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Habitat and Harvest: How the Identification of a Shark Nursery Changes Our  
Understanding of Population Demographics and Management

By

MEGHAN HOLST  
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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# Abstract

The northeastern Pacific population of the broadnose sevengill shark, *Notorynchus cepedianus*, is a large apex predator found ranging from Alaska, USA to Mexico. Like other large shark species, sevengill sharks face threats of overfishing and climate change. Threats to sevengill sharks may be exacerbated in areas considered essential for aspects of their life history, such as breeding or feeding. The northeastern Pacific population of sevengill sharks is known to frequent bays and estuaries along their range, but the only suggested nursery areas have been Humboldt and San Francisco Bays, California, USA. California has an open targeted fishery that is not restricted to size, sex, season, or location. This dissertation 1) tests whether San Francisco Bay is a nursery habitat based on published criteria, 2) evaluates sevengill physiological response to catch-and-release fishing, and 3) investigates attitudes and opinions of current sevengill management and explores the ecological knowledge of key stakeholders (charter captains and California Fish and Wildlife (CDFW)). Results demonstrate that San Francisco Bay is a shark nursery area for sevengill sharks as previously suggested. Significant physiological effects were observed during 30-minute catch-and-release stress events. Stakeholder interviews revealed positive attitudes of charter captains towards informed conservation management, with captains proactively implementing fishing restrictions more strictly than what is required of them by CDFW. Results suggest that if the population is at risk in the future, San Francisco Bay is an essential fish habitat worth exploring for protections, and would be well supported in San Francisco Bay, California, where they are frequently targeted by recreational anglers.



# Introduction

The broadnose sevengill shark, *Notorynchus cepedianus*, is a large apex predator that can be found in temperate waters in the western Pacific Ocean around China and Japan, Australia, and New Zealand; the northeastern Pacific Ocean around Canada to Mexico; and the southern Atlantic Ocean around Argentina and South Africa. Sevengill sharks typically inhabit deep ocean waters (up to 400m) but seasonally visit shallow areas in Willapa Bay, Washington, as well as Humboldt and San Francisco Bays in California, USA; Patagonia, Chile (Lucifora et al. 2005, Irigoyen et al. 2018, 2019, Bustamante et al. 2021); Argentina (Irigoyen et al. 2015); Derwent Estuary and Norfolk Bay, Tasmania (Barnett et al. 2010b); and Stewart Island, New Zealand (Housiaux et al. 2019, Lewis et al. 2020), with observations heavily biased toward females at Stewart Island (Lewis et al. 2023). The primary threat to these sharks is fishers taking advantage of these aggregations, except in the few places where there is a moratorium for fishing these sharks, such as Tasmania (Barnett et al. 2010a), and Washington state, USA (Williams et al. 2012).

The distribution and management of sevengill shark nursery areas highlight significant challenges and gaps in global population assessments and conservation efforts. Nursery areas for sevengills have been suggested in Humboldt and San Francisco Bays, California, USA; and in San Antonio Cape, Argentina, but juvenile presence has not been identified in other populations around the world. These sevengill populations do not mix (Schmidt-Roach et al. 2021), emphasizing the need to manage each individual population individually. Open fisheries exist for sevengill sharks across populations, typically as bycatch or as direct targeted fisheries for

consumption. Yet, no population assessments have been conducted for any population around the world.

Sharks can have disproportionate impacts on the ecosystem by influencing food webs from a top-down approach. When top predators are removed from the ecosystem, food web dynamics can become imbalanced (Heithaus et al., 2008). When compared with other apex predator sharks, sevengills rank the highest trophic level, including over white sharks, *Carcharodon carcharias* (Cortes, 1999), preying on mammals such as sea lions and seals, teleost fish such as salmon or ocean white bass, and other chondrichthyans, including other sevengills (Ebert 2002, Lucifora et al. 2005, Braccini 2008, Abrantes and Barnett 2011).

As their name suggests, broadnose sevengill sharks are known for their particularly broad nose and for having seven gill slits, whereas most shark species have five sets of gills (VanderWright et al., 2020). Sevengill sharks have a single, relatively small posterior dorsal fin, whereas most shark species display at least two dorsal fins with one large dorsal fin relatively above the pectoral fins. They exhibit coloration patterns that include both black and white spots, unique to each individual, similar to the fingerprint of a human. The species is known to grow to a maximum of roughly 300 cm (9.8 ft) in total length (TL), and may weigh as much as ~350 lbs.

The northeastern Pacific population ranges from Alaska, USA to Mexico frequenting Willapa Bay, Washington (Williams et al., 2012) and Humboldt and San Francisco Bays, California (Ebert, 2001). Online searches reveal public reports of sevengills sharks aggregating in La Jolla, California (Blue Water Photo, In Focus, 2024; San Diego Scuba Guide, 2024; Scuba Diver Girls, 2015). Of these shallow habitats, San Francisco and Humboldt Bays, California have been suggested as pupping and nursery areas for sevengill sharks (Ebert, 1984, 1986, 1989, 2001).

Sevengill sharks face the same threats as many other shark species. They may be particularly vulnerable to overfishing due to their slow rate of maturation and low fecundity rate (Dulvy et al., 2014, 2021). The northeastern Pacific population is frequently targeted by recreational anglers or as bycatch in rockfish, salmon, and halibut fisheries. Some of the most reported landings of sevengill sharks occur in and around San Francisco Bay, a proposed pupping and nursery habitat (Ebert, 1989). It is currently unknown how fisheries interactions may impact the sevengill population, particularly in a proposed nursery area. No formal investigation or quantitative analyses have occurred to definitively demonstrate whether San Francisco or Humboldt bay are nursery fish habitats (M. Heupel et al., 2007; NOAA, 2007).

To address some of these uncertainties, I will 1) formally test San Francisco Bay as a pupping and nursery ground to evaluate if management should consider this area as essential fish habitat, 2) evaluate the physiological stress response of sevengills to catch-and-release fishing to better understand sublethal impacts on juveniles in their proposed nursery habitat, and 3) evaluate the opinions of key stakeholders of the sevengill fishery (charter captains who target sevengills for income, and California Fish and Wildlife (CDFW) on current management and identify areas where improvement may be supported and necessary.

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# Chapter 1

Title: Identification of San Francisco Bay as a pupping and nursery ground for the apex predator, the broadnose sevengill shark (*Notorynchus cepedianus*)

## Introduction

Marine fish nursery habitats provide vulnerable juveniles with protection from predators (Jenkins and Wheatley 1998, Bloomfield and Gillanders 2005, Schaffler et al. 2013, James et al. 2019), food-rich environments that facilitate juvenile growth (Heck Hay et al. 2003, James et al. 2019), and improved recruitment to adult populations (James et al. 2019). Nurseries are typically shallow coastal or estuarine habitats that are protected from the open ocean (Heupel et al. 2007). Roving or migratory elasmobranchs have demonstrated heterogeneous use of nursery areas across geographic regions (ex: Barbato et al., 2023; López-Angarita et al., 2021; TinHan et al., 2020), enhancing the likelihood of reproductive success across changing environmental conditions. This “portfolio” of nurseries (Schindler et al. 2015) may improve the likelihood of population persistence by buffering regions with annually varying recruitment due to shifting environmental conditions (Yates et al. 2012).

Identifying nursery areas improves conservation of cartilaginous fishes because many shark species are threatened and declining (Dulvy et al. 2021), and the heightened susceptibility of densely populated juvenile habitats to human-induced pressures. Studies to delineate nursery areas for potential protection have increased in recent decades due to mandates by NOAA to

identify fish habitat that is essential for the survival of fish species (Heupel et al. 2007, NOAA 2007). Locating and identifying these habitats is challenging because many sharks are highly migratory and span large geographic regions (Heupel et al. 2007, Kinney and Simpfendorfer 2009), and nursery areas could be located anywhere along their range, potentially in remote locations.

To determine if a habitat qualifies as a shark nursery area, its relative importance for the population needs to be assessed. Merely finding juveniles in a habitat does not necessarily indicate a nursery area (Heupel et al. 2007). This misconception can lead to the protection of large coastal areas that may not significantly contribute to the recruitment of a species. Habitats are considered nursery areas if they meet the following criteria: juveniles are more frequently encountered than in other areas (Criterion 1); juveniles remain in the area for extended periods (Criterion 2); and the area is repeatedly used across years (Criterion 3; Heupel et al. 2007). These criteria have been widely adopted in the shark science community since their inception (Heupel et al. 2019) with as many as 938 citations listed in Google Scholar as of 4 June 2024. Several studies have identified nursery areas as a result of these criteria, including the bull shark, *Carcharhinus leucas* in Texas, USA (Froeschke et al. 2010) and the Critically Endangered smalltooth sawfish, *Pristis pectinata* in Florida, USA, the latter of which has informed the placement of Marine Protected Areas for species recovery (Brame et al. 2019). By evaluating these three criteria, stakeholders can accurately identify critical reproductive habitat for a given shark species, potentially leading to significant impacts on species conservation.

The broadnose sevengill shark, *Notorynchus cepedianus*, is one of the largest apex predators along the northeastern Pacific coastline and may use San Francisco Bay as a nursery area (Ebert 1989). Recent reclassification by the IUCN Red List from “data-deficient” to

“vulnerable” supports the need for demographic evaluation (Finucci et al. 2020). This species likely moves long distances (Van Dykhuizen et al. 1988, Ketchum et al. 2017), but connectivity along its range is not well understood. A mature female sevengill who was captured in Humboldt Bay and released in Monterey Bay was recaptured back at her original capture site 845 days later (Van Dykhuizen et al. 1988). Sevengill sharks have also been observed making frequent round-trip journeys between San Diego and San Francisco Bay California, spanning approximately 800 km (Ketchum et al. 2017), but the reasons behind these repetitive long-distance movements remain poorly understood. Despite these examples of long-distance movements, it is currently unclear whether migratory behavior is a population-wide behavior or occurs more on an individual basis (Dingle 2014). The northeastern Pacific population can exhibit strong site fidelity, with repeated returns to Willapa Bay in Washington (Williams et al. 2012), and Humboldt and San Francisco Bays in California (Van Dykhuizen et al. 1988, Ebert 1989). Studies suggest that San Francisco and Humboldt Bays may be pupping and nursery grounds for sevengill sharks (Ebert 1989, 2001), with juvenile presence published as early as 1948 in San Francisco Bay (Herald and Ripley 1951). Recent stock assessments for Humboldt Bay do not demonstrate sevengill presence (Chamberlain et al. 1993, Cole 2004), highlighting the importance of investigating potential nursery areas. In this study, I focus on San Francisco Bay by identifying the habitat use and residency of juvenile and adult sevengill sharks in the area. Sevengill shark residency in the Bay has been studied in adults and subadults (Ketchum et al. 2017, McInturf et al. 2019), but not in juveniles.

San Francisco Bay habitat reflects what is typically seen in elasmobranch nursery habitat. San Francisco Bay has extensive shallow water, eelgrass beds, and remnant tidal marshes punctuated by tidal sloughs. These create complex habitat structures (Colombano et al. 2020)



that support high levels of biodiversity and productivity (Robertson and Duke 1987, Blaber et al. 1989, Beck et al. 2001, Yates et al. 2012). South San Francisco Bay serves as a nursery ground for several elasmobranch species, including leopard sharks (*Triakis semifasciata*) and brown smoothhound sharks (*Mustelus henlei*; Russo 2019).

