Zoo Biology 32: 104–111 (2013)

TECHNICAL REPORT

Validation of an Ultrasound-Guided Technique to Establish a Liver-to-Coelom Ratio and a Comparative Analysis of the Ratios Among Acclimated and Recently Wild-Caught Southern Stingrays, *Dasyatis Americana*

Krystan R. Grant,[1*] Terry W. Campbell,[1] Tawni I. Silver,[2] and Francisco J. Olea-Popelka[1]

[1]*Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado*
[2]*Department of Medical Imaging, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada*

Southern stingrays, *Dasyatis americana*, are a well-represented elasmobranch species in public aquaria and other facilities throughout the world. This study was conducted at a facility that experienced some mortality and replenished the collection with wild-caught stingrays. A common necropsy finding among the stingrays was a small, dark liver. The objectives of this study were to assess the reliability of an ultrasound-guided technique for establishing a liver-to-coelom ratio by calculating the approximate length of the liver with respect to the coelomic cavity length and then to compare ratios between acclimated captive and wild-caught stingrays. The ultrasound validation phase of the study measured the distance from the caudal margin of the liver to the pelvic cartilaginous girdle and compared it to the actual distance measured during the necropsy or surgery. There was no significant difference found between the ultrasound and actual distance measurements ($P = 0.945$). This technique was then used to establish liver-to-coelom ratios and compare two groups of stingrays, presumably under different metabolic states at different periods. Liver-to-coelom ratios were established during initial examinations as well as 8 months after cohabitation in a touch pool exhibit. There were significant differences in liver-to-coelom ratios between the two stingray groups at introduction (median difference = 30.9%, $P = 0.007$) and after 8 months (median difference = 20.5%, $P = 0.008$). There were also significant differences in the liver-to-coelom ratios within each group at introduction and at 8 months (acclimated group median difference = 20.4%, $P = 0.018$; wild-caught group median difference 31%, $P = 0.008$). Zoo Biol. 32:104–111, 2013.

Keywords: stingray; liver; elasmobranch; lipid; ultrasound

INTRODUCTION

Elasmobranchs have been an attraction in public aquarium exhibits since the late 1800s [Koob, 2004]. The southern stingray, *Dasyatis americana*, is a well-represented stingray species in public aquariums throughout the world. The southern stingray is exhibited in over 48 facilities worldwide and is the second most represented marine stingray species [AES 2008; Firchau et al., 2004]. Contributing to their popularity is their commonplace presence in feeding or touch pools where the public may interact with the animals [Jeffery and Wandersee, 1996]. Due to the public involvement with these types of facilities, monitoring feedings and caloric intake for these animals is challenging. The study presented

*Correspondence to: Krystan R. Grant, Department of Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, CO 80523. E-mail: krystan.grant@colostate.edu

Received 19 October 2011; Revised 11 January 2012; Accepted 26 January 2012

DOI 10.1002/zoo.21014
Published online in 15 May 2012 Wiley Online Library (wileyonlinelibrary.com).

here will introduce a method for assessing body condition based on relative liver size and will use this method to compare stingrays in presumably different metabolic states.

The liver is a large organ in elasmobranchs and may occupy the majority of the coelomic cavity. In *Benthic elasmobranch* species, it may weigh between 1% and 6% of their body weight of which 80% may be lipid [Holmgren and Nilsson, 1999]. In some shark species, it has been noted that the liver extends to the cloaca [Walsh et al., 1993]. The liver is the primary location for triacylglycerol storage [Zammit and Newsholme, 1979]. These lipid stores provide energy between meals and, in some species, assist with buoyancy. It is suspected that under stressful situations, long periods between meals, or during times of high energy or nutritional demand, the lipid stores become depleted thereby altering the size of the liver. In addition to a decreased size, lipid depletion will decrease the echogenicity of the liver during ultrasound examination [d'Anjou, 2008; Mathiesen et al., 2002; Nyland and Park, 1983].

Animals in this study were housed in a commercial interactive pool with approximately 20 southern and cownose stingrays, *Rhinoptera bonasus*. Over a 3-year period, this facility experienced intermittent mortalities involving their recently wild-caught, adult, female southern stingrays. Necropsy records noted that all of these animals had prominent follicles and a small, dark liver upon gross necropsy, which was described as lipid or glycogen depletion in the pathology reports. Records indicated that there were no signs of illness prior to death and in many cases, the animals ate until the day before they died.

