### NORTH PACIFIC RESEARCH BOARD FINAL REPORT

# SUPPLEMENTAL FUNDING: BIOENERGETICS, MOVEMENT BEHAVIOR AND TEMPERATURE RANGES OF THE POORLY UNDERSTOOD PACIFIC SLEEPER SHARK

NPRB PROJECT 2004

Dr. Markus Horning (Lead PI) Alaska SeaLife Center 907-224-6324 markush@alaskasealife.org

Dr. Amy Bishop (Co-Investigator) Alaska SeaLife Center 907-224-6331 amybi@alaskasealife.org

#### **Dr. Christopher Lowe (Collaborator)**

California State University Long Beach 562-985-4918 clowe@csulb.edu

#### Mr. Jared Guthridge (Participant) Alaska SeaLife Center 907-224-6886 jaredg@alaskasealife.org

# Ms. Taylor Smith (Graduate Student)

California State University Long Beach <u>tsmith</u> yhs@hotmail.com

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## ABSTRACT

The Pacific sleeper shark (PSS) is a large subpolar and deep-water temperate apex marine predator which is of little economic value but frequently caught as bycatch in commercial fisheries. Given their likely longevity, unknown abundance, energetic demands, movement and foraging behavior, it is likely that PSS play a key role in arctic marine ecosystem structures that has been completely ignored. Through funding from the North Pacific Research Board (R1711), in 2018 and 2019 we successfully pioneered research on PSS behavior and physiology: we captured 23 immature sharks and equipped 10 with acoustic and satellite transmitters to begin to determine movement patterns and temperature preferences, and successfully transported two small sharks (< 2.5m TL) and maintained them in temporary captivity at the Alaska SeaLife Center, prior to release. From these animals, we obtained measurements of temperature dependent metabolic rates using a closed annular respirometer, the first such data ever obtained from a large, cold water fish species. Under this continuation award, we captured, sampled and released an additional 11 sharks. A 12<sup>th</sup> shark was captured and transported to the ASLC, and two respirometry sessions were conducted. However, this shark rapidly declined and was euthanized after two weeks in residence at the ASLC.

Blood and tissues were sampled and are being used to investigate foraging ecology and relative body condition. Field movement and associated environmental data will be correlated with estimated metabolic costs at associated temperatures to make predictions of how movement and feeding behaviors may be altered under changing temperature scenarios.

# **KEY WORDS**

bioenergetics, digestive efficiency, preferred temperatures, Q<sub>10</sub>, Routine Metabolic Rate, Temperature coefficient, temporary captivity

# CITATION

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### CHRONOLOGY

This project was initiated in 2017, and grew out of prior NPRB supported studies (projects 1011 and 1310) using life-long, satellite-linked vital rate telemetry implants (LHX tags) to determine survival and causes of mortality in juvenile western stock Steller sea lions in the Gulf of Alaska, from 2005 through 2012. Under project 1711 and starting in 2016, we began preparations for enabling captures, transportation and holding of sharks in Resurrection Bay and at the Alaska SeaLife Center. This included the custom design and fabrication of the shark Transport and Experimental Container (TEC) by a local company (Raibow Fiberglass in Seward, AK), and the fabrication of a custom lifting sling/stretcher (Sam's Custom Sewing in Seward, AK). This also included purveying of oxygen sampling equipment (Fibox by Presens) and satellite pop-up tags and acoustic tags for sharks (Wildlife Computers and Vemco/Innovasea). Based on reports by local fishermen, we anticipated and prepared for the capture and transport of mostly small sharks (<2m TL) in Resurrection Bay. We began capture operations in May of 2018, and captured our first shark on May 30th, 2018. This shark, and the next 8 sharks we caught in 2018, all exceeded the anticipated maximum length of 2m. Therefore, for 2019 we prepared for the possibility of transporting larger sharks up to 2.5m TL. In 2019 capture operations resumed and we caught an additional 14 sharks, including three that were small enough to transport back to ASLC. However, only two were transported back, since the third was captured during a period of residency of one of the other sufficiently small sharks, and planned operations called for initially not attempting simultaneous housing of multiple sharks at the ASLC. The second 'transient' shark was released back into Resurrection Bay on August 6th, 2019. The last shark under that initial effort was caught on Sept. 16th, 2019. The capture operations extending into September of 2019 meant that satellite pop-up tags attached to sharks that were set to release up to 180 days after release would detach and begin to transmit as late as early 2020. Receipt of satellite tracking and analysis of respirometry data continued through mid-2020. Therefore, we requested and were granted a no-cost extension of the award through March 2020. Overall, all goals originally proposed for R1711 were accomplished within the slightly extended time frame, though our sample size for respirometry on transient sharks remained low at n=2. We therefore requested supplemental funding to permit one more season of captures and potential respirometry trials on transient sharks.

This supplemental funding request resulted in R2004 – and allowed us to add one more field season to the shark capture efforts, in 2020. In 2020, we added 29 additional catch effort days between July 10 and September 20, bringing the total over all three years (2018, 2019, 2020) to 79 days or 316 sets. We captured 12 additional sharks in 2020, including one that was brought back to the ASLC for respirometry trials. However, this animal had to be euthanized after two weeks.

# **INTRODUCTION**

The Pacific sleeper shark (*Somniosus pacificus*, PSS) is distributed from the North Pacific past the Arctic Circle into the Bering Sea (Bright, 1959; Orlov, 1999; Orlov and Moiseev, 1999a; 1999b; Benz et al., 2004; Hulbert et al., 2006; Courtney and Sigler, 2007; Tribuzio et al., 2010). PSS are slow growing and long-lived (Ebert et al., 1987). Females reach maturity at 3.7m, males at 4m Total Length (TL) (Yano et al., 2007). Photographic estimates suggest adults may exceed 7m TL (Hart, 1973; Compagno, 1984). PSS are aplacental viviparious and may produce 200– 300 offspring per brood (Gotshall and Jow 1965). PSS range widely in the water column, influenced by latitude and season (Orlov and Baitalyuk 2014). By-catch data from 1960–2008 shows that PSS were commonly captured in 200–700 m water in high latitudes and down to 3000 m in temperate latitudes (Yano et al., 2007; Tribuzio et al., 2010, Orlov and Baitalyuk 2014). Hulbert et al. (2006) documented continuous daily vertical movements, migration to shallow waters predominantly during the night and to greater depths during the day (Hulbert et al. 2006). Individuals spent ~60% of time between 150 – 450m but ranged 0m to >700 m (Hulbert et al. 2006), traversing a range of temperatures.

Species distinction and range overlap to the North Atlantic Greenland shark (*Somniosus microcephalus*, GLS) remain unclear. PSS and a possible hybrid were reported from Greenland (J. Christiansen, pers. comm.). A GLS study suggests extreme longevity to 392 years (+/- 120), and age at sexual maturity >150 years (Nielsen et al. 2016). This and decades of sustained harvest levels through targeted fisheries and bycatch from 25,000 to 100,000 individuals per year without declines in effort-corrected catch rates (MacNeil et al. 2012; Lydersen et al. 2016) suggest possibly staggering abundance for the GLS in the North Atlantic and Arctic Ocean. A study using eDNA supports much higher GLS abundance than estimates from trawls (Thomsen et al. 2016).

Few abundance estimates exist for PSS. They are consistently caught in NMFS trawl surveys on the eastern Bering Sea shelf and slope, and along the Aleutian Islands, with combined biomass estimates from 2,000 to 30,000 mt (Tribuzio et al., 2010). PSS average annual bycatch estimates for these regions are 416 mt, accounting for 60% of total shark bycatch in the BSAI region (Tribuzio et al., 2010). Courtney and Sigler (2007) reported increasing effort-corrected bycatch estimates from sablefish longline surveys between 1988 and 1994 in the eastern Bering Sea, and between 1989 and 2003 in the Gulf of Alaska, with a drop in 1997 (driven by catch data from the Shelikof Trough near Kodiak Island). Furthermore, long-line fisheries are biased by favoring smaller and younger sharks (Orlov and Moiseev, 1999b; Yano et al., 2007) and may not reflect abundance of mature adults (Tribuzio et al., 2009).

PSS are managed as a Tier 6 shark stock complex in Alaska due to lack of data for reliable biomass estimates. This complex is segregated into Gulf of Alaska (GOA), Bering Sea and Aleutian Islands (BSAI). In GOA, the estimates of total PSS bycatch ranged between 100 - 500 mt. Bycatch peaked in 2005 and steadily declined to ~200 mt in 2013. Total estimates of PSS bycatch in BSIA declined from about 400 mt in 2003 to less than 100 mt in 2013 (Tribuzio et al. 2014). In addition to bycatch mortality from long-lining, pelagic trawling and to a lesser extent non-pelagic trawling, PSS incur mortalities from killer whales as predators (Ford et al., 2011).