Evaluating whether San Francisco Bay serves as a nursery for sevengill sharks not only aligns with mandates to protect Essential Fish Habitat (EFH; Heupel et al. 2007, NOAA 2007) but also addresses the vulnerability of the population to exploitation and environmental fluctuations. Sevengills are targeted by fishers around the world in locations where sevengills aggregate (Irigoyen et al. 2015, 2018, 2019, Bustamante et al. 2021), and there is a limited understanding of how this impacts the populations. In California, sevengill populations were likely depleted by fishing tournaments in the 1940s and 1950s (Herald 1953). Today, there is an open fishery for sevengill sharks, with a one-bag limit implemented (i.e. every person can land one shark per day), and no limitations on size, sex, or season, or location in California. No status reports have been conducted by the California Department of Fish and Wildlife (CDFW) since 2001 (Ebert 2001), yet recreational fisheries remain within San Francisco Bay where sevengills seasonally aggregate (Ebert 1989). Sevengill seasonal movements are known to be influenced by temperature (Williams et al. 2012, Stehfest et al. 2015), mating and parturition (Ebert 1989, Lucifora et al. 2005), and prey abundance (Barnett et al. 2010b, 2010a, Williams et al. 2012). However, it is currently unknown how fisheries interactions may impact movement or behavior in shallow seasonal habitats. This is particularly true in areas like San Francisco Bay, which may be a year-round nursery habitat for juveniles that may be disproportionately affected by fishing pressure. Other shark fisheries, for example the dogfish (*Squalus acanthias*) fishery off the coast of Europe have collapsed from targeting aggregations (Fordham 2006). Substantiating the

hypothesis that San Francisco Bay serves as a crucial nursery area can enhance our understanding of sevengill sharks' vulnerability to fishing pressure and future potential shifting environmental conditions.

San Francisco Bay is one of the largest and most densely populated bay-estuaries in North America (Huning and Perlmutter 2016) and is affected by shipping ports, industry, and recreational fisheries. Identifying the specific area where sevengill juveniles aggregate in San Francisco Bay will better enhance potential future protections, by minimizing economic disruptions while providing few conservation benefits. Small sevengill sharks were historically harvested near Coyote Point in San Francisco Bay (Herald 1953), deep in the south channel. In this study, I will refer to this area as 'South Bay,' where I will capture sevengill sharks using hook-and-line methods. I will compare this area with another frequently visited recreational fishing location in the Central Bay to investigate whether juveniles and adults are segregating spatially.

One way to demonstrate long-term residency (Criterion 2) of sevengill sharks is through stable isotope analysis. Stable isotope composition of animal tissues reflect their diet and environment (Peterson and Fry 1987, Carlisle et al. 2015), and have been used to demonstrate ontogenetic shifts in both factors (Estrada et al. 2006, Kim et al. 2012b, Sakamoto et al. 2023). At the base of the food web marine phytoplankton demonstrate high temporal and spatial variability (Kurle and McWhorter 2017, Ho et al. 2021), which is specific to geographic locations of isotope origins. Consumer tissues become enriched with stable isotopes of their prey (Sweeting et al. 2007, Canseco et al. 2022) and can create a history of where prey was consumed. White muscle tissue isotope signals within the muscle reflect a summary of environmental isotopes for roughly 422 days (MacNeil et al. 2006, Kinney et al. 2011, Kim and

Koch 2012). Therefore, stable isotope analysis is an effective way to demonstrate the long-term residency of sharks in a given area when compared with the isotope signals of the resident prey source.

In this study, I will test if South San Francisco Bay is a nursery using three criteria as defined above (Heupel et al. 2007) via the following hypotheses:

Hypothesis 1: I expected to find a higher occurrence of juvenile sevengills within South Bay compared to Central Bay, demonstrating that South Bay is where the nursery habitat occurs within San Francisco Bay (Criterion 1).

Hypothesis 2: I expected to find evidence of consistent occupancy of sevengills (both juveniles and adults) in the nursery habitat across years, demonstrating persistence use of the habitat across years (Criterion 3).

Hypothesis 3: I expected that the stable isotope signatures of juveniles would show differences from adults, reflecting fidelity to San Francisco Bay by juveniles and coastal migratory behavior by adults (Criterion 2).

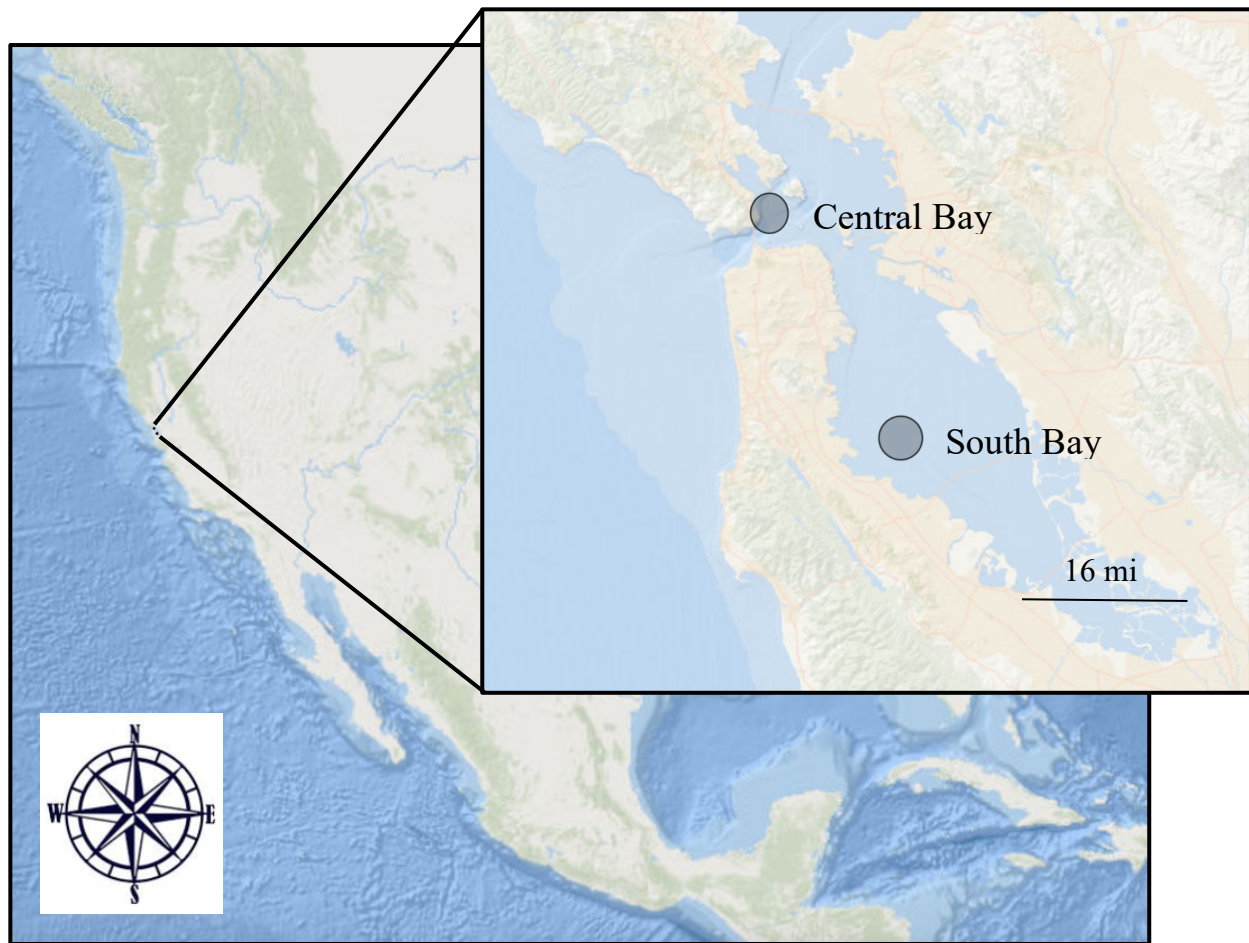
## Materials and Methods

Several methods were pursued to address all four hypotheses. First, I conducted repeated sampling and mark-recapture within and outside the potential nursery habitat. This allowed for me to determine if there was a higher occurrence of juvenile sevengills within South Bay compared to Central Bay (H1; Criterion 1) and determine consistent presence over years (H2; Criterion 3). H2 was further supported by combining the present data with a 20-year dataset of

mark recaptures from Monterey Bay Aquarium, presented in this study. H3 was investigated by acquiring white muscle tissue samples of sevengills in the field. Stable isotope analysis of muscle biopsies was then conducted to demonstrate the long-term habitat use of juvenile sharks within San Francisco Bay (i.e., residency) compared to the open ocean migrating adults (H3; Criterion 2).

### *Field Sites*

I collected data between June 2019 and December 2023 for a total of 47 sampling trips at two primary locations. I identified the adult sampling site, referred to as “Central Bay” (Fig. 1), from the literature (Ketchum et al. 2017, McInturf et al. 2019) and communication with local anglers (M. McGill, January 2018, *pers. comm.*). I also identified the juvenile sampling site, referred to as “South Bay” (Fig. 1), from the knowledge of local anglers (M. McGill, January 2018, *pers. comm.*) and mention of small juvenile sevengill sharks in the literature off Coyote Point (Herald and Ripley 1951, Herald 1953). I sampled these two sites across years to demonstrate repeated use of each habitat and limited overlap between the proposed adult and juvenile habitats within San Francisco Bay (H1 and H2).



**Fig 1:** Primary Field Sites. The primary field sites for this study were Central Bay and South Bay within San Francisco Bay, California, USA. Central Bay is located at the deepest part of the bay between Golden Gate Bridge and Alcatraz Island in San Francisco Bay. South Bay is roughly 40 km south within San Francisco Bay in shallower habitat.

*Animal handling, morphometrics, tissue collection, and tagging*

I captured sevengill sharks via hook-and-line. I fished on the incoming tide, typically two hours before high slack, an optimal period for shark fishing. My sampling methods were approved by CDFW and the animal care regulatory body at the University of California, Davis (Holst Scientific Collecting Permit #: S-223320002-22356-001; Holst IACUC #: University of

California, Davis, 22586). Once I captured a sevengill shark, they were sexed based on the presence or absence of claspers and I measured with a soft measuring tape for total length (TL, measured from the rostrum to the dorsal posterior end of the caudal fin; cm). I then used these measurements to determine life history group, as sevengill shark developmental stages are defined by length (Ebert 1986, 1989, 1996, 2002).

Table 1. Developmental stages defined for male and female *N. cepedianus*, based on previously published life-history information (Ebert 1986, 1989, 1996, 2002).

Sex	Life History Group			
	Neonates	Juveniles	Subadults	Adults
Male	<60cm TL	60 - 120cm TL	121 - 180cm TL	>180cm TL
Female	<60cm TL	60 - 180cm TL	181 - 220cm TL	>220cm TL

I obtained muscle biopsies from all sevengill sharks captured in Central Bay and South Bay between June 2019 and December 2023. Biopsies were taken using a 4mm disposable muscle biopsy punch (Integra Miltex, Princeton, New Jersey, USA), inserted dorsally from the pectoral fin. I stored muscle biopsies in microcentrifuge tubes and placed them in a cooler with ice for up to 3 hours, and then I placed them in a -20°C freezer upon return from the field.

I tagged sevengills with Floy tags (Floy Tag & Manufacturing Inc, Seattle Washington, USA) caudally of their single dorsal fin in the dorsal pit with a steel Floy applicator. Floy tags included unique sevengill identification codes, as well as callback numbers for anglers to report any recaptures of tagged animals.