Since the most evident macroscopic lesion at necropsy, in all cases, involved the size of the liver, the objective of the initial phase was to determine the reliability of accurately measuring the distance between the caudal margin of the liver and the pelvic cartilaginous girdle using an ultrasound-guided technique. This distance implies a relative liver length compared to the coelomic length and was used to establish a liver-to-coelom ratio (liver size %) to identify potentially compromised animals.

The second phase of this study compares the liver size percentages of stingrays recently wild-caught in the fall to those acclimated to captivity using the previously described ultrasound technique. Since these wild-caught stingrays several weeks into captivity are potentially more nutritionally compromised due to the season they were captured in and the stress of capture, transport, and a new environment; it is suspected that their introduction into captivity results in a more nutritionally compromised state than those acclimated to captivity.

METHODS

Validation of the Ultrasound-Guided Technique for Establishing the Liver-to-Coelom Ratio

The purpose of this phase of the study was to validate an ultrasound-guided technique to measure the liver. This phase was exploratory in which available subjects, 14 adult southern stingrays (13 females, 1 male), were used. The ultrasound exams were performed and measurements were taken on 13 deceased animals and one animal under general anesthesia for a coelomic exploratory surgery. The ultrasound exams and necropsies were performed within 24 hr of death.

All but one of these animals lived in the touch pool exhibit. The 14th stingray lived in a much larger exhibit with a variety of other animals. The exhibit and water quality parameters were consistent with recommendations for captive elasmobranchs [Mohan and Aiken, 2004]. The 12,000-gallon touch pool is located in an area of the facility where natural sunlight is filtered through skylights. The water quality parameters are maintained as follows: temperature range is 73–75 degrees Fahrenheit, the pH is 7.5–8.0, ammonia is zero parts per million (ppm), nitrite is less than 0.05 ppm, and nitrate is less than 150 ppm. The offered diet consisted of a variety of fish, such as smelt, pollock, capelin, mackerel as well as squid and shrimp. The stingrays were also supplemented with an elasmobranch vitamin (Vita-Zu®, Mazuri®, St. Louis, MO) once weekly.

Each stingray was placed in dorsal recumbency for sonography. Thirteen imaging exams were performed post mortem and one during surgery. The stingray undergoing surgery was placed in a shallow bath with recirculating saltwater treated with 100 ppm of tricaine methanesulfonate (Finquel® or MS-222®, Argent Laboratories, Redmond, WA). The length of the coelomic cavity was established by palpating and measuring the distance (in centimeters) between the pectoral and pelvic cartilaginous girdles on ventral midline (Fig. 1).

Ultrasound examinations were performed by the same ultrasonographer using a 7.5 MHz linear array transducer with a commercial ultrasound unit (Aloka SSD-900v, Aloka, Inc. Wallingford, CT). The overall gain, time gain compensation (TGC), and depth settings were adjusted to maximize image resolution and organ visualization. With the transducer in a sagittal position, on ventral midline, just

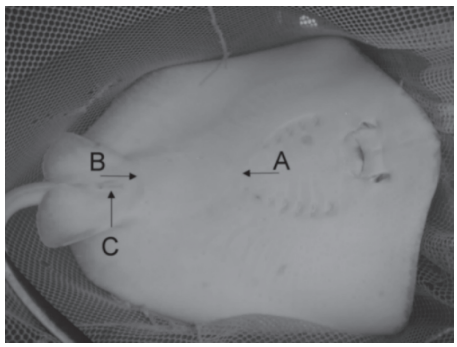

Fig. 1. Ventral view of a female southern stingray, *D. americana*: the pectoral cartilaginous girdle (A), the pelvic cartilaginous girdle (B), and the vent (C).