PSS have broad dietary habits including benthic and mid-water crustaceans, cephalopods, salmonids, gadids, flatfish, and multiple marine mammals (Bright, 1959; Compagno, 1984; Orlov, 1999; Orlov and Moiseev, 1999a; 1999b; Sigler et al., 2006; Yano et al., 2007). For mammal prey, questions persist about scavenging versus predation. Histological analysis of genetically identified stomach contents have

demonstrated PSS as scavengers of cetaceans and phocids (Sigler et al., 2006). PSS are capable of preving on live, fast-swimming prey such as salmonids and scombrids (Ebert et al., 1987). GLS have been shown to prey on live pinnipeds (Fisk et al., 2002; Leclerc et al., 2012) though these may be attacks on sleeping seals (Watanabe et al., 2012). Southern sleeper sharks (S. antarcticus) were reported to attack live southern elephant seals (Van Den Hoff and Morrice, 2008). PSS has been reported as a predator of Steller sea lions by Loughlin and York (1999, without source). Due to the decline of the western Steller sea lion (Loughlin et al., 1992) and potential habitat overlap, possible PSS predation on Stellers received special attention (Hulbert et al., 2006; Sigler et al., 2006). A modeling exercise by Frid et al. (2009) explained sea lion dive behavior through predation avoidance from transient killer whales and PSS. Model output was supported by telemetered sea lion dive behavior data from Prince William Sound. Sigler et al. (2006) found harbor seal and grey whale remains in stomachs of 198 small PSS (median pre-caudal lengths < 2.2m) caught by long-lining near Steller sea lion rookeries in the GOA, but no remains of Steller sea lions were found. However, this could be a result of the size selection-bias of long-lining and ontogenetic diet shifts in sharks including S. microcephalus, antarcticus and pacificus (Orlov and Moiseev, 1999b; Fisk et al., 2002; Yano et al., 2007; Courtney and Foy, 2012). Recently, we presented indirect evidence of S. pacificus predation on live Steller sea lions in the GOA, from implanted vital rate telemeters (Horning & Mellish 2014). However, the degree to which PSS prey on Stellers remains unclear and the impact of shark predation pressure on Stellers may be greater than previously known.

Given uncertainties in the genetic relationship between *S. microcephalus* and *S. pacificus*, knowledge deficit on stock structure, longevity, growth rate, age at sexual maturity, on their reproductive rate and trophic positioning, as well as abundance, migratory movements and distribution, it is conceivable and even likely that we are looking at a genus with a key role in arctic marine ecosystem structures that has been essentially completely ignored (McMeans et al. 2013, Lydersen et al. 2016).

### **OBJECTIVES**

(1) Develop the capacity to bring wild immature Pacific sleeper sharks (<2m TL) into temporary captivity at the ASLC for controlled experiments.

In 2018-2019, we captured 23 immature Pacific sleeper sharks in Resurrection Bay, AK. We successfully transported two small sharks (< 2.5m TL) and maintained them in temporary captivity at the Alaska SeaLife Center for a period of 2 weeks. In 2020, we captured an additional 12 immature sharks in Resurrection Bay. One small shark (1.45m TL) was transported to the ASLC for respirometry measurements. This shark however was nutritionally and physiologically compromised and was euthanized after two weeks [Chapter 1].

(2) Determine the Routine Metabolic Rate (RMR) and if possible standard metabolic rate (SMR) for immature Pacific sleeper sharks.

*RMR* and *SMR* were calculated from n=2 individuals in 2019 and we have added data from one additional shark in 2020. However, this animal was nutritionally and physiologically compromised, and respirometry measurements from this shark are therefore presented separately as standalone measurements from a compromised animal **[Chapter 2]**.

- (3) Determine metabolic  $Q_{10}$ , the temperature coefficient of the RMR/SMR  $Q_{10}$  temperature coefficient of RMR/SMR was calculated from n=2 individuals [Chapter 2].
- (4) Examine changes to RMR/SMR (metabolic Q<sub>10</sub>) following a period of acclimation at a different water temperature than at capture location.

The metabolic  $Q_{10}$  for n=2 sleeper shark individuals was estimated utilizing the difference in routine metabolic rate, but limited to a 1-2°C temperature change [Chapter 2]. While this is not the best temperature range for measuring metabolic Q10, this narrow range was used to minimize stress to animals being studied for the first time

(5) Collect tissue samples to characterize basic hematological and metabolite values for immature sleeper sharks, at onset of captivity, and prior to release.

Blood samples were collected from n=11 sharks in the field, and from the n=3 sharks that were brought into temporary captivity. Hematological and metabolite values were compared between intake and pre-release to evaluate changes in physiological state during captivity [Chapter 1].

(6) Archive tissue samples for subsequent comparative assessment of trophic positioning via stable isotope ratios.

Skin, blood, and muscle samples were collected from sleeper shark individuals and are archived at the Alaska SeaLife Center and/or California State University Long Beach. Muscle samples were collected from a total of n=22 sharks for enzyme activity assays. These assays have been completed, but data analysis on assay results is continuing [Chapter 1].

(7) Determine if sharks will consume live prey or carrion offered in temporary captivity.

Sharks in temporary captivity were offered carrion prey (e.g. salmon, squid) in 2019 but no ingestion was observed during the two-week period that each of the two sharks was in residency at the ASLC in 2019. One compromised shark in residency at the ASLC in 2020 was offered prey but did not ingest. This animal was also tube fed whole prey and fish gruel, which however it was unable to process or assimilate. Most of the fed mass was not retained. This animal was euthanized after two weeks, and post-mortem necropsy revealed it was severely nutritionally compromised, and with an atrophied liver likely unable to digest any food [Chapter 1].

(8) Collect long-term ambient temperature, movement and behavioral data on up to 10 sharks through a combination of acoustic tracking pingers and pop-up archival transmitters (PAT tags).

Pop-up archival transmitters and coded acoustic transmitters were attached to n=10 sleeper shark individuals and recorded broad movement patterns and temperature preferences [Chapter 1].

(9) Compare ambient temperature ranges and vertical movement behavior for sharks tracked during the cold season (Jan to Mar) and the warm season (Aug to Oct).

Post-release ambient temperature ranges and vertical movement behavior will be compared between seasons – this analysis will be integrated into the new NPRB-funded sleeper shark project R2010. As of the date of our previous final report, data from 2 tags was still outstanding. Data from one more tag was recovered after that, but no data returns were obtained from the final tag.

(10) Compare movement behavior and temperature ranges between animals that experienced temporary captivity and wild controls

Post-release ambient temperature ranges and vertical movement behavior will be compared between temporarily captive animals, and non-captive controls from 2018 and 2019, as well as from initial returns of new tag deployments in 2021 under new project R2010, which will allow a much larger sample size for non-captive controls.

# Chapter 1 Capture, Transport and Maintenance of Pacific Sleeper sharks in captivity

Horning M, Lowe CG, Guthridge J, Hocking R, Bishop A, Smith T

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# INTRODUCTION

This use of temporary captivity under controlled conditions has been pioneered once before at the Alaska SeaLife Center (Mellish et al. 2006) and was highly successful. Over more than a decade, more than 75 individual juvenile western Steller sea lions, an endangered species were brought to the ASLC, held in temporary captivity, and released. This has led to or enabled more than 25 important new publications, including the very finding of potential sleeper shark predation on Steller sea lions that brought us to design this project plan.

Designing and implementing methodology for transporting and maintaining *Somniosus pacificus* in temporary captivity enables measures of temperature-dependent metabolic rates, feeding behavior, metabolomics, proteomic and genomics assessments in a controlled environment that could potentially be manipulated. Past programs have attempted this with limited to poor success; therefore, we adopted an approach that sought to minimize or eliminate the most likely causes of trauma associated with transport and captivity.

# **METHODS**

# Transport and Experimental Chamber



**Figure 1.1:** The shark Transport and Experimental Container (TEC). Left panel shows the TEC with lid partially removed and a shark inside TEC. Lid fittings are used for evacuating air and flushing. Right panel shows TEC in operation with fully sealed lid and external recirculation plumbing using submerged pumps. TEC is shown during a flush cycle with water vented through lid fitting. Wires on pool side lead to DC power supply for pumps, oxygen and temperature sensors as well as infrared video monitors.

A transport and experimental chamber (TEC) was constructed for this project (Figure 1.1). The TEC system of tub with removable, sealed lid comprises fittings for external closed-circuit adjustable recirculation and

temperature conditioning, as well as intermittent flushing. Coverable viewports and two internal infrared cameras allow monitoring of animal state and behavior. Flow rates are comparable to the slow movement speeds of PSS (<=10 cm/s, see Watanabe et al. 2012 for GLS). The system can also be operated as an open flow-through system for flushing and re-oxygenation. The round bottom TEC can also be partially or fully drained to serve as a shark cradle and allow controlled access to animals for tissue sampling, and for pre-release attachment of telemetry devices

# Fishing Effort

Fishing was conducted in Resurrection Bay, Alaska over 19 effort-days from May through September in 2018, and over 33 effort days from June to September in 2019 and over 29 effort-days from July to September in 2020 (Figure 1.2). Initial attempts utilized rod and reel fishing effort, but did not result in any shark captures. Between June and September 2018, and for all effort in 2019 and 2020, we then deployed 4 sets of capture gear for 4-hour daytime soaks. Sets consisted of two 16, 18 or 20 gauge circle hooks connected via 1-2 m gangions and longline clips to the bottom of a line connecting an anchor to a buoy. Floats above gangions elevated the hooks off the bottom. Hooks were baited with salmon, halibut, sablefish or skate carcass parts, and placed between 150 and 300 m depth.



Figure 1.2 (left): Map of 2020 fishing effort. The Seward Harbor is on the upper (northern) limit of Resurrection Bay. Blue circles represent fishing effort locations, green circles represent shark captures.

Sharks caught that were > 2.5 m TL (n=32) were sampled and processed at the surface from the research vessel. Sharks caught that were < 2.5 m TL (n=3) were transferred into the 1,750 liter fiberglass TEC partially filled with seawater, placed on the deck of a 7 m landing craft. Transfer was done in a custom-designed conformal sling, upon removal of injury-minimizing circle hooks at the surface. Minimizing removal from water during transport, and all other severe manipulations, helps eliminate trauma and addresses the primary reason why past efforts by others to bring PSS/GLS into captivity have not been successful. The vessel then transported the container with shark to the ASLC (Figure 1.3).



**Figure 1.3:** Transferring the shark from the water into the partially filled TEC aboard the research vessel R/V *Jubatus* (left) and transporting the TEC and shark to the harbor (right) minimized time spent out of the water.