## *Spatial analysis*

To assess the likelihood of encountering juveniles at South Bay relative to Central Bay (H1; Criterion1), I employed logistic regression analysis (Nad'ó and Kaňuch 2018). The binary outcome variable represented whether an observation belonged to the juvenile category or another age class. The main predictor of interest was the sampling site, a categorical variable that included two levels: South Bay and Central Bay.

A logistic regression model was fitted using the generalized linear modeling framework. The model formulation was as follows:

$$\text{logit}(\pi_i) = \beta_0 + \beta_1 * \text{Sample.Site}(y) + \epsilon_i$$

Here,  $\pi_i$  represents the probability of encountering a juvenile shark for the  $i$ th observation. The term  $\beta_0$  denotes the intercept, while  $\beta_1$  represents the coefficient associated with the sampling site variable for South Bay. The variable `Sample.Site` is a binary indicator, taking the value 1 if the sampling site is South Bay and 0 otherwise.  $E_i$  represents the error term.

The logistic regression coefficients estimated the effect of sampling site on the likelihood of encountering juvenile sharks. A positive coefficient for `Sample.Site (South Bay)` would indicate a higher likelihood of encountering juveniles in South Bay compared to Central Bay. Statistical significance of the logistic regression coefficient associated with `Sample.Site (South Bay)` was assessed using a p-value threshold of  $\alpha = 0.05$ . Statistical analyses were conducted using the R programming language (2023.12.1+402) with the 'glm' function from the 'stats' package.

### *Mark-Recapture data*

I used tag mark-recapture to investigate the persistent use of San Francisco Bay by sevengill sharks across years (H2) and support isotope data demonstrating long-term residency of juveniles (H3). To achieve this, I combined a 20-year dataset on sevengill shark catch and recapture provided by Monterey Bay Aquarium of Central Bay (2003-2023) with data from the present study (2019-2023). This dataset illustrates long-term habitat utilization and increases recapture counts data for comprehensive analysis within our study. All sharks captured by Monterey Bay Aquarium followed the same hook-and-line methods and were captured in Central Bay. All data collected from Monterey Bay Aquarium were approved (Ezcurra permit #: S-190810004-21007-001).

Of the sevengills tagged by both Monterey Bay Aquarium and me for this study ( $n = 432$ ), ~10% were recaptured ( $n = 42$ ). Recaptures were dependent on individuals who fished or found sevengill sharks with spaghetti tags and called the associated phone number to report the sighting. Data acquired upon recapture (i.e., total length) was dependent on individuals having a measuring tool for accurate measurements, which was not always possible and resulted in an unknown total length measurement for some recaptured animals ( $n = 11$ ).

### *Stable Isotope Analysis*

I used stable isotope analysis of sevengill white muscle to demonstrate that juvenile sharks remain in San Francisco Bay for multiple years (H3; Criterion 2). The discrimination factor (or trophic placement) is a biological parameter that must be incorporated for comparative analysis between species. This process standardizes isotope signals across species and/or studies that allow for isotope signals to be directly compared. When an organism consumes prey, there is



enrichment in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  in the consumer's tissues relative to prey tissues. This discrimination factor is calculated as  $\delta X_{\text{consumer}} - \delta X_{\text{prey}} = \Delta^{13}\text{C}$  or  $\Delta^{15}\text{N}$ . For this study, I used the discrimination factor of leopard sharks (*Triakis semifasciata*) as a proxy for sevengill sharks, as this is the most robust discrimination factor study in elasmobranchs. These discrimination factors were  $3.7\text{‰} \pm 0.4$  for nitrogen and  $1.7\text{‰} \pm 0.5$  SD for carbon (Kim et al. 2012a).

I compared carbon and nitrogen isotope signals from sevengill with current literature for various potential prey or key food web species, and compared between San Francisco Bay and coastal isotope signals (Table 3; Cloern et al., 2002). I expected adult sevengills to have a more coastal isotope signal than juveniles, reflective of ontogenetic habitat shifts in sevengill sharks.

Before stable isotope analysis, I chemically removed lipids and urea from muscle samples since these compounds affect  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results. For lipid removal, I placed muscle samples in glass scintillation vials with foil-lined caps and ~10ml of petroleum ether, and sonicated them for 15 minutes (Dobush et al. 1985, Newsome et al. 2010, Kim and Koch 2012). I then removed the supernatant and the process was repeated two more times for a total of three rinses (Kim and Koch 2012). To remove urea, I added 10 ml of deionized water to the scintillation vial and sonicated for 15 minutes; I then repeated this process twice more for a total of three deionized water rinses (Kim and Koch 2012). Muscle samples were placed in a  $-20^{\circ}\text{C}$  freezer overnight and lyophilised overnight.

I measured elemental and isotopic compositions of carbon and nitrogen in the Stable Isotope Ecosystem Laboratory at the University of California, Merced. I placed samples between 500-700 $\mu\text{g}$  into 8 x 5mm tin capsules and combusted in a *Costech 4010 Elemental Analyzer* coupled with a *Delta V Plus Continuous Flow Isotope Ratio Mass Spectrometer*. I corrected results for instrument drift, and mass linearity and standardized to the international VPDB ( $\delta^{13}\text{C}$ )

and AIR ( $\delta^{15}\text{N}$ ) scales using the USGS 41a and USGS 40 standard reference materials (Trayler et al., 2023). Mean isotope compositions for reference materials were USGS 40 =  $-15.3 \pm 0.1\text{‰}$  ( $n = 20$ ) and USGS 41a =  $38.4 \pm 0.1\text{‰}$  ( $n = 12$ ) for  $\delta^{13}\text{C}$  values, and USGS 40 =  $-15.3 \pm 0.1\text{‰}$  ( $n = 20$ ) and USGS 41a =  $45.3 \pm 0.1\text{‰}$  ( $n = 12$ ) for  $\delta^{15}\text{N}$  values, respectively. I determined elemental carbon and nitrogen contents via linear regression of  $\text{CO}_2$  and  $\text{N}_2$  sample gas peak areas against the known carbon and nitrogen contents of USGS 40, USGS 41a, and costech acetanilide.

Despite our removal of chemical lipids, simple linear regression demonstrated some samples had significantly elevated C:N ratios that correlated to  $\delta^{13}\text{C}$  values. I applied arithmetic lipid corrections to all  $\delta^{13}\text{C}$  values following (Hoffman and Sutton 2010) for deep-sea fishes:

$$\delta^{13}\text{C}_{\text{protein}} = \delta^{13}\text{C}_{\text{bulk}} + (-6.39\text{‰} \times (3.76 - \text{C:N}_{\text{bulk}})) / \text{C:N}_{\text{bulk}}$$

Shark-specific approaches have not been developed, but a recent study (Shipley et al. 2017) validated this correction as more accurate than others. Lipid-corrected values resulted in a non-linear relationship between  $\delta^{13}\text{C}$  and C:N ratio, indicating the isotopic effect of lipids was removed as a confounding factor.

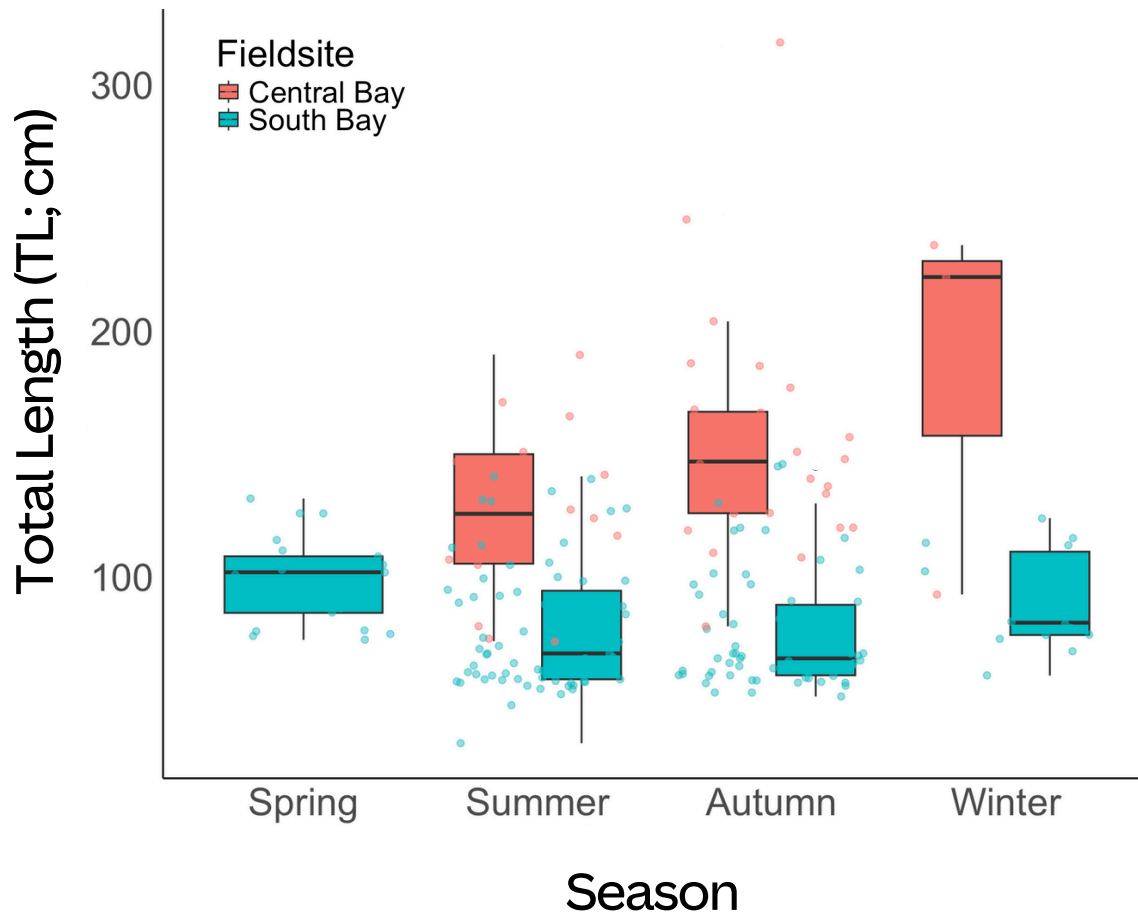
Standard ellipse analysis was conducted for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between sexes and life history groups, with a standard 40% data representation.