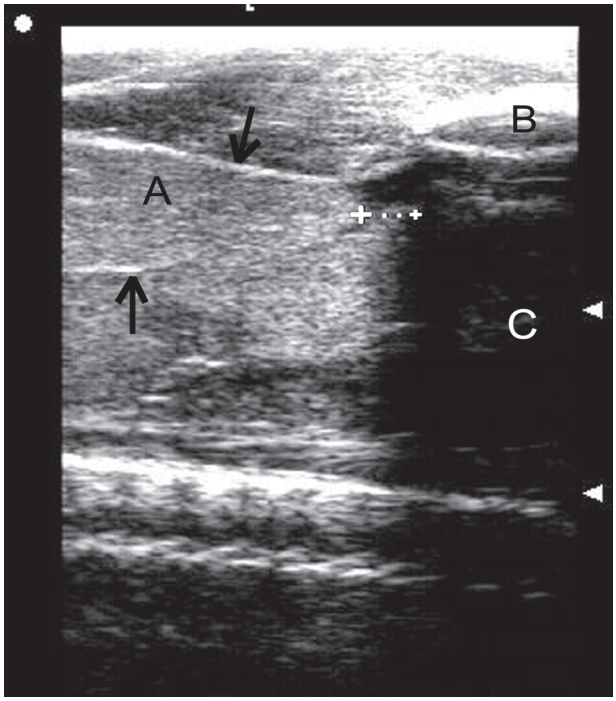

Fig. 2. Ultrasound image of the caudal, mid coelom in a female southern stingray, *D. americana.* Note, the caudal margin of the liver (A) and the pelvic cartilaginous girdle (B). Similar to that of bone, the cartilage produces a distal acoustic shadow (C). The black arrows denote the dorsal and ventral margins of the liver. The distance between them can be measured using the ultrasound unit as noted by the dotted line in the image. The large "+" symbol is at the caudal margin of the liver and the small "+" symbol is at the cranial edge of the pelvic girdle.

caudal to the pectoral cartilaginous girdle, the liver was identified. The caudal margin of the liver was located with the ultrasound along the ventral midline. The pelvic cartilaginous girdle was identified by palpation just cranial to the vent (Fig. 1). If the cartilage and the caudal liver margin were captured within the same view, then the distance between the two landmarks was measured using the ultrasound unit (Fig. 2). If the two landmarks were not captured within the same image, then the caudal edge of the transducer was aligned with the caudal margin of the liver and the distance between the caudal edge of the transducer and the cartilage was measured with a ruler. The estimated liver length is calculated by subtracting the distance between the liver and pelvic cartilage from the coelomic cavity length. The liver size is expressed as a percent of the coelom (or liver-to-coelom ratio) by dividing the estimated liver length by the coelomic cavity length.

The necropsy was performed by making a circular incision along the border of the cartilage surrounding the coelomic cavity. The contents of the coelom were exposed during necropsy. The distance between the caudal margin of the liver on ventral midline and the pelvic cartilaginous girdle just cranial to the vent was measured in centimeters. This distance and the distance obtained using the ultrasound provided two measurements for each stingray (paired data).

Comparative Analysis of Ratios among Acclimated and Wild-Caught Stingrays

This phase was prospective using a cohort of 20 female southern stingrays. Nine of the stingrays were acclimated to the touchpool exhibit for a minimum of 2 years (acclimated stingrays) and 11 of the stingrays were recently introduced to the exhibit after being captured from the wild (wild-caught stingrays). Initial examination of the acclimated stingrays occurred 1 month prior to the introduction of the wild-caught stingrays. Upon arrival to the aquarium, the wild-caught stingrays were treated with 2 ppm of praziquantel (Fishman Chemical, LLC, Ft. Pierce, FL) for 5 days while quarantined for 2 weeks. The 11 wild-caught stingrays were examined on two different occasions within 1 month of arrival.

Physical examinations of both groups consisted of placing the animal in dorsal recumbency for measurements and ultrasound imaging. The stingrays were captured with a large nylon net and manually turned into dorsal recumbency. Although the barbs are clipped due to public interaction, careful handling by trained personnel ensured the safety of those involved with the examinations. Measurements recorded included wingspan (largest distance from wing tip to wing tip), snout-to-vent length, length of coelomic cavity (pectoral to pelvic cartilaginous girdle measurement), and liver length (using the ultrasound-guided technique). Liver size percentages were established for each stingray. An ultrasound image comparing liver and spleen echogenicity was also recorded.

The wild-caught stingrays were introduced into the touchpool exhibit approximately 2 weeks after arrival. The husbandry and diet for the stingrays were identical to those described previously. In an effort to monitor the health status of the collection, physical and ultrasound examinations were performed twice yearly. Therefore, 8 months after introduction, examinations were repeated.

STATISTICAL ANALYSES

Validation of the Ultrasound-Guided Technique for Establishing the Liver-to-Coelom Ratio

StatTools (Palisade Corporation, Ithaca, NY) was used for statistical analysis. The Wilcoxon signed rank test was used to compare the median distances measured from the caudal liver margin to the pelvic cartilaginous girdle using the ultrasound-guided technique and the measurement taken during necropsy among each subject (paired data). We tested the null hypothesis that there will be no difference between the liver-to-cartilage distance measurements when using the ultrasound-guided technique compared to the measurement taken during necropsy or surgery.