The time between the shark appearing at the surface and release ranged from 9-69 min in the field (mean = 34 min). For all sharks (\*except one shark that came off the hook at the surface prior to assessment), morphometrics, sex, and general condition were recorded. Skin samples were collected and frozen/archived for future genetic and stable isotope analysis (n=30). Muscle samples were collected from the epaxial muscle via appropriate sterile biopsy large-bore needles (n=22). Blood samples (10 - 30 mL) were collected via caudal venipuncture (n=13). Sharks that were brought to the ASLC were weighed once during captivity. Mass was estimated for all other sharks following Nielsen et al. (2014)'s regression between body mass ~ TL.

### ASLC captive facility and shark husbandry

At the ASLC, each shark was removed from the TEC via sling into the largest outdoor holding tank at the center, ODL8, which has two levels and can skim at each level. It is 2 m deep at the shallow end and 3 m at the deep end and is a 16 m x 9 m oval. It holds 282,588 liters of water with a surface area of 130 m<sup>2</sup>. The tank is supplied with water from a mid-water intake in Resurrection Bay. The temperature within ODL8 remained within 1°C of the temperature at which the sharks were caught (~6°C). A removable floating pool cover was deployed to reduce surface light intensity. Sharks were offered carrion prey (e.g. salmon, squid).

Behavior (e.g. swimming patterns, activity) and health of the shark (e.g. abrasions, hematological and metabolite values) were monitored throughout the duration of the captivity. During captivity, additional blood samples (5ml) were collected via caudal venipuncture following experimental testing and prior to release. While it would be of interest to attempt to quantify stress from manipulation and captivity in our subjects, studies have shown that even within elasmobranchs, responses to manipulation in multiple parameters considered (including plasma electrolytes, glucose, lactate, and hematocrit) vary widely (Marshall et al. 2012). Therefore, our initial approach to this question was descriptive, comparing these

metrics across the period of captivity and different sampling and experimental events. Blood chemistry was conducted in-house at the ASLC. Hematology was sent to an external lab for analysis (National Aquarium) in 2018, with 2019 samples pending. Additionally, a small sample of blood was processed in-field immediately after draw with an i-STAT (CG8+).

# Experimental conditions

For duration of experimental determination of the metabolic rate [Chapter 2], sharks were confined to the TEC. After in-water transfer to TEC, water circulation, conditioning and monitoring equipment was attached to the TEC, with the TEC partially submerged to improve temperature control. Displacement volume of the shark is needed for mass-specific metabolic rate measurements (volume of TEC (1,700L) - volume of water with shark in TEC). For both transient sharks caught in 2019, the TEC volume was reduced through addition of solid displacement modules, to improve resolution of oxygen consumption rates (Svendsen et al. 2016a). Shark displacement volume was measured at the end of an experiment using a flow meter. The transient shark caught in 2020 was even smaller, and additional displacement modules had to be built. Even so, there was a greater mis-match between shark and TEC volume for SP20-05 in 2020, and this also resulted in the shark being able to move around and turn around much more liberally within the TEC.

# Release and Post-release tracking

Two of the three captive animals (SP19-04 and SP19-08) were each transported in the TEC to their capture location and released in the water. Prior to release in the field or from captivity, sharks (n=33) were outfitted with 2 identification spaghetti tags (1 nylon and 1 wire core), provided by collaborators at NOAA (C. Tribuzio, Figure 1.4).



Figure 1.4: Identification 'spaghetti tag's attached laterally along the dorsal midline

A subset of animals also received additional post-release tracking tags. Vemco (now called Innovasea) V16-3H coded 69 kHz acoustic tracking transmitters (Innovasea / Vemco Corp., Bedford, NS, Canada) were attached to sharks in 2019 only (n=10) via subdermal titanium anchors. These transmitters have a range of about 0.7 - 1.2 km. Transmitters were programmed to pulse at 30-60 sec for 2 years (>700 d). The coded 69 kHz transmitters can be detected by an acoustic receiver array already installed at 'entries' into Prince William Sound by the Ocean Tracking Network hosted by Dalhousie University.

Wildlife Computers (Redmond, WA) MiniPAT-348A towed archival satellite transmitters, secured via tether to subdermal titanium anchors were attached to captive and free-ranging sharks in 2018 and 2019 (n = 10). The MiniPAT is a pop-up archival transmitting tag (PAT tag, also known as a PSAT), a combination of archival and Argos satellite technology. PAT tags are designed to track the large-scale movements and behavior of fish and other animals which do not spend enough time at the surface to allow the use of real-time Argos satellite tags. Sensor data are collected during deployment and archived in onboard memory. Then on a preset date, the tag releases from its host animal, surfaces, and uploads a summary of the archived data to Argos satellites" (from www.wildlifecomputers.com). PATs log and later transmit data on ambient temperature, depth (pressure), and light-levels. Time series data of depth and temperature were programmed at a 10 min sampling rate, which typically allows up to 8-month long records to be recorded and subsequently transmitted.

Both sharks released from captivity received all three types of tags. One shark in captivity (SP20-05) had to be euthanized after 2 weeks.

# RESULTS

### Catch per unit effort (CPUE)

Please note that the previously reported CPUE was calculated differently, and we have updated the way we calculate CPUE here, still indicated in units of sharks caught per hook per hour. CPUE in 2018 was 0.00937 sharks/hook/hr over 19 fishing days and 0.00964 sharks/hook/hr in 2019 over 33 fishing days. In 2020, CPUE was 0.00853 sharks/hook/hr over 29 fishing days.

We used two hooks per set, and these two hooks are not independent from one another. Another common CPUE measure is animals caught per set-hour. These values of course are simply the above listed CPUE in sharks/hook/hr multiplied by two. Thus, CPUE in sharks caught per set-hour was 0.01874 in 2018, 0.01928 in 2019 and 0.01706 in 2020.

In 2018 bycatch was 6 halibut, in 2019 bycatch was 10 halibut, 3 Pacific cod, 1 spiny dogfish, 1 sablefish, and 1 longnose skate. In 2020 we had no bycatch. We fished in many different locations to determine the best area to catch PSS and in 2019 found "4th of July Beach" across from Seward, AK was the most successful location, as well as ideal for a short transport time to the ASLC. In 2020, we concentrated our fishing effort around this area (Fig. 1.2). In 2018-19, 21 sharks > 2.5 m TL were caught (mean TL = 2.8 m (range 1.6 to 3.5)). In 2019, two sharks 1.6 m and 2.0 m TL respectively were caught and transported to the ASLC for temporary captivity. In 2020, 12 sharks were caught including one small animal (TL 1.45m) that was transported to the ASLC (Table 1.1).

**Table 1.1:** Summary data for Pacific sleeper sharks captured 2018-2020 in Resurrection Bay. Sampling (skin, muscle (Mus), blood) and tag attachment are indicated as yes = 1, no = 0. The three sharks brought into temporary captivity are highlighted in light green. SP19-07 was captured and brought to the surface, but broke free before it could be sampled, measured, or have sex determined. \**mass estimated via Nielsen et al. 2014 mass to TL regression.* ^ *Precaudal length indicated when TL not available* 

Shark ID	Release	Sav	TL (cm)	Mass (kg)	Est. Mass*	Skin	Mus	Plaad	PAT	Vemco
	05/30/18	F	282	(kg)	211.6	1	nius.	1	1	1 ag
SP18-02	08/22/18	F	282		211.0	1	0	1	1	0
SP18-02	08/22/18	F	280		180.0	0	0	0	0	0
SP18-03	08/22/18	Г Г	272		139.0	1	0	1	1	0
SD18-04	00/20/18	M	239		236.1	0	0	1	0	0
SP18-06	09/20/18	M	292		256.1	1	0	0	0	0
SP18_07	09/25/18	M	205		243.7	1	0	1	0	0
SP18-07	09/25/18	M	328		339.8	1	0	0	0	0
SP18_00	00/26/18	F	250		145.1	1	0	1	0	0
SP10_01	06/05/19	F	315		299.4	1	1	0	0	0
SP10_02	06/10/10	F	354		299. <del>4</del> 431.7	1	1	1	1	1
SP10_03	06/12/10	F	208		451.7	1	1	1	1	1
SP10_04	07/15/19	M	100	84.5	71.0	1	0	1	1	1
SP19_05	07/08/19	M	239	04.5	126.0	1	1	1	1	1
SP19-05	07/24/19	F	263		120.0	1	1	1	1	1
SP19_07	07/25/19	1	205		170.1	0	0	0	0	0
SP19-07	08/06/19	М	162	40	37.2	0	0	1	1	1
SP19-09	07/31/19	F	330	-10	346.4	1	1	1	0	0
SP19-10	07/31/19	F	300		256.9	1	0	0	0	0
SP19-11	07/31/19	M	280		207.0	1	1	0	0	0
SP19-12	07/31/19	F	280		207.0	1	1	0	0	0
SP19-13	09/05/19	F	288		207.0	1	1	1	1	1
SP19-14	09/16/19	M	300		256.9	1	1	0	1	1
SP20-01	07/10/20	F	274		193.4	1	1	0	0	0
SP20-02	07/11/20	F	336		364.8	0	1	1	0	0
SP20-03	07/15/20	M	259^		501.0	1	1	0	0	0
SP20-04	07/29/20	F	235			1	1	0	0	0
SP20-05	08/02/20	F	145	21	26.3	1	1	1	-	-
SP20-06	08/02/20	M	289	21	228.5	1	1	0	0	0
SP20-07	08/02/20	F	333		356.4	1	1	ů 0	0 0	Ő
SP20-08	08/21/20	F	278		202.4	1	1	0 0	Ũ	0 0
SP20-09	08/27/20	F	351		420.3	1	1	0	0 0	Ő
SP20-10	08/27/20	F	309		281.9	1	1	0	0 0	Ő
SP20-11	09/04/20	F	313		293.5	1	1	0	Õ	0 0
SP20-12	09/04/20	М	253		150.6	1	1	0	0	0

### Overview of transport and captive husbandry for controlled access experiments

SP19-04 and SP19-08 were individually maintained for 14 and 12 days, respectively at the ASLC (Figure 1.5a,b). Although neither individual ate during their period of captivity, both seemed to be in good condition and there were no apparent behavioral responses of subjects during any stage of manipulation or during temporary captivity. Both sharks did present with some minor abrasions that increased in size or number throughout the duration of their captivity (Figure 1.5c,d). Results of blood analysis indicated that the hematocrit for SP19-04 during captivity increased from 18% at time of capture to 24% after second attempt at a respirometry run (4 days later); however, decreased again to 20% prior to release. Similarly, for SP19-08, the hematocrit increased from 15% at time of capture to 23% prior to the first respiratory run (2 days later) and decreased to 19% after the run. The mean hematocrit values for sharks caught in the field and released was 17.5% (n=7).



