronic stress (lasting days to weeks) and may prolong the differences in blood values depending on the severity of the stressor and the time it takes to acclimate (Manire et al. 2007; Skomal and Bernal 2010). Osmoregulatory function is affected by stressful events and may not immediately respond nor quickly stablise (Eddy 1981); however, it has also been reported that increased sodium and chloride from marine fish, undergoing capture stress, normalised within 24 hours (Eddy 1981; Cliff and Thurman 1984; Wells et al. 1986). The increase of sodium and chloride is mainly attributed to an increase of water outflow. Potassium also remained elevated in previous studies presumably from muscle (intracellular) leakage (Cliff and Thurman 1984; Wells et al. 1986). The stingrays in this study probably experienced relatively different degrees of stress during the entire process from capture to exhibition. Although the examinations were performed weeks after their arrival, they were disrupted during their time in quarantine and during the move from quarantine to exhibit. The examination process between the two groups was the same; however, the acclimated rays were much more accustomed to human interaction and routine examinations.

Other hematological or plasma biochemical values in fish shown to be affected by either chronic or acute stress include glucose, leukocyte counts, bicarbonate, PCV, protein, lactate, hemoglobin and cortisol (in teleosts) (Evans et al. 2004; Smith et al. 2004; Clauss et al. 2008; Ross and Ross 2008; Roberts et al. 2010; Stoskopf 2010). In this study, lactate, hemoglobin and cortisol (not applicable) were not analysed and no signficant differences were seen in glucose or with the leukocyte counts. Measuring cortisol in elasmobranchs is not applicable since it does not exist. The major stress hormone in elasmobranchs is considered 1α-hydroxycorticosterone (1α-OH-B) and is difficult to measure (Manire et al. 2007; Skomal and Mandelman 2012). Although corticosterone (also from the interrenal or adrenocorticoid gland), a 1α-OH-B precursor, has also been found in serum and faeces when studying stress response, the amount of increased concentrations and cross-reactivity with 1α-OH-B, support that it is not likely a primary stress hormone (Karsten et al. 2003; Manire et al. 2007; Anderson 2012; Smokal and Mandelman 2012). The glucose results in this study may not be reliable given the duration between collection and analysis. The samples were not centrifuged to separate cellular components from plasma at the time of collection and therefore were vulnerable to glucose consumption (generally at a rate of 10% per hour) (Weiser 2012). The glucose results in this study were similar to those in captive and wild southern stingrays (Phillips et al. 2016; Cain et al. 2004) and were lower compared to captive cownose stingrays (Ferreira et al. 2010). A stress leukogram in elasmobranchs is similar to that of other fish in that it is represented by a general leukocytosis with lymphopenia and relative granulocytosis (Clauss et al. 2008; Roberts et al. 2010; Campbell 2015; Grant 2015). With lymphocytes being the most abundant white blood cell in elasmobranchs, an inverse heterophil:lymphocyte ratio may also be indicative of a stressful event or disease response as seen in other species (Walsh and Luer 2004; Alexander et al. 2016; Dove et al. 2010). There was not a significant difference in these cell counts and, although the recently captive rays had a slightly higher leukocyte count, there was a median increase in lymphocytes and decrease in granulocytes compared to the acclimated rays (Table 1). Extrapolating from other elasmobranch species and their hemogram response, the hemogram response here is uneventful but more research is warranted in this area to understand elasmobranch hemograms as well as species-specific responses. Overall, the WBC counts in the pooled data after 8 months of cohabitation (Table 2) appeared subjectively higher. The values for WBC counts are similar compared to other reports from wild caught free-ranging Atlantic sharpnose sharks (*Rhizoprionodon terraenovae*), bonnethead sharks (*Sphyrna tiburo*) and spiny dogfish (*Squalus acanthias*), but higher in captive cownose stingrays (*Rhinoptera bonasus*) (Ferreira et al. 2010; Haman et al. 2012). When comparing the total leukocyte count to a study done on white-spotted bamboo sharks (*Chiloscyllium plagiosum*), the values are most similar to the pre-operative males with traumatic clasper wounds, although the heterophil:lymphocyte was not inversed as it was with the bamboo sharks (Alexander et al. 2016). The stingrays in this study had presumed bite wounds as a result of mating behavior which may have induced an inflammatory response but studies on cytochemical analysis for southern stingrays, and in elasmobranchs in general, are needed.