Comparative Analysis of Ratios among Acclimated and Wild-Caught Stingrays

The liver size median percentages were compared between the two stingray groups (acclimated vs. wild-caught) using the Wilcoxon rank sum test at the time of introduction and after 8 months of cohabitation. The liver-to-coelom percentages were compared between time periods (introduction vs. 8 months) within each stingray group (paired data) using the Wilcoxon signed rank test using SPSS 17.0 for Windows, release 17.0.2 (SPSS Inc., Chicago, IL). We tested the null hypotheses that liver size percentages between stingrays groups and time points were not different. Statistical significance (rejection of the null hypotheses) was considered at $P < 0.05$.

The wild-caught stingray examinations were completedwithin 1 month of arrival, 2 weeks apart. The liver size percentages were subjectively evaluated between the two exams and showed no difference.

RESULTS

Validation of the Ultrasound-Guided Technique for Establishing the Liver-to-Coelom Ratio

Table 1 represents the data for 14 southern stingrays. The minimum and maximum actual liver-cartilage distances were 0 and 15 cm, respectively. The minimum and maximum differences between the ultrasound-guided measurement and actual distance were 0 and 2 cm, respectively. Six of the 14 stingrays had a distance measurement of 0 cm between the ultrasound guided and actual distances. Two of the 14 stingrays had distance differences of 2 cm. The remaining six observations had differences between measurements of 0.31, 1.37, 1.40, 1.48, and two at 1.67 cm. The median difference between the measurements of the two methods for the liver-cartilage distance was not statistically significant (median difference = 0.84 cm, $P = 0.945$, Table 1).

TABLE 1. Measurements from the caudal liver edge to the pelvic girdle in southern stingrays using an ultrasound-guided technique (US) and those taken during necropsy or surgery (N/S)

Stingray	US (cm)	N/S (cm)	|Difference|
1	14.5	14.5	0
2	0	0	0
3	1.67	0	1.67
4	0.31	0	0.31
5	0	0	0
6	1.4	0	1.4
7	3.52	5	1.48
8	0	0	0
9	0	0	0
10	3.37	2	1.37
11	1.67	0	1.67
12	13	15	2
13	0	0	0
14	7	9	2
Median	1.54	0	0.84
Mean	3.32	3.25	0.85
Standard deviation	4.85	5.53	0.86

Comparative Analysis of Ratios among Acclimated and Wild-caught Stingrays

The results from the comparisons between the two groups of stingray liver size percentages are shown in Table 2. The median liver size percentages of the acclimated stingrays and wild-caught stingrays at introduction were significantly different ($P = 0.007$) at 90.9% and 60.0%, respectively. Likewise, comparing the groups after 8 months of cohabitation, the median liver size percentages of the acclimated and wild-caught stingrays showed a contrasting significant difference ($P = 0.008$) at 70.5% and 91.0%, respectively.

Liver measurements were also compared within groups. The values for the acclimated and wild-caught stingrays at introduction were compared to values obtained 8 months later. There was a significant difference for liver size within the wild-caught stingray group between time periods (median difference = 31%, $P = 0.008$) and for the acclimated group (median difference = 20.4%, $P = 0.018$).

DISCUSSION

The purpose of this study was to assess the accuracy of measuring an estimated liver length relative to the coelom using an ultrasound-guided technique and to use this technique to compare stingrays acclimated to a captive environment to those recently wild-caught. The small difference between measurements in the ultrasound validation phase confirmed the accuracy of the ultrasound-guided measurements. We did not have a predefined hypothesis to test regarding the difference between the two measurements. Although small (median difference = 0.84 cm, $P = 0.945$), there were observed differences between ultrasound-guided and actual measurements in some stingrays; however, we did not find a clinical/anatomical relevant difference between the measurements between the two methods in our study. Considering the variability in our data, a post-hoc power analysis indicated our sample ($n = 14$) would be sufficient to detect a significant difference of 1 cm between methods if that difference existed, with a power of 80% and 95% confidence.