**Figure 1.5: Behavioral and health observations for Pacific sleeper sharks in temporary captivity** (a) SP19-04 swimming near the surface in ODL at the Alaska SeaLife Center. We routinely observed the shark alternating between swimming at depth and at the surface. (b) the pool cover over ODL8 to provide shading and darkness, (c) minor abrasions were noted on the dorsal midline and (d) trailing edge of the dorsal and caudal fins.

#### SP20-05 case report

In 2020, the smallest shark we caught in the three years of the project at 145cm TL was captured and transported to the ASLC, on Aug 2nd. The animal was initially maintained like the previous two transient sharks, in ODL8. Two respirometry sessions were conducted, and during the second session one eye of the shark was injured by suction from a water outlet port. This was likely a result of the greater mismatch between shark size and non-displaced TEC volume, which allowed the shark to more freely move and turn around within the TEC. The shark was treated successfully and returned to ODL8. However, over the course of the next few days, the shark became increasingly lethargic with minimal movements, while often swimming in a head-up, more vertical orientation. The shark was offered food which was not consumed. Due to the visible decline in activity, response to stimuli, and body condition, the shark was intubated and fed whole fish, and when these were not retained it was fed fish gruel. Other veterinary measures followed (see attached report). Blood collected at intake had a PCV of only 10%. Subsequently, blood glucose values in archived intake samples were measured at 28 mg/dl. After one week, PCV had increased slightly to 11%, but blood glucose had dropped to below 10 mg/dl. Following IV administration of fluids with dextrose, blood glucose levels rose to 24 mg/dl, but then dropped again below 10. PCV dropped to 4%. The shark continued to decline so rapidly that the decision was made to euthanize the animal on 8/16/20. The subsequent necropsy revealed an advanced stage of emaciation with likely a shut-down alimentary tract. The liver was measured at just under 5% of the total body mass of 21kg, when in healthy sharks it is thought to comprise 25 to 35% of body mass. This indicates that likely the animal was already severely nutritionally compromised at intake.

The respirometry data from this shark will still be analyzed and published with mention of the known limitations of these data originating from a severely compromised individual.

A detailed veterinary case report prepared by Ashley Kirby, DVM, as well as a pathology report prepared by Alvin Camus, Veterinary Pathologist, are attached as an appendix to this project report.

### Post-release tracking: survival

Post-release tracking for seven of the sharks, including the first shark brought into temporary captivity (SP19-04) concluded with programmed release of the mini-PAT after 90/180 days. Sharks dispersed widely around the Gulf of Alaska/Prince William Sound (Figure 1.7). Three of miniPAT tags detached prematurely. The telemetry tag for the second shark (SP19-08) detached prematurely after 116 d, and data from this tag suggests premature failure of the subdermal skin anchor rather than mortality caused by our treatment or fishing-related mortality. Tags for SP18-01 and SP19-01 both detached after 15 d, the former of which was attributed to predation by offshore killer whales [Case Report: Killer whale predation] while the latter is inconclusive.

#### Post-release tracking: behavior and environment

Post-release ambient temperature ranges and vertical movement behavior data have been received from 9 of 10 deployed mini-PATs, one mini-PAT never reported for undetermined reasons. These data will be compared between temporarily captive animals, and non-captive controls from 2018 and 2019, and future deployments under a new project. They will also be compared between the cold and warm seasons when additional data from new deployments will be received in 2022 (Objectives 9 & 10).



**Figure 1.6:** Map of locations where 10 tagged Pacific sleeper sharks were released in Resurrection Bay(upper left insert); where 8 of these tags popped up in the Gulf of Alaska/Prince William Sound (main panel), and in Resurrection Bay (lower right insert). Corresponding locations for individual sharks are coded in the same color.

# DISCUSSION

We demonstrated the viability of our new approach for capture, transport and maintenance of Pacific sleeper sharks in temporary captivity for controlled access experiments. However, we also encountered issues that highlight the challenges associated with captive maintenance and husbandry of an understudied, poorly understood species. Across three summers, we captured 35 immature Pacific sleeper sharks. Based on our average CPUE values across the three years, we found the best time of year to fish for PSS is May and September. The best time of year to fish for small PSS, however, was in July. These findings can facilitate future efforts to understand free-ranging PSS behavior and efforts that require access to small size-class individuals for controlled access studies.

Quantifying metabolic rates, temperature coefficients, plasticity and adaptability, feeding behavior, and digestive processes all require access, ideally under highly controlled conditions. In 2019, we successfully transported two small sharks (< 2.5m TL) and maintained them in temporary captivity at the Alaska SeaLife Center prior to release. This effort directly enabled controlled access and allowed us to obtain measurements of temperature dependent metabolic rates in these two sharks using a closed annular respirometer, the first

such data ever obtained from a large, cold water fish species **[Chapter 2].** There were no apparent behavioral or health responses of subjects during any stage of manipulation or during temporary captivity beyond minor abrasions. We evaluated hematocrit values for sharks sampled in the field, and samples from TS animals before, during and prior to release from captivity. There appeared to be a slight elevation after intake relative to in-field measures that subsequently went back to initial levels. While this provides some initial information on physiological responses to the transient paradigm, it is inconclusive in literature whether stress increases hematocrit levels in sharks or not.

In 2020, we brought one additional small shark into temporary residence at the ASLC. However, this animal rapidly declined and was euthanized after two weeks. Post-mortem necropsy revealed that this animal was likely nutritionally compromised at capture/intake with chronic malnutrition and likely hepatic failure, and suspected tail bone fracture – possibly from prior fishing gear interactions. This suggests that intake selection criteria should be developed and established, around field-measurable parameters that could be indicative of these potential conditions. For example, minimum values for PCV, blood glucose and blood lactate could be set. It may be possible to grossly assess an approximate liver volume through ultrasonography, or it might be possible to compare actual body mass to mass predicated for a given TL, with too great a discrepancy being a criterion for not transporting a subject.

By further developing a novel research tool in the form of controlled accessibility to wild PSS, this project may jumpstart a coordinated series of important studies. For example, while there were no observations of ingesting food during temporary captivity with the two animals in 2019, digestive passage time and digestive efficiency in PSS can be determined if the animals ingest food. We also expect this concept to extend into work with larger and older individuals. This could lead to innovative comparative studies within and between species, including ontogenetic comparisons.

As part of NPRB Project R1711 we hosted a workshop at the ASLC in 2018 with a core group of internationally recognized experts to plan subsequent studies to use this new experimental paradigm, including the international research group that recently reported on the extreme longevity in the GLS (Dr. John Steffensen). From this workshop, proposals are being developed; and the proposed supplemental field season and experimental activities will continue to enhance and facilitate collaborations that take advantage of the accessibility to PSS.

# Chapter 2 PSS Routine and Standard Metabolic Rate, and Metabolic Temperature Coefficient Q<sub>10</sub>

## Smith T, Lowe CG, Horning M, Bishop A,

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## **INTRODUCTION**

The Pacific sleeper shark (*Somniosus pacificus*, PSS) is an understudied, poorly understood cold water apex predator that could have significant effects on ecosystem structures due to possibly considerable, but unknown abundance. Amongst the tens of thousands of PSS bycaught in Alaskan groundfish, pelagic and trawl fishery, only five sexually mature females have been reported (Tribuzio et al. 2010). There is no knowledge of growth rates, longevity, reproductive rates or reproductive migrations or locations for these cryptic, difficult to observe sharks. As a result, PSS are managed as a Tier 6 species in the Alaskan Shark complex and stock trends are undetermined.

Essential data lacking for this species includes estimates of metabolic rates, food consumption rates, caloric requirements, and temperature characteristics of PSS habitat. Determination of phenotypic and physiological plasticity are of prime importance. With changing ocean climate, PSS could experience habitat shift and displacement towards higher latitudes and/or greater depths (Lynghammar et al. 2013). A latitudinal displacement may be slowed north of the Pacific – Bering Sea transition due to greater episodic mixing and complex transport mechanisms past this boundary, and further north at the Bering Strait (Lynghammar et al. 2013). Such slowing could in turn result in habitat compression, and locally increased PSS abundance along the Aleutian Island transition zone. This could have profound effects on ecosystem structures if PSS are primarily active predators rather than scavengers. Studies that characterize prey search, hunting, feeding and ingestion behavior are also of very high importance

Few published studies have measured the oxygen consumption rates of species larger than 1.5 m, and none on elasmobranchs measured at temperatures below 10°C. Conducting initial measurements of the metabolic rate of Pacific sleeper sharks can enhance deep-sea and polar physiology knowledge by filling gaps in respirometry research for large, arctic elasmobranchs.