The remaining analytes (bicarbonate, PCV and protein) potentially affected by stress were significantly different between the two groups. The direction of the change in bicarbonate showed the acclimated rays having higher levels of bicarbonate compared to the recently captive rays. Hyperactivity from stress may cause an acidosis thereby decreasing the bicarbonate (Smith et al. 2004). The acidosis may be from respiratory or metabolic mechanisms in the recently captive rays. The type of acidosis in elasmobranchs appears to vary among species and is caused by a relative hypoxia or an increase in anaerobic activity (Skomal and Bernal 2010). Either type of acidosis is a potential cause for a decrease in bicarbonate in this study but exercising to fatigue is more probable especially upon entering quarantine and the exhibit. The decreased PCV and protein in the recently captive rays may be a result from stress, age or life stage, diet, or disease. The median PCV for this group was greater than 20% and therefore would not be classified as an anemia (Clauss et al. 2008; Campbell 2015). Stress from acclimating to captivity, starvation and confinement are known to decrease the PCV in fish which certainly may have been the case here (Roberts et al. 2010; Stoskopf 2010). A study evaluating blood analytes between wild southern stingrays in a tourist site versus a non-tourist site resulted with lower PCV and protein levels in the tourist site rays which was attributed to those rays being in a poorer state (Semeniuk et al. 2009). Although being in a poorer state is subjective, this is possibly the case of the recently captive rays in this study. It is likely that they were tightly confined during transport in suboptimal water conditions with a lack of nutritional support. They were in a negative metabolic state after arriving to the facility based on the small liver sizes (Grant et al. 2013). The lack of nutritional support may also explain the difference in plasma cholesterol.

Another cause for a lower PCV and protein in stingrays is blood loss from parasites. There was no apparent blood loss from the recently captive rays during examination; however, mild blood loss from parasitism is possible. Wild elasmobranchs have been reported with a number of different external and internal parasites (Ruhnke 1994). Naturally, these animals may not succumb to the infestation of such parasites, but in a stressful situation, proliferation and detriment may occur. These stingrays were not specifically tested for any parasites when they arrived but as part of the aquarium's protocol for new animal arrivals and introductions, the rays were held in quarantine and treated with praziquantel. The exams were performed after treatment but if parasites contributed to the lower PCV, then perhaps not enough time lapsed for adequate red blood cell regeneration.

After the 8 month cohabitation period, there were no differences in plasma biochemistry values between the acclimated and recently captive rays (Table 4). These results were similar to those reported for wild caught bonnethead sharks (*Sphyrna tiburo*) and captive smooth dogfish (*Mustelus canis*) (Harms et al. 2002; Persky et al. 2012).

The hematologic and plasma biochemisty profiles each provided values for protein. A difference in protein values existed as the plasma protein reported in the hematological report was measured using a refractometer whereas the total protein from the plasma biochemistry profile was measured using spectrophotometry, the biuret method in this case. It was expected that the refractometer would overestimate the protein values as this method is based on the refractive index of the fluid and other solutes may contribute (Stoskopf 2010; Weiser and Allison 2012). The positive correlation between methods has been previously demonstrated in wild southern rays as well as in other species (George and O'Neill 2001; Harms et al. 2002; Cain et al. 2004; Cray et al. 2008).

Conclusion

The capture, confinement, transport and environmental and nutritional changes were probable factors involved with the differences in hematology and plasma biochemistry values found in the stingrays in this study. Although differences were noted around the time of introduction, there were no differences after 8 months and it appeared that the recently captive rays were acclimated to their new environment. The results presented in this study may serve as a hematological and plasma biochemical baseline for southern stingrays maintained in similar environmental conditions.

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