## Results

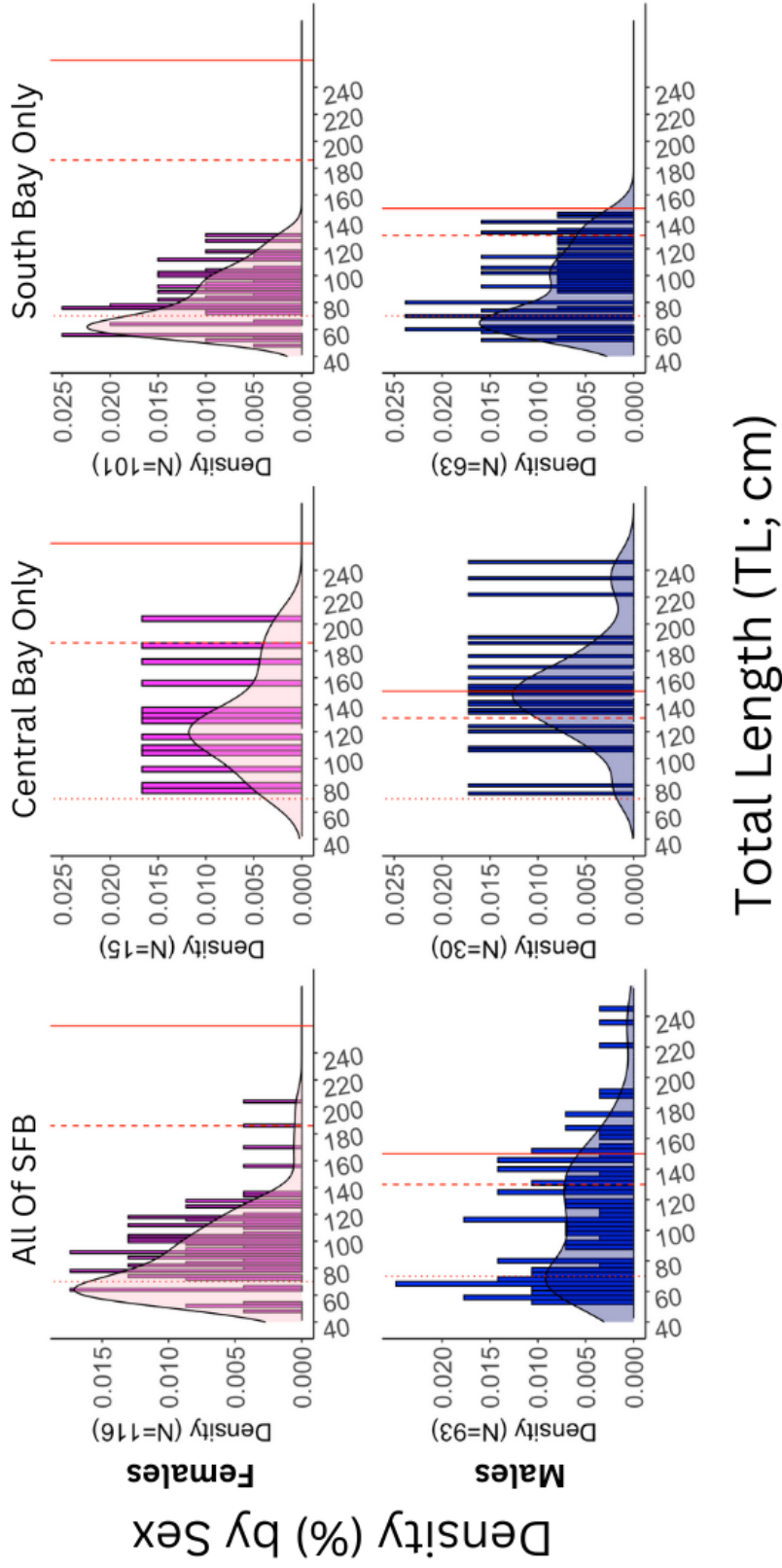
I observed seasonal variations of size representation during this study (Fig. 3). Juveniles were present at both field sites in San Francisco Bay, with year-round presence in South Bay (Fig. 3). I was not able to sample Central Bay during spring months due to both complications from the COVID-19, and historical experience of little presence of sharks at this site in spring months (M. McGill, January 2018, *pers. comm.*). I observed neonates and the smallest juveniles

most frequently during summer and autumn (Fig. 3). Larger animals were more prevalent during winter months (Fig 3).

There is representation of all life history groups for both sexes during this study, with a slight bias in neonate and juvenile females over males (Fig. 4). Neonates and smaller juveniles (<150cm TL) occurred more frequently in South Bay compared to Central Bay (Fig. 4). 100% of neonates were captured at the South Bay site (Fig 4), and 100% if adults were captured at Central Bay (Fig. 4). Adult females were not sampled during this study.



**Fig 3:** Total Length Distribution by Field Site and Season. Boxplots show the mean and standard error of total lengths (cm) of elasmobranchs captured at South Bay (blue) and Central Bay (red). Points are individual observations and colors represent sex. South Bay captures tended to overlap with neonate and juvenile size limits. Central Bay captures tended to overlap with subadult and adult size classes. Juveniles were present year-round, while adults were mostly absent in winter and spring.



**Fig 4:** Total Length Distribution of Female and Male Sevengills Across Field Sites. Rows: sex; columns: site. Dotted, dashed, and solid red vertical lines represent the thresholds for neonate, juvenile, and subadult/adult stages, respectively (Ebert 1989). Density plots are overlaid on histograms to visualize the distribution of total lengths at each sampling site and for each sex.

A total of 221 sevengill sharks were sampled in this study (2019-2023), with a slight bias towards female catch (93.116.0; Table 2). The juvenile life history group was most prevalent in density of any other life history group (n=98; Table 2). I observed few adults in this study, with no adult females captured and only 6 male adults captured. There was a strong bias in male representation for the subadult and adult groups, whereas females were more prevalent in the neonate and juvenile life history groups. As juveniles were the most abundant life history group, it makes sense that this would bias the overall density of female sharks in San Francisco Bay compared to males.

**Table 2:** Counts of sevengill sharks sampled for this study, categorized by sex and life history group. Sample sizes for each life history group and totals are delineated.

Sex	Life History Group			
	Neonates	Juveniles	Subadults	Adults
Female	49	65	2	0
Male	25	33	29	6
Totals	74	98	31	6
<b>Grand Total: 221</b>				

The logistic regression demonstrated a significant ( $p < 0.001$ ) effect of sampling site on the log odds of encountering juvenile sharks (Table 3). Specifically, the odds of encountering juveniles in South Bay were estimated to be approximately  $e^{1.7147} = 5.55$  times higher compared to Central Bay. The model's goodness of fit was assessed using the null and residual deviance values. The null deviance, representing the deviance when only the intercept is included in the model, was 564.16 on 406 degrees of freedom. The residual deviance, indicating the deviance after fitting the model, was reduced to 499.60 on 405 degrees of freedom.

**Table 3:** Logistic Regression Results for Likelihood of Encountering Juvenile Sharks in South Bay vs. Central Bay.

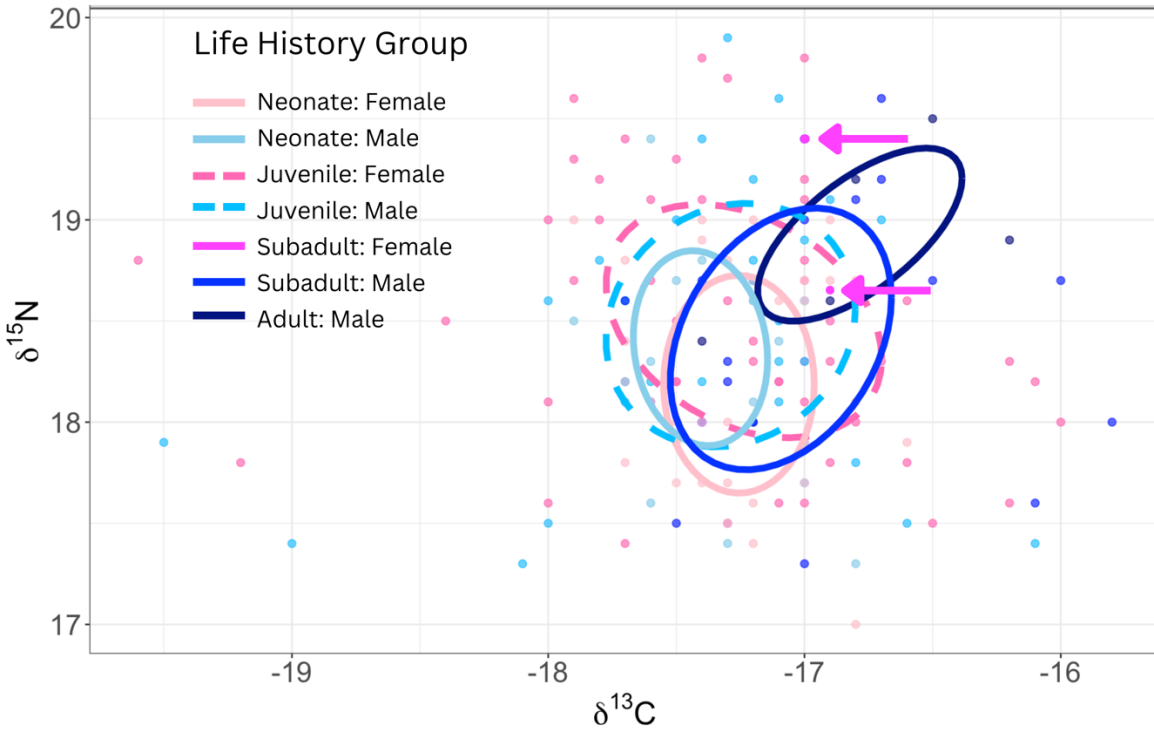
Variable	Estimate	Std. Error	z value	Pr (> z )
Sample Site: South Bay	1.7147	0.2251	7.618	<0.001

**Table 4:** Counts of sevengill sharks sampled for stable isotope analysis, categorized by sex and life history group.

Sample sizes for each category are delineated.

Sex	Life History Group			
	Neonates	Juveniles	Subadults	Adults
Female	30	52	2	0
Male	15	30	22	5

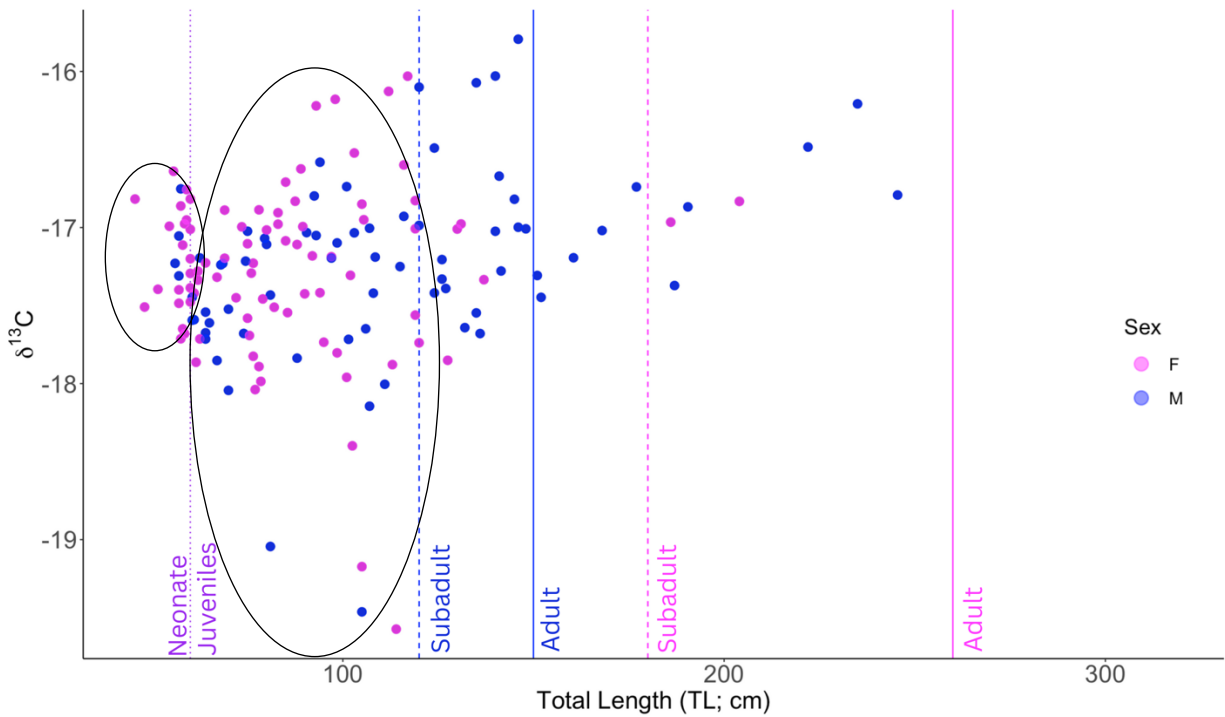
I processed a total of 156 shark white muscle biopsies for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope analysis (Table 4). Life history groups overlapped in isotopic niches as represented by C/N ratios (Fig. 5). Neonate males and females heavily overlap, with male neonates slightly more enriched in  $\delta^{15}\text{N}$  than females. Niches enlarged for male and female juvenile sevengill sharks, which heavily overlap with neonate niches but are more enriched in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signals. Male subadults overlap with all niches, with a slight shift with more enriched  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signals, particularly  $\delta^{13}\text{C}$ . Male adults' niche ellipses overlap the least with other life history groups, becoming even more enriched with  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Two subadult female data points could not be represented with an ellipse because of limited N but fall in a similar area to the adult males, suggesting similar enrichment patterns.