### **METHODS**

In July 2019, two immature Pacific sleeper sharks (SP19-04: 1.6 m TL, SP19-08: 2.0 m TL) were caught in Resurrection Bay, Alaska and transported to the Alaska SeaLife Center for temporary captivity and controlled experimental access. These two animals were maintained for about two weeks before being released back into the wild. During these two weeks, multiple experimental sessions were conducted. In August of 2020, one additional immature shark (SP20-05) was caught in Resurrection Bay and transported to the ASLC. Two experimental sessions were conducted, but this animal rapidly declined and was euthanized after two weeks. \**For capture, transport and captive husbandry information, see Chapter 1* 

#### Experimental setup for respirometry experiments

Sharks spent approximately 1 day acclimating in the outdoor holding pool (ODL8) before RMR and SMR experiments were conducted. ODL8 is supplied with water from a mid-water intake in Resurrection Bay. The water temperatures may, depending on the season, vary relative to capture depth temperatures. Inflow temperature during the period of temporary captivity for both sharks was consistently  $6.8^{\circ}$ C, which was within the natural temperature range (mean capture temperature at depth from data-logger =  $6.25^{\circ}$ C). This suggested there was limited exposure to thermal shock.

On the day of a respirometry trial, sharks were transferred in-water into the TEC within ODL8. Once in the TEC, prior to a respirometry trial, the second shark, SP19-08, was fitted with a tri-axial accelerometer data logger (TechnoSmArt, Axy 4) to record periods and degree of activity during the trial. The data-logger was not available for SP19-04 or SP20-05. Once the lid was placed on the TEC, it was then filled with water from ODL8 and sealed without air space. Water was circulated, conditioned and monitored in this closed system. Circulation speed were able to be adjusted to promote mixing and provide a current to potentially induce voluntary swimming (periods of routine activity). The TEC is opaque but includes viewports that can be covered, and the TEC lid was been outfitted with an infrared video camera for monitoring of shark behavior (Figure 2.1).



**Figure 2.1:** Prior to starting a respirometry trials, T. Smith assesses the infrared camera's ability to monitor an animal in the closed TEC (left). Additional viewports that could be covered were added to the TEC (right).

During a respirometry trial, several parameters were continuously measured via in situ sensors. Dissolved oxygen concentration (mg O2/L) was recorded via a Presens Fibox 4 oxygen meter with FTC-PSt3 fibre-optic flow through sensor (Presens, Germany). pH was monitored via a Loligo WTW 3310 pH meter with Sentix HWD probe, and temperature, conductivity and salinity were monitored via a YSI 63 recording meter. Respirometry trials lasted until oxygen levels dropped by 10-20%, providing total saturation did not drop below 60% (Figure 2.2). This reduction is expected to not pose a problem for this species with a high tolerance for hypoxic and hypercapnic conditions (Bernal et al., 2012). Following the 10-20% reduction in oxygen concentration, the TEC was flushed with ambient water from ODL8 to reduce accumulation of metabolites. SP19-04 had 2 trials, 3 replications each, and SP19-08 had 3 trials, with 5, 6, and 3 replications each. SP20-05 had 2 trials, with 2 and 1 replication each.

Total experimental time in the TEC for a single trial ranged from 3.3-22hrs (Figure 2.2, Table 2.1). At the conclusion of the final replication, the shark was released back into ODL8. Subsequent trials for the same individual were conducted after 3-9d of re-acclimation to the OLD8 (Table 2.1).

\*In two of the experiments (SP19-04 Trial 3, SP19-08 Trial 1) the TEC lid was opened between replications to check on the status of the shark (e.g. shark had moved to part of TEC where it was not observable by infrared camera).



**Figure 2.2:** Raw data traces for three respirometry trials for SP1904 and two trials for SP1908 showing changes in dissolved  $O_2$  concentrations over time. Each trial consisted of several replications to determine the rate at which dissolved  $O_2$  decreased by 15%, relative to the starting saturation. In the traces, the steep inclines following these periods of declining dissolved  $O_2$  represent a flushing of the system with seawater and re-oxygenation of the system prior to the start of another replication.



Fig. 2.3 (above): Raw data traces for two respirometry trials for SP20-05 conducted in 2020 showing changes in dissolved  $O_2$  concentrations (in mg/L) over time, as well as water temperature in the TEC (°C).

Shark	Trial	Trial	Duration of Trial		Start	End	Change in DO2	Lid	Min Temp	Max Temp
ID	Date	Number	(h)	Rep	Time	Time	(%)	opened	(°C)	(°C)
SP19-04	07/07/19	1	4	1	11:52	12:52	10	-	7.5	7.9
				2	13:17	14:22	10	No	7.9	8.3
				3	14:40	15:50	10	No	8.5	8.8
SP19-04	07/11/19	2	6.5	1	9:53	11:25	10	-	6.9	7.1
				2	11:56	13:35	15	No	6.9	7.3
				3	15:09	16:12	10	No	7.2	7.5
SP19-04	07/14/19	3	7	1	9:24	10:48	15	-	6.8	6.9
				2	11:19	12:20	10	No	6.7	6.8
				3	12:39	14:04	10	Yes	6.8	7.0
				4	14:31	15:05	5	No	6.9	7.0
				5	15:29	16:15	10	Yes	6.8	7.0
SP19-08	07/27/19	1	22	1	11:24	12:55	10	-	7.0	7.3
				2	13:35	16:15	15	Yes	7.0	7.4
				3	16:37	19:20	15	Yes	7.2	7.4
				4	19:52	0:52	20	Yes	7.0	7.2
				5	1:25	6:25	20	Yes	6.8	6.9
				6	7:00	9:45	10	No	6.7	6.9
SP19-08	08/05/19	2	13	1	11:07	12:52	10	-	7.2	7.8
				2	13:35	17:55	20	No	7.4	8.4
				3	18:42	0:05	20	No	7.5	7.8
SP20-05	08/05/19	1	6.5	1	08:23	11:10	10	-	6.0	6.6
				2	11:37	15:00	10	No	6.3	7.6
SP20-05	08/09/20	2	3.3	1	07:53	11:10	10	-	7.4	7.8

**Table 2.1:** Summary of respirometry trials for three immature Pacific sleeper sharks. (Duration of trial is approximate). Flushes occurred between replications.

#### Determining Routine and Standard Metabolic Rate (RMR & SMR)

Overall dynamic body action was calculated from the accelerometer data by subtracting the static acceleration from the X, Y, and Z axis and then summing the resulting values of all three axis (IGOR Pro, WaveMetrics). From this, periods where the shark was behaviorally 'Resting' or 'Active' were determined by visual inspection (Figure 2.3). Changes in oxygen consumption rate during periods of resting, were used to determine estimates of SMR, while oxygen consumption rates during period of activity were used to estimate RMR. We then calculated mass-specific RMR and SMR (mg O<sub>2</sub>/h) from the rate of decline in dissolved oxygen (mg O<sub>2</sub>/(L × min)) and the volume of the system, relative to the mass of the individual as per Steffensen (1989).



**Figure 2.3:** Acceleration graphs for SP1908. Overall dynamic body action is calculated by subtracting the static acceleration from the X, Y, and Z axis and then summing the resulting values of all three axis. The blue sections highlight periods of inactivity used for standard metabolic rate measurements, while the remaining periods are used for routine metabolic rate measurements.

#### Determining the temperature coefficient $Q_{10}$

SMR measurements were recorded across acute temperature changes throughout the respirometry trials (range 0.1-1°C within a replication). This gives a rough  $Q_{10}$  calculation through slightly elevated resting metabolic  $Q_{10}$ .  $Q_{10}$  values were then calculated by following the methods of Schmidt-Nielsen (1997). SMR data collected at each experimental temperature was fitted to the equation (1) MR= a × Mb via a nonlinear, iterative Gauss-Newton regression (Brill 1987). This gives more accurate parameter estimates than log-transformed linear regression (Glass 1969).

### RESULTS

The average routine metabolic rate (RMR) for the two 2019 individuals at  $7.3^{\circ}C \pm 0.5$  was calculated at  $18.9 \pm 5.9 \text{ mg O}_2/\text{kg/hr}$ , while standard metabolic rate (SMR) for the second individual at  $7.4^{\circ}C \pm 0.5$  was calculated at  $14.0 \pm 2.9 \text{ mg O}_2/\text{kg/hr}$ . The metabolic  $Q_{10}$  for the two sleeper shark individuals was estimated at  $\sim 3.3$  utilizing the difference in routine metabolic rate between a  $1 - 2^{\circ}C$  temperature change. We are still evaluating the best way to present the data collected from SP20-05, due to the compromised nature of this animal.

#### DISCUSSION

Few published studies have measured the oxygen consumption rates of species larger than 1.5 m, and none on elasmobranchs measured at temperatures below 10°C. We provide the first, albeit limited, assessment of RMR and SMR from n=2 Pacific sleeper shark individuals. Based on studies of other large polar fishes, our preliminary data suggests that juvenile sleeper sharks have relatively low metabolic rates compared to that of other comparable-sized sharks (Bernal et al. 2012, Bernal and Lowe 2015), though not so extremely low as to explain the potentially extreme longevity observed in the GLS. These initial measurements of the metabolic rate of Pacific sleeper sharks enhance deep-sea and polar physiology knowledge by filling gaps in respirometry research for large, arctic elasmobranchs and will benefit from future research expanding comparisons to other species. Estimates of metabolic rates, combined with future determinations of digestive efficiency, feeding behavior and feeding rates, as well as estimates of migration, distribution and abundance, will be essential for any future energetics based ecological modeling of the role and impact of sleeper sharks in changing arctic marine ecosystems.