The accuracy of taking liver to cartilage distance measurements may vary depending on whether or not the image captures both the caudal liver margin and the pelvic girdle. If the ultrasound image captures both landmarks, then the measurement can be taken directly with the ultrasound unit and the only variability is probe position. The variability in the actual distance measured when both landmarks are captured within the image is identifying a clean border on the pelvic girdle. The cartilage is clearly defined on the ultrasound image as it produces a distal acoustic shadow

TABLE 2. Descriptive measurements among recently wild-caught southern stingrays and acclimated southern stingrays and comparisons of liver-to-coelom ratio (liver size percentages)

	Acclimated stingrays						Recently wild-caught stingrays						Wilcoxon rank sum test
	n	Min	Max	Median	Mean	SD	*n*	Min	Max	Median	Mean	SD	*P*-value
Liver size introduction (percentage)	7	80	104	90.9	92.9	7.1	11	30	85	60.0	59.5	17.1	***0.007***
Liver size 8 months (percentage)[a]	8	53	83	70.5	69.9	9.5	11	58	100	91.0	86.9	12.6	***0.008***
Wilcoxon sign rank test *P*-value	–	–	–	***0.018***	–	–	–	–	–	***0.008***	–	–	–
Liver size 1 year (percentage)[b]	4	92.6	96.9	94.4	94.6	1.8	6	90	100	100	98.3	4.1	–

[a]The second measurement taken after 8 months of cohabitation. Wilcoxon sign rank tests were used to compare median liver sizes at different time points among the same stingrays. The Wilcoxon rank sum test was used to compare median liver size among different groups of stingrays (at two different time points). *P*-values (bolded) indicates significance (<0.05)

[b]The third measurement taken after 1 year of cohabitation. Ten stingrays randomly collected from the exhibit were examined to evaluate health status. Only descriptive statistics done, no analysis.

(Fig. 2). During necropsy, the soft tissue is needed to be removed in order to establish a definite point of measurement on the cartilage. If the liver is small and not imaged with the cartilage, then measuring the distance requires an external measuring device and a well-positioned probe. The caudal edge of the probe must be aligned with the caudal margin of the liver at which point the distance is measured from the caudal edge of the probe to the palpated pelvic girdle. The margin of error may involve the probe position, the variability of the point at which to measure from the probe, accurately palpating the pelvic girdle, and the variability of the point at which to measure to the cartilage. Again, if the probe is not on midline, this may alter the distance measured as well. The other factor that may add to the variability is movement by the animal.

A liver with decreased fat stores may not only decrease in size but also display a decreased echogenicity when imaged with an ultrasound unit. The liver may have a similar echogenicity or appear hypoechoic when compared to the spleen (Fig. 3) or epigonal organ. In cases where it is difficult to discern organs, identifying the liver to measure the distance from the caudal margin to the pelvic cartilage may be challenging. A comparison of the echogenicity, gross appearance, and corresponding histology of a liver with depleted lipid stores and a normal lipid-filled liver are shown in Figures 3 and 4, respectively. The ultrasound image of the lipid-depleted liver (Fig. 3) shows a small liver ventrally with similar echogenicity compared to the spleen. The corresponding gross image from necropsy shows the small, dark liver extending to the curvature of the stomach. The spleen is dorsal to the liver and therefore is not seen. The darker color of the liver during necropsy is an indication of depleted liver stores [Rossouw, 1987]. Histologically, this liver showed marked depletion of fat from the hepatocytes. Although there were some fat vacuoles present, the overall liver was severely lipid depleted which is apparent when compared to the histology of a normal liver (Fig. 4). The ultrasound image of the lipid-filled liver shows a large and hyperechoic

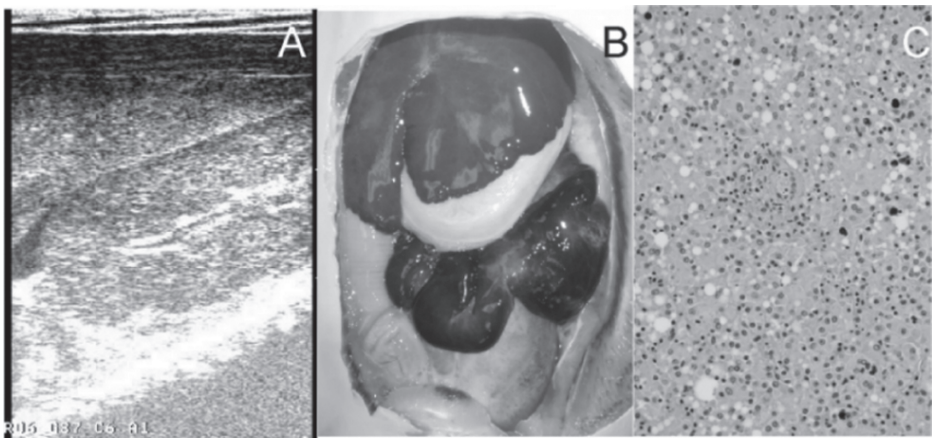

Fig. 3. These images are from the same southern stingray, *D. americana*, with a lipid-depleted liver. (A) This ultrasound image is captured with the linear transducer in a sagittal position, mid-to-cranial coelom on ventral midline. The liver is ventral (top of image) to the spleen (middle of image). Note, the similar echogenicity between the liver and the spleen. (B) This image shows the open coelom during necropsy. The caudal margin of the liver does not extend beyond the curvature of the stomach. (C) Histology of the liver shows some fat vacuoles but is severely depleted overall, HE stain.