**Fig 5:** Standard Ellipses by Sex and Life History Group. Standard ellipses for C/N ratios with a 40% representation were created to evaluate if niches between life history groups and sexes occurred. Male adults showed the least overlap with all other life stages. Female subadult samples are indicated with arrows, suggesting enrichment similar to adult males.

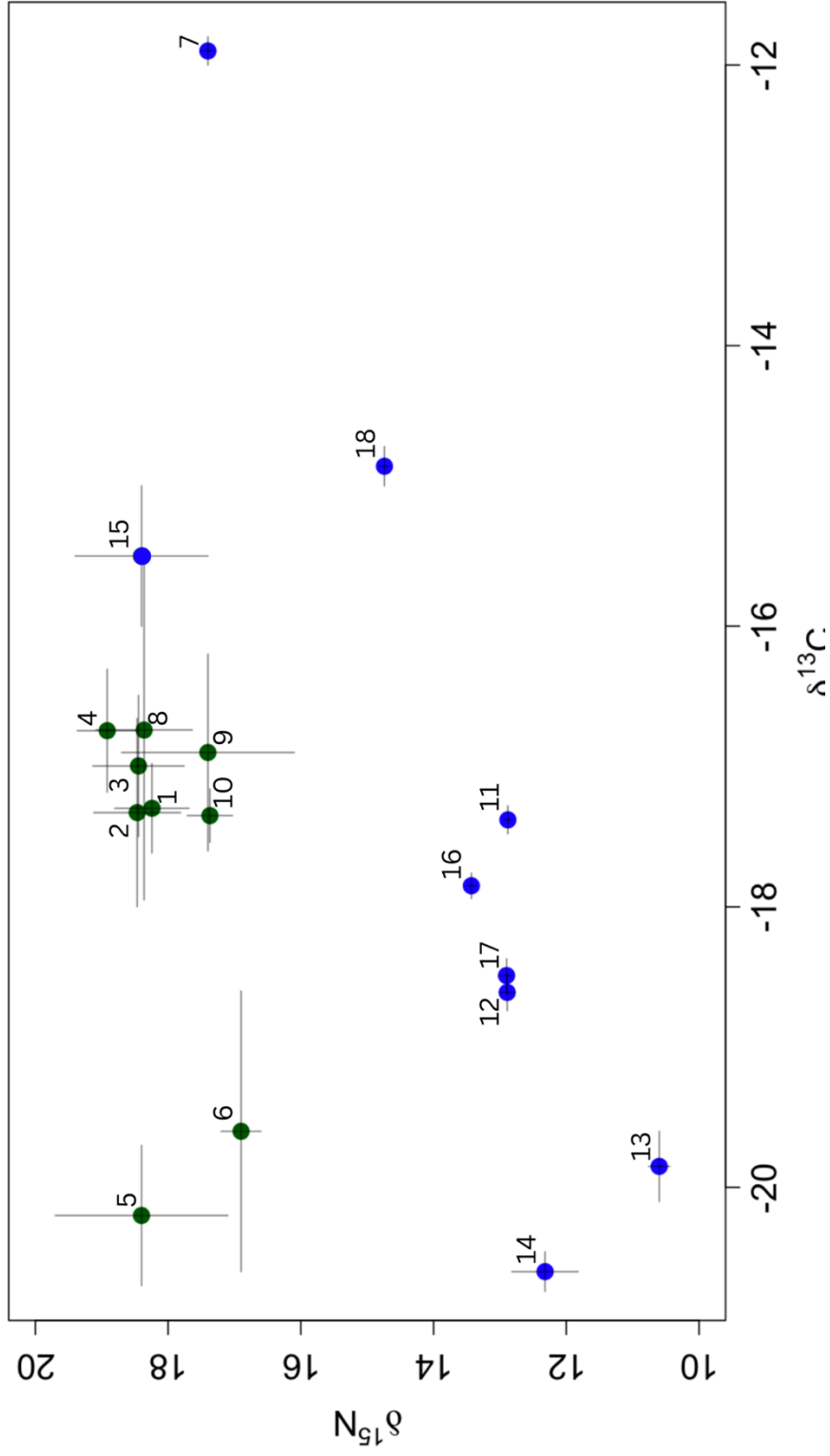
Isotope data for sevengill neonates are tightly clustered for  $\delta^{13}\text{C}$  values (Fig. 6). The  $\delta^{13}\text{C}$  variation increases as sevengills grow to the juvenile life history stage. The variation in  $\delta^{13}\text{C}$  quickly expands and is highly variable for juveniles. Subadults and adults appear to increase in  $\delta^{13}\text{C}$  enrichment as they age and are more tightly clustered than juvenile sevengill sharks.





**Fig 6:**  $\delta^{13}\text{C}$  Signals by Total Length and Sex. Relationship between  $\delta^{13}\text{C}$  isotopic values and total length (TL) of sevengill sharks, categorized by sex and life history stages (Neonate, Juvenile, Subadult, Adult). Vertical lines denote typical TL thresholds for each life stage, with corresponding labels. Points represent  $\delta^{13}\text{C}$  values, color-coded by sex (blue for males, magenta for females).

I compared mean isotope signals of each sevengill life history with mean isotopic signals of potential prey and other key food web representatives from the literature, adjusted for discrimination factor (Fig. 7, Table 5). The mean isotopic composition of animals inside of the San Francisco Bay region was highly enriched with  $\delta^{15}\text{N}$  relative to coastal species except for sea lions, white sharks, and California mussels.



**Figure 7.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of each sevengill shark life history group (neonate, juvenile, subadult, adult) and other key species within the San Francisco Bay and California coast food webs. Samples of animals found in San Francisco Bay are colored green, and coastal species are colored blue. Each point represents the isotopic mean for a species, with standard deviations depicted with bars for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Individual species are labeled according to Table 5. All species values are adjusted to account for discrimination factor (Kim et al. 2012a). Error in discrimination factors was propagated into error of mean species values.

**Table 5.** Isotopic composition of sevengill sharks with other key species in the San Francisco Bay and coastal California food webs.

<b>ID</b>	<b>Species</b>	<b><math>\delta^{13}\text{C}</math> Mean</b>	<b><math>\delta^{13}\text{C}</math> SD</b>	<b><math>\delta^{15}\text{N}</math> Mean</b>	<b><math>\delta^{15}\text{N}</math> SD</b>	<b>Region</b>	<b>Tissue</b>	<b>N</b>	<b>Reference</b>
1	Neonate sevengill ( <i>Notorynchus cepedianus</i> )	-17.3	0.7	18.2	0.7	South SFB	Muscle	45	Present study
2	Juvenile sevengill ( <i>Notorynchus cepedianus</i> )	-17.3	0.3	18.5	0.6	SFB	Muscle	82	Present study
3	Subadult sevengill ( <i>Notorynchus cepedianus</i> )	-17.0	0.5	18.5	0.7	Central SFB	Muscle	24	Present study
4	Adult sevengill ( <i>Notorynchus cepedianus</i> )	-16.7	0.4	18.9	0.5	Central SFB	Muscle	5	Present study
5	South Bay particulate organic matter	-20.2	0.5	18.4	1.3	South SFB	Baseline/ whole organism	12	Kuntz, unpublished data
6	Central Bay particulate organic matter	-19.6	1.0	16.9	0.3	Central SFB	Baseline/ whole organism	8	Kuntz, unpublished data
7	California mussel ( <i>Mytilus californianus</i> )	-11.9	0.1	17.4	0.1	Coastal SFB	Muscle	5	(Vokhshoori et al. 2014, Vokhshoori and McCarthy 2014)
8	Marine-estuarine phytoplankton	-16.7	1.21	18.4	0.7	SFB	Whole organism	31	(Cloern et al. 2002)