We also found that Pacific sleeper sharks have a  $Q_{10}$  of approximately 3.3; however, this was only measured across a 2°C range. It is likely that PSS experience greater temperature ranges and therefore more work is needed to provide a realistic measure of metabolic  $Q_{10}$ . This comparably high value (>3) could make this potentially long-lived species vulnerable to climate change-related ocean temperature rises. Sleeper sharks may occasionally forage in shallower or surface conditions to take advantage of higher quantify food availability (e.g. pinnipeds) but may face greater energetic costs of venturing into these warmer conditions. Therefore, then rising global SSTs could force redistribution of sleeper sharks to deeper cooler waters.

# Case Report Evidence from telemetry and observations for offshore killer whale predation on Pacific sleeper sharks

{In prep: Animal Biotelemetry}

Horning M., Olsen D., Wildes S., Lowe C.G., Smith T., Tribuzio C., Bishop A., Sattler R., Guthridge J.,

### Hocking R.

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Offshore killer whales (*Orcinus orca*) represent one of at least three lineages (also referred to as ecotypes or forms) of killer whales in the North Pacific, with resident and transient killer whales being more commonly observed forms (Morin, 2015; Parsons 2013). These sympatric ecotypes are reproductively isolated and genetically distinct. Offshore killer whales were the most recently recognized form (since the late 1980's), and are rarely observed and poorly understood, primarily due to their preference for waters of the outer continental shelf. Evidence of tooth wear and multiple observations of predation on Pacific sleeper shark (*Somniosus pacificus*) were an early indication of diet for this ecotype (Ford, 2011). Analyses of offshore blubber biopsies for stable isotope ratios, fatty acid signatures and concentrations of organic pollutants suggested distinct prey habits for offshores, with a possible focus on fish with high trophic positioning (Herman et al. 2005, Krahn et al. 2007). Elasmobranchs consist of the vast majority of identified prey of Offshores (93% of 40 samples), which include Pacific sleeper shark, Pacific Spiny dogfish (*Squalus sucklei*), and Blue shark (*Prionace glauca*) (Ford, 2014, technical report).

Here we report on a unique opportunity to link telemetry data obtained from a tagged immature female Pacific sleeper shark that died by predation, to direct observations of hunting and feeding behavior of a group of offshore killer whales in Resurrection Bay, Alaska.

We captured a 2.46 m pre-caudal length (2.8m total length) immature female Pacific sleeper shark in Resurrection Bay of the Gulf of Alaska region, using baited bottom hook and line setups. The animal was measured, sampled, tagged with a single Wildlife Computers mini-PAT satellite transmitter and released at the capture location. The mini-PAT was programmed to release and report after 180 days, or after exceeding a preset pressure threshold, or five days after detecting mortality of the host. The mini-PAT started reporting 15 days after release. Initial tag emergence location and subsequent movement were tracked via the Argos system, and the tag recovered after it washed ashore near the City of Seward, Alaska. Downloaded accelerometer data suggested a predation-related mortality 10 days after release, at a depth of 240 m. At this same time and the subsequent emergence location, hunting behavior was observed for a group of 8 offshore ecotype killer whales. Long submergence indicating deep diving coincided with tissue appearing at the surface. A genetic analysis of the tissue grossly visualized as a shark spleen revealed a 1 in 1,369 chance that this tissue is from a different shark than the tagged individual, based on a single microsatellite marker. The mini-PAT data in connection with direct visual observations and genetic tissue analysis provides strong added evidence of offshore ecotype killer whale predation on sleeper sharks in this region, and near the sea floor at depths in excess of 200 m.

#### CONCLUSIONS

Here, we demonstrated the viability of our new approach to estimate metabolic rate and its temperature dependence in temporarily captive, wild Pacific sleeper sharks. We captured 23 immature sharks and equipped 10 with acoustic and satellite transmitters to determine movement patterns and temperature preferences. We collected blood and tissue samples and are analyzing these to investigate foraging ecology and relative body condition. We successfully transported two small sharks (< 2.5m TL) and maintained them in temporary captivity at the Alaska SeaLife Center, prior to release. From these animals, we obtained measurements of temperature dependent metabolic rates using a closed annular respirometer, the first such data ever obtained from a large, cold water fish species. We are continuing to collect the last few field movement and associated environmental data, and these will be correlated with estimated metabolic costs at associated temperature scenarios. Overall, all originally proposed goals were accomplished, though our sample size for respirometry on transient sharks remains low at n=2. We therefore requested a small amount of supplemental funding to hopefully permit one more field season of captures and potential respirometry trials on transient sharks. It is our hope to then use this continuation effort to bring the sample size of transient sharks up to 3-5 animals in total. This request is currently pending.

#### MANAGEMENT AND POLICY IMPLICATIONS

The Pacific sleeper shark (PSS) is an understudied, poorly understood cold water apex predator that could have significant effects on ecosystem structures due to possibly considerable, but unknown abundance. Amongst the tens of thousands of PSS bycaught in Alaskan groundfish, pelagic and trawl fishery, only five sexually mature females have been reported (Tribuzio et al. 2010). There is no knowledge of growth rates, longevity, reproductive rates or reproductive migrations or locations for these cryptic, difficult to observe sharks. As a result, PSS are managed as a Tier 6 species in the Alaskan Shark complex. Stock trends are undetermined. The 2010 BSAI and 2014 GOA (Tribuzio et al. 2010, 2014) stock assessment reports raise concerns about declining rates, but Courtney and Foy (2012) summarized increased PSS catch rates in fisheries-independent surveys in the eastern North Pacific. Identified research priorities include defining migration patterns, population genetics, life history and eco-physiological parameters for use in (bioenergetics) models.

This project 1711 directly addresses essential data gaps including estimates of metabolic rates, and temperature characteristics of PSS habitat. Our preliminary data on RMR, SMR and  $Q_{10}$  suggests with changing ocean climate, PSS could experience habitat shift and displacement towards higher latitudes and/or greater depths (Lynghammar et al. 2013). A latitudinal displacement may be slowed north of the Pacific – Bering Sea transition due to greater episodic mixing and complex transport mechanisms past this boundary, and further north at the Bering Strait (Lynghammar et al. 2013). Such slowing could in turn result in habitat compression, and locally increased PSS abundance along the Aleutian Island transition zone. This could have profound effects on ecosystem structures if PSS are primarily active predators rather than scavengers.

We also successfully demonstrated the viability of transporting and maintaining PSS in temporary captivity to facilitate access for controlled experiments. This novel research tool provides the framework for jumpstarting important studies on the PSS and could lead to innovative comparative studies within and between species, including ontogenetic comparisons. As part of Project R1711, we hosted a workshop at the ASLC in 2018 with a core group of internationally recognized experts to plan subsequent studies to use this new experimental paradigm, including the international research group that recently reported on the extreme longevity in the GLS (Dr. John Steffensen). From this workshop, proposals are being developed;

and the proposed supplemental field season and experimental activities will continue to enhance and facilitate collaborations that take advantage of the accessibility to PSS.

### PUBLICATIONS

{In prep} Horning M., Olsen D., Wildes S., Lowe C., Smith T., Tribuzio C., Bishop A., Sattler R., Guthridge J., Hocking R. "Consummate and consumed predators: evidence from telemetry and observations for offshore killer whale predation on Pacific sleeper sharks in an Alaskan glacial fjord". Animal Biotelemetry

#### PRESENTATIONS

- Smith, T., Horning, M., Guthridge, J., Bishop, A., Hocking, R., Lowe, C. 2020. "Oxygen consumption rate of Pacific sleeper sharks (*Somniosus pacificus*). Oral Presentation, Northeast Pacific Shark Symposium, La Paz, Mexico.
- Horning, M., Bishop, A., Guthridge, J., Hocking, R., Lowe, C., Smith, T. 2019. "Black is the new orange: bringing the poorly understood Pacific sleeper shark into temporary captivity for controlled access studies." Poster Presentation, Alaska Marine Science Symposium, Anchorage, AK.
- Smith, T., Horning, M., Guthridge, J., Bishop, A., Hocking, R., Lowe, C. 2019. "Oxygen consumption rates of two immature Pacific sleeper sharks (*Somniosus pacificus*). Poster Presentation, Alaska Marine Science Symposium, Anchorage, AK.
- Horning, M., Olsen, D., Wildes, S., Lowe, C., Smith, T., Tribuzio, C., Bishop, A., Sattler, R., Guthridge, J., Hocking, R. 2018. "Consummate and consumed predators: evidence from telemetry and observations for offshore killer whale predation on Pacific sleeper sharks in an Alaskan glacial fjord". Poster Presentation, Alaska Marine Science Symposium, Anchorage, AK.

#### DATA AND METADATA

This project generated original raw data on catch-effort, shark morphology, blood chemistry and hematology, and respirometry for Pacific sleeper sharks in Alaska. All data will be saved as original, unprocessed data files, uploaded to the NPRB Research Workspace, and made available publically after March 2022. We also generated data from post-release tracking, on shark behavior and habitat (e.g. temperature). The original raw data was transmitted via Argos satellite, downloaded weekly in DIAG format, converted to a standard format (csv), and is available on the Wildlife Computers Portal upon request.

## OUTREACH

#### Media

We generated 21 blogs and 1 video-blog entry that are hosted on the ASLC's <u>60° N Science</u> blog website, a series of educational mini-lectures on ALSC YouTube account (<u>here, here</u>, and <u>here</u>). All were shared via social media platforms. The materials covered the research process, and conveyed the scientific, management, and stakeholder importance to the general public. These posts collectively totaled over 6,500 views.

We also interacted directly with the community in which our organization is located (Seward, AK) through presentations to the general public at the Seward Science Café, National Ocean Science Bowl, and at the ASLC. More broadly, presentations were given to a National audience via platforms like "Road Scholars".