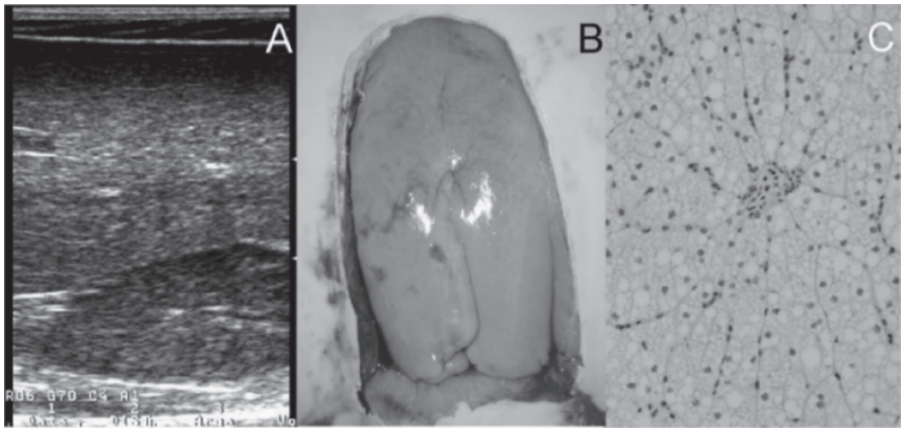

Fig. 4. These images are from the same southern stingray, *D. americana*, with a lipid-filled liver. (A) The ultrasound image is with the linear transducer in a sagittal, mid coelom, ventral midline position. The liver is occupying the majority of the image (top half of image A) and is hyperechoic compared to the spleen (bottom of image A). (B) The open coelom during necropsy. Only the liver can be seen, as it is large and lipid-filled. (C) Histology (HE stain) of the lipid-filled liver with the majority of the hepatocytes containing fat vacuoles.

liver compared to the spleen, which is dorsal to the liver (Fig. 4). While in elasmobranchs it represents a normal condition, increased echogenicity is consistent in other animals with abnormal fatty infiltration to the liver [Nyland et al., 2002; Stetter, 2004]. The histology of this normal stingray liver shows the majority of hepatocytes with fat vacuoles, which is similar to hepatic lipidosis in other animals [Cebra et al., 1997; Cooper, 2002]. On necropsy, this liver is large and of a light tan color (Fig. 4).

To further evaluate the liver size using this technique under presumably different metabolic states, two groups of stingrays were compared. One group originated from the wild and had been in captivity for at least 2 years while the other group was recently acquired from the wild. The results provided evidence to support the hypotheses that wild-caught stingrays' liver size percentages are significantly different compared to the acclimated stingrays between the two groups at introduction. The liver size percentages were significantly different when analyzed between groups at introduction and after 8 months of cohabitation as well as within both groups. The smaller liver size percentages in the wild-caught stingrays at introduction were expected due to possible stress of the capture and long transport, stress from the new and unfamiliar environment, and anorexia. Southern stingrays reside in the western Atlantic [Cain et al., 2004; Chapman et al., 2003] and Gulf of Mexico [Lytle and Lytle, 1994; Semeniuk et al., 2007] so the transport distance to this facility was over 2,000 miles. The exact time that lapsed from capture to arrival is unknown but it is likely that these animals relied on their fat stores for energy during the majority of this process. Their liver sizes were unknown at time of capture, so livers may have been depleted in the wild. Regardless of nutritional status prior to capture, the transport and anorexia likely contributed to their negative metabolic states. This study only confirmed that they arrived with relatively small livers.