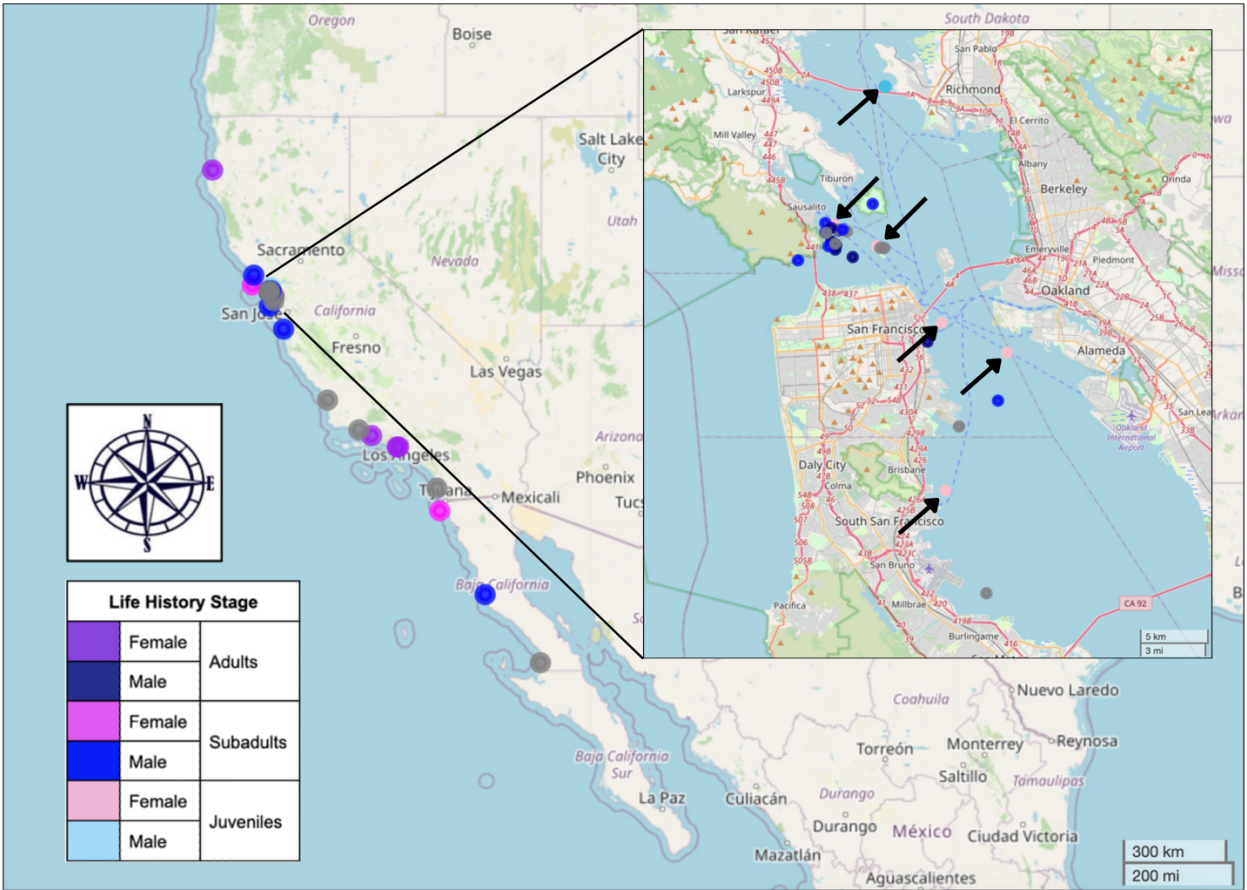
9	Harbor seal ( <i>Phoca vitulina</i> )	-16.9	0.7	17.4	1.3	SFB	Blood serum	17	(Germain et al. 2012)
10	California sea lion ( <i>Zalophus californianus</i> )	-17.4	0.2	17.4	0.3	Monterey Bay	Liver	8	(Bernstein et al. 2021)
11	Sardine ( <i>Sardinops sagax sp.</i> )	-17.4	0.1	12.9	0.1	Coastal	Muscle	21	(Bernstein et al. 2021)
12	Juvenile rockfish ( <i>Sebastes sp.</i> )	-18.6	0.13	12.9	0.1	Coastal	Muscle	46	(Bernstein et al. 2021)
13	Krill ( <i>Euphausia pacifica</i> and <i>Thysanoessa spinifera</i> )	-19.9	0.3	10.6	0.2	Coastal CA	Whole organism, calculated to muscle	10	(Bernstein et al. 2021)
14	Prawn ( <i>Sergestidae sp.</i> )	-20.6	0.1	12.3	0.5	Coastal CA	Muscle	1	(Bernstein et al. 2021)
15	White shark ( <i>Carcharodon carcharias</i> )	-15.5	0.5	18.4	1.0	Coastal SFB	Muscle	21	(Carlisle et al. 2012)
16	California anchovy ( <i>Engraulis mordax</i> )	-17.9	0.1	13.4	0.1	Coastal CA	Muscle	21	(Bernstein et al. 2021)
17	Market squid ( <i>Doryteuthis opalescens</i> )	-18.5	0.1	12.9	0.1	Coastal CA	Muscle	28	(Bernstein et al. 2021)
18	Dungeness crab ( <i>Metacarcinus magister</i> )	-14.9	0.1	14.7	0.1	Coastal CA	Muscle	29	(Bernstein et al. 2021)

### *Recapture results*

Of the sevengill sharks that were recaptured and measured by fishers ( $n = 30$ ), 100% of juvenile sevengill sharks ( $n=12$ ) were recaptured within San Francisco Bay (Table 6, Fig. 8). Time at liberty for these juveniles ranged from 0.2 to 3.9 years. In contrast, 100% of measured sharks recaptured coastally outside of San Francisco Bay were either subadults or adults ( $n = 10$ ).

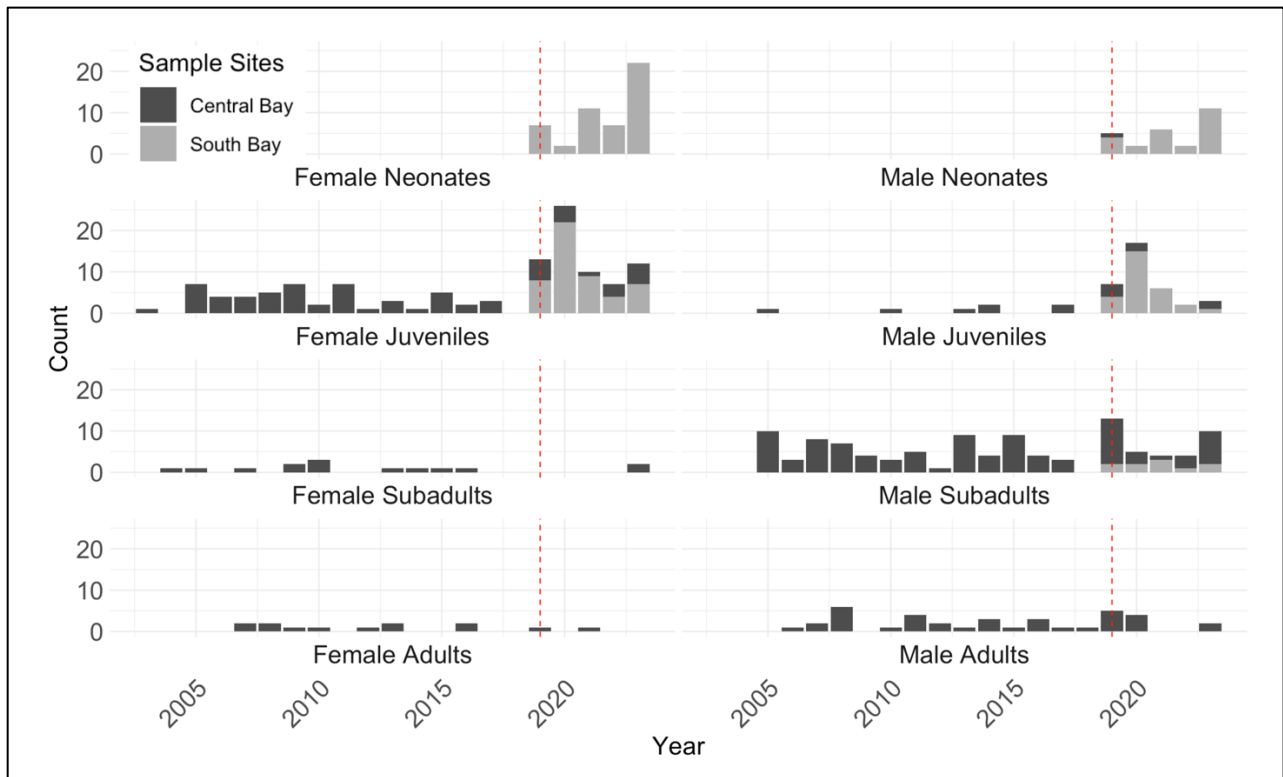
**Table 6:** Recapture data of combined datasets from this study (2019-2022) and data shared by Monterey Bay Aquarium (2003 - 2023). Of individuals tagged ( $n= 432$ ), ~10% were recaptured ( $n=42$ ). Individuals without total length recorded were removed for evaluating life history demographics. The remaining recaptures ( $n=30$ ) demonstrate that 100% of juvenile recaptures occurred within San Francisco Bay, while 100% of recaptures that occurred along the coast were all either subadults or adults.

<b>Life History Group</b>	<b>Sex</b>	<b>Coastal</b>	<b>San Francisco Bay</b>
Adults	Female	4	0
	Male	0	7
Subadults	Female	2	0
	Male	4	7
Juveniles	Female	0	5
	Male	0	1



**Fig 8:** Recaptures that occurred outside of San Francisco Bay were either subadults or adults, ranging from Humboldt Bay, California, USA to Laguna Manuela, Mexico. Arrows identify all re-captured juvenile age classes, which all occurred inside of San Francisco Bay.

Sevengill presence was consistent from 2003-2023 in San Francisco Bay from combined datasets for this study ( $n = 222$ ) and Monterey Bay Aquarium's data ( $n = 211$ ; Fig. 9). There is a higher frequency of male subadult and adults captured than females. More female juveniles are captured than males at the Central Bay sample site, but because females mature to a juvenile life history group at a smaller TL than males (Table1). No neonates were captured before 2019 because Monterey Bay Aquarium did not sample in South Bay, the only place where neonates occur (Fig. 4).



**Fig. 9:** Total Catch of Each Life History Group from 2003-2023. Datasets between this current study (2019-2022), and data collected by Monterey Bay Aquarium (2003-2023) were combined to demonstrate the long-term use of San Francisco Bay, California of all life history groups. Catch counts were visualized between Central Bay and South Bay. Catch counts increased starting in 2019 (indicated with a red dashed line) when these two datasets were combined, and sampling began in South Bay. Scales on the y-axis for each group vary to increase visualization.

## Discussion

I applied a formal test using multiple strands of evidence to show that San Francisco Bay provides a nursery ground for sevengill sharks. Juveniles are more frequently encountered in South Bay than Central Bay (Criterion 1, supported by H1); juveniles remain in the area for extended periods (Criterion 2, supported by H3 and recapture data); and the area is repeatedly used across years (Criterion 3, supported by H2), supporting that San Francisco Bay is a nursery area for the sevengill shark (Heupel et al. 2007).

Catch data provided by Monterey Bay Aquarium demonstrate persistent sevengill use of San Francisco Bay across decades (H2; Fig 9). This is reflected with juveniles, subadults, and adult life history groups continuously encountered during opportunistic sampling by Monterey Bay Aquarium in Central Bay from 2003-2023. Additionally, 100% of recaptured juveniles were captured within San Francisco Bay (Fig. 8), supporting that juveniles may stay in these areas between recapture events. Year-round sampling between 2019-2023 demonstrates presence of juveniles across seasons in South San Francisco Bay (Fig. 3), further supporting persistence occupancy of sevengills in the nursery area across years.

Extended residency of juveniles is supported with stable isotope analysis, where juvenile isotope signals directly align with isotope signals of other species within San Francisco Bay (Fig. 7). The  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope area for juveniles is larger than observed for neonate sevengills (Fig 5.). Further investigation of  $\delta^{13}\text{C}$  signals by TL would also suggest a highly variable environment for juvenile sevengills when compared to neonate sevengills (Fig 6). These data would support a shift in isotope signals between neonate and juvenile sevengill sharks.



All isotopic signatures within the bay, including pelagic (particulate organic matter) and benthic (marine-estuarine phytoplankton) baseline signals, are highly enriched with  $\delta^{15}\text{N}$  compared to coastal species signatures, indicating foraging within San Francisco Bay. The only exceptions of highly enriched  $\delta^{15}\text{N}$  outside of San Francisco Bay are sea lions, white sharks, and California mussels. Sea lions were sampled in Monterey Bay, and white sharks were sampled between Monterey and Bay and the Farallon Islands, just outside of San Francisco Bay. Mussels were collected in Pacifica, also just outside of San Francisco Bay. The close or overlapping proximity of these species to San Francisco Bay potentially reflect some of this  $\delta^{15}\text{N}$  enrichment. Other coastal species were collected from various locations between San Diego and Humboldt and do not exhibit the same  $\delta^{15}\text{N}$  enrichment as seen within or just outside of San Francisco Bay (see Table 5 references for more information). Furthermore, consumers  $\delta^{13}\text{C}$  are typically reflective of  $\delta^{13}\text{C}$  of pelagic and benthic carbon baselines (Duffill Telsnig et al. 2019). In this study, neonate through subadult mean  $\delta^{13}\text{C}$  signatures all land between -17.0‰ to -17.3‰ with a max SD of  $\pm 0.7$  (Table 5). The San Francisco Bay pelagic baseline (particulate organic matter), range from -19.6‰ to -20.2‰ with a max SD  $\pm 1.21$  (Table 5). The San Francisco Bay benthic baseline (marine-estuarine phytoplankton) is  $-16.7\text{‰} \pm 1.0$  (Table 5). Sevengill neonate through adult signature land between these two baselines, with a closer association with the benthic baseline (Fig. 7). This makes sense, as prey species of sevengills within San Francisco Bay will primarily be consuming prey from the benthos, as opposed to filtering pelagic organic matter. Sevengill signatures landing between the pelagic and benthic baselines of San Francisco Bay suggests that juvenile sevengills are acquiring their food sources from within the Bay during their residency. This is because consumer tissues become enriched with stable isotopes of their prey (Sweeting et al. 2007, Canseco et al. 2022) and can create a history of where prey was

consumed. Isotopic signals within white muscle have an estimate of 422 days for metabolic turnover to 95% of new diet equilibrium (MacNeil et al. 2006), demonstrating over a year's worth of consumer history. This long-term storage of isotopes allows researchers to examine isotopes within the white muscle tissue of sharks that reflects 422 days of the geographic source of their prey.