Lastly, in 2019 National Geographic Wild filmed research conducted under this project as part of their chew, "Alaska Arimal Passaus" which sized in April 2020



show "Alaska Animal Rescue" which aired in April 2020 to a national and international audience.

#### **Public Interpretive Exhibit**

In 2019, an exhibit was designed at the ASLC that describes the research, provides information about PSS biology, and displays audio-visual story-telling about the work with the sharks while in temporary captivity (Figure 1). The ASLC has an annual average of 35,465 Alaska residents and 132,777 foreign and domestic out of state visitors.



Figure 1: Science exhibit room at the ASLC

#### **SYNOPSIS**

{online form}

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#### APPENDIX A - SP20-05 CASE REPORT

#### SP20-05

Pacific sleeper shark (*Somniosus pacificus*) Female, age unknown/juvenile

### HISTORY:

The shark, female approximately 145 cm and 21 kg, was caught (baited shark hook ~400-1000 feet deep) and transported back to the center on 8/2/20. She was housed in a 53' x 30' oval, 6 to 10 foot deep pool (total capacity of 75,725 gallons when full but lower when staff were working with her) at the Alaska SeaLife Center with similar conditions to the two previous sharks that were housed for metabolic studies. This was the smallest sleeper shark to be housed at the center. Upon arrival, the shark displayed abnormal buoyancy and swimming pattern - listing to the side, intermittently bobbing vertically at the surface, rarely swimming ventral side up. After a few days and with a tarp over the deep end of the pool, she swam in a slightly more coordinated manner in a relatively dorsum up posture but still listed some. Previous sharks also displayed some unusual behaviors that decreased with acclimation to the housing. A copepod was present OD, which occurs commonly with sleeper sharks. Blood was collected by the Shark Team at admit, which revealed a PCV of 10% and plasma was archived. Chemistries were later run on 8/10/20 on our IDEXX system and blood glucose was 28 mg/dl, additional diagnostic results below and attached. **Shark Team, please fill in additional data from intake if you have it.** 

#### OS CASE SUMMARY:

Research procedures began on 8/5/20 with the shark being placed in the research TEC (~1000 L volume, 90x24x30 inch dimensions) for metabolic measurements to be done during the day. Several checks during the day did not reveal any problems, however once the run was complete and the shark was about to be moved out, staff noticed that the left eye had proptosed. The eye had likely been extruded from the socket after lodging against the outflow valve of the research vessel, which also produced multifocal "sucker" marks (white discoloration/abrasions) on the skin. While surrounding tissues were very swollen, the globe appeared to be intact. Swelling was reduced by topically applying hypertonic solutions, medical honey and 50% dextrose. Army navy retractors and manual pressure was used to replace the eye and a single suture (3-0 PDS) was placed in the eyelid. A drop of ketorolac was administered to the eye as well. When the suture was removed on 8/9/20, some blood accumulated along the ventral aspect of the lens, but otherwise appeared similar to other eye and the globe stayed intact though eye function is difficult to evaluate in this species. Additionally, mild swelling along the base of one dorsal fin was present though was reported to have been seen previously.

8/9: The shark's behavior had returned to baseline prior to the suture removal and the researchers proceeded with a metabolic trial in the research container. Several hours into the run, the shark had become listless, sinking to the bottom, and in general nonreactive. She was released into the larger pool and displayed behaviors similar to when first admitted. Previous sharks had also demonstrated some unusual behaviors, appearing disoriented upon release from the research container and took up to 24 hours to return to baseline.

8/10-15: The following day, the shark continued to appear weak, was barely responsive, and had more pronounced swellings at the base of all fins. Sampling for diagnostic tests and treatments were initiated,

details below, and for a couple days the shark appeared to improve, becoming more active, assuming a better orientation in the water, and decreased degrees of swellings around the base of fins. However, on 8/13 she started vomiting fish that had been fed two days previously and later vomited fish gruel. For the next four days attempts at supportive care did not reverse her decline and she became increasingly inactive. Ultimately, she was euthanized on 16 Aug due to the poor prognosis and poor quality of life.

# GENERAL CASE SUMMARY

# EXAM FINDINGS

- 1. Vomiting of whole undigested fish (officially noted starting 8/13) and fish gruel through gills before/after tube feeding (8/15)
- 2. QAR to depressed attitude (sitting at bottom)
- 3. Abnormal swimming listing to one side, circling, vertically bobbing, difficulty righting (staff initially reported listless behavior and trouble righting on 8/9)
- 4. Abnormal tail movement with kinking and swelling/edema
- 5. Edema at base of fins and vent
- 6. Large hook wound right jaw
- 7. Small superficial wound on left side of jaw developed into a small ulcer by 8/11
- 8. Multifocal skin discoloration (sucker marks) as well as erythema/abrasions

# DIAGNOSTICS

8/10: PCV 11%, TS 3.8, BG <10, lactate low, blood smears (very few WBC seen, saved for official review)

8/11: BG (post fluid administration) - 24, Hemocue hemoglobin 0.2 (not registering)

8/14: Ultrasound - liver significantly small and NOT hyperechoic (spleen not identified/differentiated), visualized heart, spiral colon, enlarged gallbladder, and gi tract (brief exam to limit handling)
8/16: Lactate 1.0, BG low, hemoglobin low, PCV 4%, TS 3.3%, BCL 0.5%, blood smears made for official review (very low cellularity)

# TREATMENTS

- Dexamethasone 0.4 ml administered IM by Shark Team on 8/10
- Fluids NaCl with 2.5% dextrose and vitamin B (discussed with Dr. Clauss since Hanks solution was not available) IV in dorsal sinus and caudal vein; 150 ml on 8/11 and 120 ml 8/15
- Ceftazidime 20mg/kg IM right epaxial (8/11, 8/14)
- Dexamethasone SP 0.5 mg/kg IM left epaxial (8/11, 8/14)
- Vitamin E/Selenium 0.065 mg/kg IM left epaxial (8/11)
- Wound care: Saline flush, 1 ml topical (ophthalmic) tobramycin flush, 30 sec laser (8/11, 8/14)

# NUTRITIONAL SUPPORT

- Orogastric tube feeding: herring fish gruel diluted with electrolyte solution/saline (150 ml 8/11, 50 ml 8/15 stopped due to vomiting/regurgitation)
- Mazuri Shark Tab (vitamins) absorption unlikely as fish were regurgitated
- Whole fish started feeding 8/11, regurgitated 8/13

# EUTHANASIA

- Through previous group discussion involving veterinary, husbandry, and research staff, a two step euthanasia process was agreed upon (MS-222 followed by mechanical method). Additional available drugs (propofol, pentobarbital, KCL) were declined due to concerns about interfering with subsequent tissue analysis in support of research aims as well as the lack of documented use of these drugs in this species.
- Shark transported by stretcher to holding tank under hangover
- Initial buffered MS-222 dose of 100 mg/L produced short excitement phase, shark remained slightly responsive to manipulation after 15 minutes
- Total dose buffered MS-222 of 468 mg/L effective for stopping of gilling and all response to manipulation and painful stimuli
- Removed from anesthetic water after approximately 30 minutes and spine was severed using a sharpened knife

# NECROPSY - ABNORMAL FINDINGS

- EXTERNAL: Very thin body condition noted when out of water, additional edema/congestion ventrally (suspect post mortem), edema at base of dorsal fins and vent remained present, feathered/ulcerated tips of caudal and dorsal fins, multifocal erythema and abrasions; inflammation deep to hook wound wound appeared to have fresh healthy tissue, but was still patent with no evidence of significant healing progress, approximately 5mm ulcer ventral jaw side opposite to hook wound. Multiple circular (sucker) skin discolorations. Eyes cloudy (suspect potentially normal for species) and globes intact OU
- INTERNAL: Generally pale, fair postmortem condition
  - <u>STOMACH</u>: enlarged and thickened, appeared to be necrotic with gel-like smooth mucosal surface (no papillae or crevices), one small ulcer seen distally, contents included octopus beak, a single minimally digested small fish, and parasites
  - <u>LIVER-</u>microhepatica (less than 5% bw), while texture was uniform it was subjectively less fatty than normal, enlarged bile-filled gallbladder consistent with chronic anorexia
  - <u>INTESTINES</u>: mild amount of fluid, mild necrosis/autolysis, significant number of segmented worms in intestine and spiral colon.
  - <u>MUSCULOSKELETAL</u>: suspected caudal vertebral fracture where kink was; cartilage generally soft (unknown if due to nutritional status or if appropriate for age/life stage), some teeth were missing in general hook wound area
  - <u>KIDNEYS/SPLEEN</u>: Subjectively enlarged, texture different from other elasmobranchs.
  - <u>CNS/SENSORY</u>: Both optic nerves and globes were intact. There was more erythema seen deep to the eye that was treated for prolapse. The eyes and surrounding tissues were collected by the Shark Research Team as part of an aging study.