Within 1 month of introducing the recently wild-caught group to the touch pool, the amount of food provided to the exhibit was 6 kg daily. This food was given in addition to the amount provided by the public. There were no stingrays lost during this transition and they were re-evaluated after 8 months. Unexpectedly, there was an inverse relationship with liver size percentages between groups after 8 months. The wild-caught group's median liver size percentage was significantly higher than the acclimated group's median liver percentage. One explanation for the decreased liver size in the acclimated group is competition. The acclimated group may have become accustomed to daily feedings whereas the wild-caught group was accustomed to foraging in the wild and therefore capitalized on the opportunity. One study conducted in Grand Cayman southern stingrays found that there were behavior changes between tourist sites and non-tourist sites [Semeniuk and Rothley, 2008]. The stingrays in the tourist sites appeared to display more aggressive competitive behaviors and exhibited more injuries compared to stingrays in non-tourist sites [Semeniuk and Rothley, 2008]. The stingrays in this study may have had similar behavioral differences when competing for food although the stingrays in the recently wild-caught group were seemingly the more aggressive feeders. One year after introduction, an informal examination of 10 randomly selected stingrays from the total collection in this study (six were from the wild-caught group and four from the previously acclimated group) showed all liver percentages between 90% and 100% (Table 2). This was an indication that despite the presumed initial competition, the stingrays in this collection were adapting.

Another possible explanation for the significant difference in liver size percentages is that the acclimated group is overconditioned or overfed. It is difficult to accurately measure individual stingrays' intake due to the design of the touch pool exhibit with public interaction. The amount of feed purchased by the public may be tracked;

however, this is still not an accurate account of how much each stingray is ingesting. There are seasonal changes in attendance and routine daily feed is adjusted accordingly. The length of the liver was the only measurement considered in this study, but taking depth into consideration may provide a better overall anatomical size of the liver in future studies.

Based on the wingspan measurements of the wild-caught group, it is suspected that these stingrays were younger. The median wingspan difference between groups at introduction and 8 months later was 21 cm and 20.5 cm, respectively. These differences were significant ($P < 0.001$). Both groups increased in size similarly over the 8-month cohabitation period with median wingspan differences of 9.5 cm in the acclimated group and 10 cm in the wild-caught group. The wild-caught group was significantly smaller which may imply that these stingrays were not yet sexually mature and therefore the nutrient demand during folliculogenesis was absent. Vitellogenic precursors originate from the liver [Hamlett and Koob, 1999; Hamlett et al., 2005]. A decreased amount of lipid in the liver may possibly contribute to small follicles. This correlation between liver size and follicle size has been shown in other elasmobranchs [Walker, 2005]. Follicle size was not recorded during the examinations due to difficultly visualizing them in many of the stingrays within the wild-caught group. Since they were not likely undergoing folliculogenesis, there was a decreased demand from the liver's lipid stores for this process. This provided stored energy for other metabolic needs during transport, which may have aided with their successful transition into captivity.

Animals that have undergone a potentially stressful event, such as transport, and that are anorexic may be in a vulnerable condition that possibly predisposes them to opportunistic pathogens or other immunosuppressive diseases. It is important to quarantine, examine, and provide additional nutritional support through the capture and transport transition. Ultrasound is a noninvasive approach for evaluating the liver-to-coelom ratio and hepatic echogenicity in recently captured elasmobranchs. This technique can be easily incorporated into the routine physical examination and will provide insight into the nutritional status of the animals. Routine examinations with established collections are also necessary to gain more accurate health assessments. Stingrays in these types of exhibits are often difficult to monitor feedings and many of the animals may appear to be eating when actually they are mouthing or playing with the food. Further investigation is necessary to evaluate the dynamics and physiology of the elasmobranch liver during different metabolic states.

CONCLUSIONS

1. The ultrasound serves as a useful tool in approximating the relative length of the liver when compared to the cartilaginous borders of the coelomic cavity.
2. The ultrasound-guided technique for establishing a liver-to-coelom percentage showed a significant difference between relative liver size percentages among stingrays recently wild-caught compared to stingrays that were acclimated to captivity.
3. Further studies are needed to determine the liver-to-coelom percentage at which intervention is necessary and to better understand the dynamics of the elasmobranch liver.

ACKNOWLEDGMENTS

We would like to thank the Downtown Aquarium at Denver for their contribution and support with this study.

REFERENCES

American Elasmobranch Society [AES]. 2008. AES international captive elasmobranch census. Available at: http://elasmo.org/census.php. Accessed 06/09/11.

Cain DK, Harms CA, Segars A. 2004. Plasma biochemistry reference values of wild-caught southern stingrays (*Dasyatis americana*). J Zoo Wildlife Med 35:471–476.

Cebra CK, Garry FB, Getzy DM, Fettman MJ. 1997. Hepatic lipidosis in anorectic, lactating Holstein cattle: a retrospective study of serum biochemical abnormalities. J Vet Int Med 11:231–237.

Chapman DD, Corcoran MJ, Harvey GM, Malan S, Shivji MS. 2003. Mating behavior of southern stingrays, *Dasyatis americana* (*Dasyatidae*). Environ Biol Fishes 68:241–245.