Male sevengill adults had the least overlapping isotopic niche of any other life history group (Fig. 5) and was the only life history group of sevengill sharks to not fall in between the San Francisco Bay pelagic and benthic baselines (Fig. 7), although they are in close proximity. The male sevengill adult signals are more closely aligned with white sharks (Carlisle et al. 2012) and California mussel (Vokhshoori et al. 2014, Vokhshoori and McCarthy 2014) signals than other sevengill life history groups, both of which were sampled just outside of San Francisco Bay. This likely reflects an ontogenetic shift in habitat use, where adult sevengill sharks are beginning to shift into a more coastal environment than younger age classes that are resident to San Francisco Bay. I only sampled two subadult female sevengill sharks in this study, which overlapped with the isotopic range of male sevengill adults, potentially demonstrating that females experience an ontogenetic shift in habitat earlier in their life histories than males. More samples of both subadult females, adult females, and adult males are needed to thoroughly examine this overlap.

The northeastern Pacific population of sevengill sharks likely mixes along the United States coastline (Larson et al. 2015). I showed that individual sevengill sharks tagged within San Francisco Bay travel extensively, ranging as far north as Humboldt Bay, California, and south to at least Laguna Manuela, Mexico (Fig. 8). San Francisco Bay is centrally located within the northeastern Pacific range of sevengill sharks. The long-distance movements of adult sharks

from San Francisco Bay suggest that this area may serve as a nursery habitat, potentially supplying sevengill sharks throughout the entire northeastern Pacific range. Chondrichthyans generally have slow growth rates, slower sexual maturation, and longer generation times (Cailliet et al. 2005), making them vulnerable to depletion and slow for recovery. Considering this study demonstrates year-round aggregations of juveniles, and persistent use of adults each year (Fig. 9), this makes sevengills prone to depletion if they are heavily targeted. This could have disproportionate impacts by depleting apex predators that have shown to migrate long distances, impacting ecosystems that they inhabit at various parts of their life history. Sevengill sharks have high litter sizes of an average of 80 pups per litter (Ebert 1989), which may be a unique life history strategy that provides higher population rebound potential, compared to other shark species, which typically have between 1-15 pups per gestation period, and typically less than 5 for apex predator sharks (Smith et al. 1998) . This is the first demonstration of year-round use of this habitat of neonate and juvenile sevengills (Fig. 3), a unique life history strategy not seen in other populations of sevengill sharks around the world (Barnett et al. 2010b, Jaureguizar et al. 2022). Further investigation is needed to determine whether this concentrated area of juvenile sevengill sharks poses a threat to the population when the species also demonstrates high fecundity, and therefore strong rebound potential.

Pupping and nursery grounds in other populations of broadnose sevengill sharks around the world demonstrate different habitat use than I have observed in the northeastern Pacific and San Francisco Bay. The only other suggested broadnose sevengill nursery ground is in the nearshore waters of Uruguay and northern Argentina (Jaureguizar et al. 2022), a similar habitat to San Francisco Bay. Juveniles there occur within San Antonio Cape, Argentina seasonally from September - May, and then disperse to unknown locations. A more formal assessment is needed

to evaluate if San Antonio Cape serves as a seasonal nursery area. Researchers in Tasmania have also attempted to identify broadnose sevengill nursery areas (Barnett et al. 2010b). The habitats they investigated included Norfolk Bay and Derwent Estuary, habitats offering similar protection and complexity as San Francisco Bay, and which function as nursery grounds for other shark species including the gummy shark, *Mustelus antarcticus*, and school sharks, *Galeorhinus galeus* (Olsen 1954, Stevens and West 1997, Barnett et al. 2010b). Similar seasonal adult presence was observed in previous studies conducted in both California, USA (Ebert 1989), and north Patagonia, Argentina (Lucifora et al. 2005). Despite these similarities in habitat and adult presence, there was no indication of juvenile presence. Widespread knowledge about juvenile habitat use in the Tasmanian population remains limited, with the conclusion that juveniles here may inhabit various locations across their range or in nursery areas yet to be identified. (Barnett et al. 2010b).

The importance and implications of identifying nursery areas extend beyond understanding their spatial distribution, but also identify vulnerable areas prone to heightened risks, potentially leading to adverse impacts on the population. Range shifts resulting from climate change potentially expose animals to different environmental cues. Nursery areas are particularly at risk from climate change (Heupel et al. 2007, Chin et al. 2010, Matich and Heithaus 2012, Matich et al. 2020, Rummer et al. 2022). Shark and other marine species distributions are expected to shift poleward in response to warming temperatures (Rummer et al. 2022). White shark (*Carcharodon carcharias*) juvenile range has moved northward from southern to central California (Tanaka et al. 2021). Warming temperatures in the Atlantic Ocean have facilitated novel bull shark (*Carcharhinus leucas*) recruitment to Pamlico Sound, North Carolina (Bangley et al. 2018). Temperature decreases signal to sharks to depart nursery areas

(Heupel et al. 2007), while future climate-induced temperature increases may disrupt the natural cues of sevengill sharks (Rosa et al. 2014, Crear et al. 2020). The displacement of sharks may impact phenological cycles and seasonal patterns of distribution due to a shift in light and temperature cycles (Servili et al. 2020, Rummer et al. 2022). This change could impact parts of shark life history, such as breeding, migrating, and reproduction (Mull et al. 2008, Abrahms et al. 2018, Rummer et al. 2022).

The impact of increasing temperatures on nursery grounds for sevengill sharks can manifest in two distinct but potentially detrimental ways. Elevated temperatures in the nursery ground for sevengill sharks could have a disproportionate impact on population viability by displacing the theorized pupping and nursery ground outside of the protected San Francisco Bay. While displacement can allow for immediate survival, having juveniles shift to coastal waters may not necessarily improve long-term survival and recruitment (Knip et al. 2010). This has been demonstrated in Bull sharks (*Carcharhinus leucas*), where the young moved out of their protected nursery area in Florida due to a sudden decrease in salinity within their environment. While moving to an area with appropriate salinity increased immediate survival, it also exposed juveniles to predation (Simpfendorfer et al. 2005). Climate change could cause the San Francisco Bay habitat to be less suitable for broadnose sevengill shark survival, but strong philopatry in this population could cause them to continue to use the Bay regardless of suitability and therefore threaten recruitment. Return of shark species to unsuitable habitats has been demonstrated in lemon shark nursery areas (*Negaprion brevirostris*; Stump 2013) and for bull sharks when decreased water temperatures led to their death (Snelson and Bradley Jr 1978). Identifying and confirming shark nurseries is critical to understanding the complex dynamics

between temperature changes, salinity and habitats and providing safeguards to long-term viability of broadnose sevengill shark populations in San Francisco Bay.

Several limitations in this study highlight the need for further research to better understand the residency, habitat preferences, and genetic connectivity of juvenile broadnose sevengill sharks in the San Francisco Bay. One major limitation of this study was the lack of acoustic telemetry to confirm the long-term residency of juveniles (Heupel et al. 2007, 2019). Future studies would benefit from evaluating the residency of South Bay specifically, identifying when sharks leave the Central Bay to open ocean, and identifying when sevengill sharks return. The results of this study would be further advanced with analysis of adult female sevengill sharks, and increased representation of subadults and adults of both sexes. Isotopes between adult female sharks and neonates should be evaluated in the future for maternal influence (Niella et al. 2021) to better understand the source of neonate isotopic signals. Future inclusion of sulfur  $\delta^{34}\text{S}$  isotopes would also strengthen the comparison of samples from within San Francisco Bay compared to coastal waters (Munroe et al. 2018). This study confirmed the presence of juvenile sharks in the San Francisco Bay but did not investigate the factors influencing their habitat selection. Future studies would benefit from comparing habitat and water quality differences between Central and South Bay, and how this differs from other nursery areas for sevengill sharks (i.e. Jaureguizar et al. 2022). While San Francisco Bay serves as a nursery area for juvenile sharks, further evaluation of alternative nursery areas such as Humboldt Bay is necessary to assess its importance for this population. Future studies should also thoroughly examine the genetic connectivity of sevengills along their range. While a study has occurred directly comparing San Francisco Bay, California, and Willapa Bay, Washington, USA (Larson et al. 2015), only 13 microsatellites were used for analysis, and the population probably ranges

from Mexico to Alaska. Further and more intensive SNPS genetic analysis would more precisely elucidate the connectivity of this population along its range. Ideally, the genetic analysis would be complemented with acoustic telemetry data to confirm the connectivity of sevengills along the northeastern Pacific coastline. Addressing these limitations and conducting further comprehensive studies will not only enhance our understanding of broadnose sevengill shark ecology in the San Francisco Bay but also inform more effective conservation and management efforts for this vulnerable species.

### *Conclusion*

Understanding and protecting nursery habitats becomes increasingly urgent in the face of climate change and anthropogenic-induced disruptions. The substantial presence of neonate and juvenile sevengill sharks within South San Francisco Bay compared to Central Bay (Table 3) suggests the importance of South Bay as a nursery habitat for the population (H1, Fig 4). While juvenile presence existed in both the South and Central Bay areas, no adults were encountered in South Bay, and no neonates were encountered in Central Bay. Protecting essential fish habitat can significantly enhance species recruitment and recovery (Melaku Canu et al. 2021). For deep-sea fishes, modeling efforts to identify essential fish habitat have shown promise in improving ecosystem-based management strategies (Moore et al. 2016). Given that San Francisco Bay is consistently reported as the primary area for juvenile presence and has been identified as a nursery area, it is crucial to recognize it as an essential fish habitat in management decisions. Decisions regarding habitat management in San Francisco Bay could thus have broad implications for the conservation of sevengill sharks across their entire range, spanning political borders. Precautionary broadnose sevengill shark management strategies are essential to mitigate

potential threats, particularly in highly anthropogenically influenced areas such as San Francisco Bay. The recognition of San Francisco Bay as a nursery area, and therefore essential fish habitat, should prompt a reevaluation of management practices and regulations across regions. When comparing fishing regulations between California and Washington, California enforces a one-bag limit with no restrictions on size, sex, season, or location, while Washington has implemented a moratorium on sevengill fishing. There are limited potential locations that provide habitat similar to the San Francisco Bay on the west coast of the USA. A lack of pupping opportunities due to nursery habitat constraints makes the population vulnerable to environmental perturbations, warranting careful management strategies to ensure continued viability.