# PROBLEM LIST

1. Maldigestion/absorption (vomiting of whole undigested fish and gruel, necrosis in stomach with lack of normal mucosa, thin body condition, anemia, hypoglycemia) - suspect secondary to chronic anorexia and liver failure

- 2. Poor if any liver function/failure (hypoglycemia, abnormal swimming, small size, abnormal echogenicity) suspect secondary to chronic anorexia > infectious, toxin
- 3. Tail kinking with suspected fracture previous injury vs pressure sore/injury from poor buoyancy control
- 4. Edema hypoproteinemia, salt imbalance
- 5. Systemic shock anemia, lactic acidosis, likely hypovolemia, depressed
- 6. Abnormal swimming poor liver function, previous injury to tail, stress/behavioral component, hypoglycemia component
- 7. Large hook wound right jaw (sustained from catch for this project)
- 8. Marked gastrointestinal parasitosis suspect secondary to immunocompromised state
- 9. Ulcer left jaw initial catch trauma vs previous traumatic event uncertain
- 10. Multifocal skin discoloration (sucker marks) from outflow of research vessel
- 11. Multifocal erythema and abrasions likely secondary bacterial infection from environmental stress or trauma from initial catch as well as handling, ms-222 component, potential UVB exposure effect
- 12. Prolapsed OS appeared to be resolving

# ASSESSMENT

Suspect gastrointestinal shut down due to chronic anorexia and liver failure. Shark was likely debilitated at intake. It's history of abnormal buoyancy and hypoglycemia is consistent with liver failure, which was likely due to chronic anorexia (although other causes such as toxins cannot be ruled out at this time). Additional treatment would have no to very little chance of success and euthanasia was appropriate. While a small number of parasites are normal for wild animals, there was a significant number and likely secondary to the shark's immunocompromised state. They may have contributed to anemia as well. Tail injury and/or previous capture (prior to study) is a potential cause of chronic anorexia. Redness on skin and fins is likely due to secondary bacterial infection due to underlying disease/stress as well as handling.

# ADDITIONAL DIAGNOSTIC PLAN

- 1. Histopathology Dr. Al Camus (UGA, does a lot of elasmobranch work)
  - a. Sent biopsy samples collected from approximately 17 tissues, preserved in formalin
  - b. Samples from SP1701 if still available Let us know if you would like these sent as we do not have these samples, they were turned over to research staff.
- 2. Chemistry/BUN/Osmolality Sent to Dr. Jill Arnold (Zooquatics)
  - a. Elasmobranch chemistry panel includes BUN, osmolality) Intake and Pre-Euthanasia
  - b. BUN and osmolality only 8/11
- 3. Differential and est. WBC count from blood smear review
  - a. 8/11 and 8/16 (no smears available from intake)

We will let you know when we receive results.



Owner:

### Alaska SeaLife Center

301 Railway Ave Seward, AK 99664 Phone 919-810-7002 ashleyk@alaskasealife.org

#### RDVM:

### Ashley Kirby

Clinic:

### Alaska SeaLife Center

301 Railway Ave Seward, AK 99664 Phone 919-810-7002 ashleyk@alaskasealife.org

# Pathology

## Mail-In Multiple Tissue Pathology Report

### Gross Pathology

Received for histologic evaluation wrapped in formalin-soaked towels are multiple fixed tissues from a 145 cm, 21 kg, female Pacific sleeper shark (SP20-05). Reported necropsy findings included a poor body condition, edema at fin bases, multiple abrasions and ulcerations, a deep hook wound, a gastric ulcer, microhepatica, soft cartilage, possible vertebral fracture, and multiple parasites. The shark was euthanized due to declining condition and poor quality of life two weeks after collection. Findings observed while trimming include dark parallel bands of tissue, resembling hemorrhage, vertically apposed to the vertebral centra. A 1.5 cm, pale yellow nodule is present within the gastric submucosa. Another piece of gastric mucosa contains a 1.5 cm raised area with a central 0.4 cm ulcer from which thread-like nematodes extend. Cross sectioning reveals an approximately 1.5 x 1.0 cm, dark, multinodular focus containing additional nematodes. In the presumed hook lesion, an approximately 1.5 cm wound extends into subadjacent muscle. Representative sections of each tissue were trimmed into cassettes 1-18 and processed routinely.

# Histopathology

Overview: Collagenous tissues throughout the body, including submucosal, subserosal, interstitial, and perivascular sites, have a wispy, loosely organized aerolar appearance.

Vertebral column and spinal cord: The dark tissue observed grossly corresponds to symmetrical vascular plexuses bordering the vertebral centra. The spinal cord is microscopically normal, but the meninges and extrameningeal connective tissues lining the spinal canal are infiltrated by small to occasionally moderate numbers of fine eosinophilic granulocytes.

Hook wound (presumed): Extensive fields of hemorrhage disrupt bands of dense regular collagen, as well as interdigitate interstitial areas of skeletal muscle to isolate variably sized groups of myofibers. Affected collagen bundles are hypereosinophilic, fragmented, and fibrillated. Multiple groups of myofibers as necrotic, as evidenced by loss of tinctorial properties, morphologic detail, and sarcomeric cross striations. Small to moderate numbers of fine eosinophilic granulocytes are distributed throughout areas of hemorrhage and occasionally enter the sarcoplasm of necrotic myofibers.

College of Veterinary Medicine University of Georgia Athens, GA 30602 Phone: 706-542-5837 Fax: 706-542-5828

Department of Pathology

Accession Number: M21-00011 Case Coordinator: CAMUS, ALVIN C. Received: Sep 03, 2020 Finalized: Oct 14, 2020 Species: Fish - Shark Breed: Pacific Sleeper Shark Sex: Female Age: unknown Animal ID: SP20-05 Specimen: Fixed Tissue

# Pathology

Stomach: The epithelium contains a sharply demarcated, umbilicated ulcer with adhered margins. A broad zone of necrotic cellular debris infiltrated by moderate numbers of fine eosinophilic granulocytes extends from the ulcer surface laterally beneath the epithelium and deeply into the subadjacent muscularis. Beneath this is an expansile, nodular area of mixed fibrosis, necrotic debris, and variable small to moderate numbers of granulocytes and lymphocytes surrounding small cavities containing numerous nematodes. The nematodes are characterized by a smooth cuticle, coelomyarian-polymyarian musculature, compact lateral cords, and a tri-radiate esophagus lined by tall epithelial cells.

Liver: Individual hepatocytes have more sharply defined cell borders and subjectively contain slightly less cytoplasmic lipid vacuolation than is typical or normal healthy elasmobranchs. Sinusoidal prominence is markedly increased.

Heart: Subepicardial and subendothelial sites have an aerolar appearance with abundant clear space as noted in the overview above. Cardiac myofibers are subjectively small in diameter. The above observations are most notable in the atrium. The ventricular outer compact layer of the myocardium is thin in comparison to other elasmobranch species.

Kidney: Abundant hematopoietic tissue resembling the epigonal and Leydig organs is diffuse beneath the renal capsule and scattered within the sinus zone.

Pancreas: Interstitial areas contain multiple, small, loosely organized collections of lymphocytes.

Stomach and esophagus: Low, broad folds form the gastric mucosa. Epithelial surfaces and gastric glands ae microscopically normal. The submucosa, presumed proximal, contains a circumscribed area of hematopoietic tissue typical of epigonal and Leydig organs. The esophageal section examined lacks a defining mucosal surface but possesses a thick muscular wall containing skeletal muscle. The tissue is bordered on its internal surface by hematopoietic tissue typical of the Leydig organ.

Intestinal worms: The worms are identified as cestodes, as evidenced by an acellular tegument and parenchymatous body with numerous calcareous corpuscles. Scolices were not present for further characterization.

The following tissues were examined microscopically, but contained no significant changes: brain, rectal gland, intestine, spiral intestine, spleen, skin, skeletal muscle, and gill.

### Diagnosis

Spinal cord: Meningitis and perimyelitis, granulocytic, acute, mild Hook wound: Hemorrhage and cellulitis, granulocytic, focally extensive, acute, moderate Stomach: Gastritis, ulcerative, necrotizing, chronic and active, focally extensive, severe, with fibrosis and intramural nematodes Liver: Hepatocellular atrophy, diffuse, chronic, mild

Intestinal worms: Unidentified cestodes

### Comments

Although no specific definitive cause for the shark's decline was determined microscopically, a number of potential contributing factors were observed. While subjective, evaluation of the liver is consistent with consumption of energy stores, mild atrophy, and poor feeding in captive elasmobranchs. Although no fracture was identified, granulocytes were infiltrating the spinal canal and meninges in a section taken from the presumed fracture site. In the absence of organisms, most importantly bacteria, it is uncertain whether the granulocytes were responding to an infectious process or were attracted to an area of clinically suspected tissue damage. Two dark bands adjacent to the vertebral centra, presumed at the time of trimming to be areas of hemorrhage, were identified microscopically as vascular plexuses and presumed normal structures. Their function is unknown, but possibly involved in heat exchange to the nervous system. Examination of the hook wound revealed

# Pathology

extensive hemorrhage, localized tissue damage, and granulocytic inflammation, but no organisms were observed. Although acute bacterial sepsis associated with the hook wound cannot be entirely ruled out, there were no additional changes beyond the spinal canal and meninges to suggest a systemic infectious process. Also potentially contributing to the shark's poor condition was the presence of parasitic gastritis in association with a focal ulcer and bundles of encysted nematodes within the gastric wall. Similar gastric lesions are seen in Atlantic cod with anasakid nematode infections, but I found no similar descriptions in elasmobranchs.

The significance of a number of additional findings is unclear, although they are assumed normal tissue variations in a species for which little literature is available. Compared to other elasmobranch species in which tissues generally appear dense and compact, collagenous tissues throughout the body were loosely organized, almost spongy in appearance. Cardiac myofibers were small in diameter and while potentially atrophic are presumed normal for a less active shark species. Hematopoietic tissue was more widely distributed than is seen in most elasmobranchs. In addition to the epigonal organ, this species has a large Leydig organ that extends into the proximal wall of the stomach. Most notably, hematopoietic tissue was also present in the renal intersitium as is typically seen in teleosts. Any comparisons here to "extramedullay hematopoiesis" in mammalian species would be entirely speculative. Lymphocytic infiltrates in the pancreatic interstitium are common in elasmobranchs and considered an insignificant finding. Lastly, trematode and cestode parasites are common in the intestine, particularly the spiral intestine, of elasmobranchs but are generally of low pathogenicity.

Reported By

Alvin C. Camus Anatomic Pathologist camus@uga.edu