Cooper BJ. 2002. Disease at the cellular level. In: Slauson DO, Cooper BJ, editors. Mechanisms of disease: a textbook of comparative general pathology. St. Louis (MO): Mosby Inc. p 16–75.

d'Anjou MA. 2008. Liver. In: Penninck D, d'Anjou MA, editors. Atlas of small animal ultrasonography. Ames (IA): Blackwell Publishing. p 217–262.

Firchau B, Pryor W, Correia JP. 2004. Census of elasmobranchs in public aquariums. In: Smith M, Warmolts D, Thoney D, Hueter R, editors. The elasmobranch husbandry manual: captive care of sharks, rays, and their relatives. Columbus (OH): Ohio Biological Survey, Inc. p 515–519.

Hamlett WC, Koob TJ. 1999. Female reproductive system. In: Hamlett WC, editor. Sharks, skates, and rays: the biology of elasmobranch fishes. Baltimore (MD): The John Hopkins University Press. p 398–443.

Hamlett WC, Jones CJP, Paulesu LR. 2005. Placentatrophy in sharks. In: Hamlett WC, editor. Reproductive biology and phylogeny of chondrichthyes shark, batoids and chimaeras. Enfield (NH): Science Publishers, Inc. p 463–502.

Holmgren S, Nilsson S. 1999. Digestive system. In: Hamlett WC, editor. Sharks, skates, and rays: the biology of elasmobranch fishes. Baltimore (MD): The John Hopkins University Press. p 144–173.

Jeffery KR, Wandersee JH. 1996. Visitor understanding of interactive exhibits: a study of family groups in a public aquarium. Louisiana State University: research report. 14 pages.

Koob TJ. 2004. Elasmobranchs in the public aquarium: 1860–1930. In: Smith M, Warmolts D, Thoney D, Hueter R, editors. The Elasmobranch Husbandry Manual: captive care of sharks, rays, and their relatives. Columbus (OH): Ohio Biological Survey, Inc. p 1–14.

Lytle JS, Lytle TF. 1994. Fatty acid and cholesterol content of sharks and rays. J Food Composition Analysis 7:110–118.

Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, Fryden A, Bodemar G. 2002. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Digestive and liver disease: official of the Italian Society of Gastroenterology and the Italian Association for the study of the liver 34:516–522.

Mohan PJ, Aiken A. 2004. Water quality and life support systems for elasmobranch exhibits. In: Smith M, Warmolts D, Thoney D, Hueter R, editors. The elasmobranch husbandry manual: captive care of sharks, rays, and their relatives. Columbus (OH): Ohio Biological Survey, Inc. p 69–88.

Nyland TG, Park RD. 1983. Hepatic ultrasonography in the dog. Vet Radiol 24:74–84.

Nyland TG, Mattoon JS, Herrgesell EJ, Wisner ER. 2002. Liver. In: Nyland TG, Mattoon JS, editors. Small animal diagnostic ultrasound. Philadelphia (PA): W.B. Saunders Company. p 93–127.

Rossouw GJ. 1987. Function of the liver and hepatic lipids of the lesser sand shark, *Rhinobatos annulatus* (Muller and Henle). Comp Biochem Physiol 86B:785–791.

Semeniuk CAD, Rothley KD. 2008. Costs of group-living for a normally solitary forager: effects of provisioning tourism on southern stingrays *Dasyatis americana*. Marine Ecol Progress Ser 357:271–282.

Semeniuk CAD, Speer-Roesch B, Rothley KD. 2007. Using fatty-acid profile analysis as an ecologic indicator in the management of tourist impacts on marine wildlife: a case of stingray-feeding in the Caribbean. Environ Manage 40:665–677.

Stetter MD. 2004. Diagnostic imaging of elasmobranchs. In: Smith M, Warmolts D, Thoney D, Hueter R, editors. The elasmobranch husbandry manual: captive care of sharks, rays, and their relatives. Columbus (OH): Ohio Biological Survey, Inc. p 297–306.

Walker TI. 2005. Reproduction in fisheries science. In: Hamlett WC, editor. Reproductive biology and phylogeny of chondrichthyes shark, batoids and chimaeras. Enfield (NH): Science Publishers, Inc. p 81–127.

Walsh MT, Pipers FS, Brendemuehl CA, Murru FL. 1993. Ultrasonography as a diagnostic tool in shark species. Vet Radiol Ultrasound 34: 213–218.

Zammit VA, Newsholme EA. 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem J 184:313–322.