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# Chapter 2

Title: Physiological stress response of juvenile sevengill sharks, *Notorynchus cepedianus*, in the nursery grounds of San Francisco Bay, California

## Introduction

Recreational shark fishing can have serious consequences for shark populations (Gallagher et al. 2017). These encounters have the potential to result in mortality or sublethal effects of sharks from these interactions. Mortality rates are often linked to the intensity and duration of the angling event (Mandelman and Skomal 2009, Ellis et al. 2017) but even sublethal effects can have detrimental impacts on shark health and survival (Pickering 1981, Wedemeyer et al. 1990, Pickering and Pottinger 1995, Skomal and Mandelman 2012, Jerome et al. 2018). Physiological indicators can provide valuable insights on the effects of angling. Changes in blood chemistry or stress hormone levels indicate the stress response of sharks during fishing and associated intense physical activity, hooking and/or handling injuries, and air exposure. By quantifying these physiological responses, researchers can gain a clearer understanding of the impact of fishing activities on sharks to help inform conservation and management efforts aimed at mitigating these effects.

Assessing stress through the endocrine response is a common practice in vertebrates, allowing for a deeper understanding of the physiological mechanisms underlying stress perception and response (Bouyoucos et al. 2021). There are primary, secondary, and tertiary

responses to stress driven by endocrine mechanisms underlying stress perception and physiological adaptations (Pickering 1981, Wedemeyer et al. 1990, Pickering and Pottinger 1995, Wendelaar Bonga 1997, Bouyoucos et al. 2021). The primary stress response is fight-or-flight response, involving the brain's initial perception of a stressor and the subsequent release of catecholamines (e.g. epinephrine and norepinephrine), along with energy metabolites (Wendelaar Bonga 1997). These hormones and metabolites provide the animal with immediate physiological changes needed for rapid response to stress, such as increased heart rate. Prolonged stress events in ray-finned fishes typically lead to the subsequent release of cortisol as the primary glucocorticoid in response to stressful events (Wendelaar Bonga 1997). In elasmobranchs the prevailing corticosteroid is  $1\alpha$ -hydroxycorticosterone ( $1\alpha$ -OHB), although whether it serves as a glucocorticoid or functions as a stress hormone remains elusive (Idler and Truscott 1967, Anderson 2012, Wheaton et al. 2018, Schoen et al. 2021). Further examination of  $1\alpha$ -OHB is necessary to better understand its role in elasmobranchs and if it is involved in the stress axis.

The secondary stress response affects tissues, leading to increased gluconeogenesis, lactate production, acidosis, and other physiological changes (Wendelaar Bonga 1997). Blood acidosis is marked by a decline in pH, and is coupled with an increase in lactate as a result of anaerobic glycolysis that occurs during intense exercise (Brooks 2007). Interestingly, sharks metabolize ketone bodies (e.g. Beta-Hydroxybutyrate;  $\beta$ -HB) as an energy source in white muscle during intense exercise in addition to glucose (Richards et al. 2003). Glucose, lactate, and pH are often the most common blood parameters measured for stress in sharks (Hoffmayer and Parsons 2001, Frick et al. 2010, Marshall et al. 2012, Fuller 2019). Blood acidosis creates an imbalance of electrolytes and osmolytes in the cells that can lead to difficulties in osmoregulation, and can be measured as osmolarity (Marshall et al. 2012). Mineral imbalances

may result in insufficient oxygen delivery to tissues, prompting some animals to augment their red blood cell size or numbers. This adaptation facilitates efficient oxygen provision during intense exercise. An increase in red blood cells can be measured as hematocrit (Hct). Buffy Coat (BC) is often measured alongside hematocrit during blood analysis, which primarily consists of white blood cells (leukocytes) and platelets. An increase in white blood cell count can represent an immune response in many vertebrate species, including as a response to stress (Pedersen et al. 1990). Quantifying these parameters in blood is an effective way to measure the physiological stress sharks experience under various conditions.

Tertiary stress encompasses long-term sublethal effects, where physiological stress impacts an animals overall health and fitness (Bouyoucos et al. 2018). Conservation management can be improved when the significance of physiological stress during fishing interactions is considered in habitats essential for their life history (Metcalf et al. 2012). Juvenile sharks often rely heavily on nursery habitats for protection and survival, making these areas critical for their development and long-term population persistence (M. R. Heupel et al., 2007a). Investigating the physiological stress experienced by juvenile sharks in their nursery grounds provides valuable insights into the potential impacts of tertiary stress.

Quantifying physiological responses of juveniles to fishing in nursery habitats is important for determining if protections are needed in these areas. In the northeastern Pacific range, San Francisco Bay is the primary known pupping and nursery ground for the broadnose sevengill shark, *Notorynchus cepedianus* (Holst, Chapter 1). This is defined as an area where juveniles (1) are more commonly encountered than in other areas; (2) they remain in the area for extended periods; and (3) the area is used repeatedly across years (M. R. Heupel et al., 2007b). Recreational fishing is a popular and economically important activity in San Francisco Bay, and



sevengill sharks are often targeted or caught as bycatch during salmon, halibut, and rockfish fisheries. The extent to which fisheries interactions generate strong physiological responses in sevengills, potentially leading to mortality or harmful sub-lethal effects, is unknown. The long-term physiological consequences of human-shark interactions is highly context-dependent, including angler behavior (e.g. time of fight, length of handling out of water) and environmental conditions (Cooke et al., 2013). Fishing interactions of large sharks often involve extended fight times of reeling the animal in and additional handling by anglers (Cooke et al. 2013). Additionally, the frequency of fishing interactions is important to understand how often sevengill sharks experience fisheries interactions. Although population estimates for sevengill sharks are limited, I evaluated fishing pressure through surveys of charter captains, offering insights into the persistent physiological stressors faced by these sharks, in Chapter Three.

Here I explore the physiological impacts of acute fishing events on juvenile sevengill sharks on their nursery ground. Juvenile sharks, due to their smaller size, offer a unique opportunity to rapidly reel them in and obtain baseline representation of their blood before the initiation of significant endocrine responses. Subsequently, changes in the blood can be assessed by simulating prolonged fight times experienced by larger sharks, allowing the fish to continue swimming, or ‘fighting’, on the line. Prolonging the fight time of juvenile sharks will replicate longer fishing interactions, during which they may swim for extended periods or be handled by anglers for a longer duration. This facilitates the measurement of the endocrine response over time. I test the hypothesis that fishing interactions induce significant physiological stress responses, evidenced by changes in blood physiology (blood gasses, lactate, glucose, pH, BHB, Osmo, Hct, BC) over a 30-minutes.  $1\alpha$ -OHB will also be measured during this study to further examine the presence of this hormone during acute stress events.

## Materials and Methods

Thirty-one juvenile sevengill sharks were captured via hook-and-line in the southern portion of San Francisco Bay near Coyote Point from September to November 2021. All methods of data collection for this study were approved by CDFW and the animal care regulatory body at the University of California, Davis (Holst permit #: S-223320002-22356-001; Holst IACUC #: University of California, Davis, 22586).

Once an animal was hooked, a timer was started to measure “fight” and handling time. If the animal was identified as a sevengill shark at the surface, it was immediately placed aboard the vessel. Once aboard, a PVC manifold was placed in the animal’s mouth, which pumped fresh saltwater over their gills for ventilation. An initial ~1 ml blood draw was taken immediately to provide a physiological baseline. Blood was taken via the ventral-caudal tail vessel, immediately posterior to the anal fin with a 2” 18-gauge needle. All initial blood samples were taken <2 minutes from the time fish were detected on the line.

The hook either remained in the jaw during this time, or the animal was rehooked after the initial blood draw if the original hook placement posed a risk to the animal (e.g., cutting the gills or eye). The animal was then placed on the side of the vessel to actively swim for a thirty-minute trial while remaining on the line to replicate the fight they may experience from recreational anglers.

During the trial, the animal was retrieved back onboard and ventilated for three subsequent ~1 ml blood draws randomly drawn at 5, 10, and 15 minutes apart for a total of 30 minutes (i.e., the interval order was randomized. Ex: 10, 15, 5 minutes). For each blood sample, 0.1ml of whole blood was used to measure blood gasses immediately on board. The remainder of

the blood sample was then stored on ice for a maximum of 5 hours until it could be stored in a -20°C freezer.

After the 30-minute timed trial, sharks were sexed and measured with a soft measuring tape for total length (TL, measured from the rostrum to the dorsal posterior end of the caudal fin; cm).

### *Blood processing*

Whole blood aliquots of 0.1ml were analyzed with a hand-held point-of-care device (Abbott i-STAT Alinity; Heska Corp, Fort Collins, Colorado) for blood gasses and pH using a CG8+ cartridge (Table 1), which has been validated in sharks (Harter et al., 2014). These samples were processed immediately after collection (<1min). The remaining whole blood was then transferred to a microcentrifuge tube coated with lithium heparin. Heparinized blood was then drawn into two capillary tubes and centrifuged at 12,600g for three minutes for determination of hematocrit values (TRIAC Clay Adams, Parsipanny, NJ USA). The remainder of the sample was spun at 1,500g for five minutes. Plasma was then decanted into clean microcentrifuge tubes and stored at -20°C for later analysis of glucose, blood metabolites, and hormone analysis.

### *1 $\alpha$ -hydroxycorticosterone and corticosterone assays*

Serum extractions, processing, and enzyme immunoassays (EIAs) were performed at Disney's Animal Kingdom Science Center. Lab protocols closely followed the methods described in Schoen, Treberg, et al. (2021), Schoen, Bouyoucos, et al., (2021), and Wheaton et al. (2018). Briefly, 0.5ml plasma samples were extracted in 0.75ml ice-cold acetonitrile, evaporated, and then reconstituted in an assay buffer. Concentrating (1 $\alpha$ -OHB) or diluting

(corticosterone) plasma adjusts hormone measurements into the readable range of standard detection curves (Wheaton et al., 2018). Samples with concentrations below the detectable limit of the assays were considered to have a zero concentration. All chemicals (purchased from Sigma Aldrich) and equipment remained standardized unless otherwise stated. Samples were randomly assorted on the assay plates and respectively assayed in triplicate (1 $\alpha$ -OHB) and duplicate (corticosterone).

**Table 1:** Blood variables measured for this study, with units and measurement methods listed. TC Indicates measurement was temperature corrected for pH, PO<sub>2</sub>, and PCO<sub>2</sub> using Abbott *i*-STAT Alinity; Heska Corp., Fort Collins, CO; CG8+ cartridge.

Blood Variables	Units	Measurement Method
<b>Blood Gasses</b>		
pH <sub>TC</sub>	NA	i-STAT Alinity <sup>®</sup>
PCO <sub>2,TC</sub> (partial pressure of carbon dioxide)	mmHg	i-STAT Alinity <sup>®</sup>
PO <sub>2,TC</sub> (partial pressure of oxygen)	mmHg	i-STAT Alinity <sup>®</sup>
TCO <sub>2</sub> (total carbon dioxide)	mmol.L <sup>-1</sup>	i-STAT Alinity <sup>®</sup>
HCO <sub>3</sub> (bicarbonate)	mmol.L <sup>-1</sup>	i-STAT Alinity <sup>®</sup>
BE <sub>ecf</sub> (Base Excess deficit)	mmol.L <sup>-1</sup>	i-STAT Alinity <sup>®</sup>
SO <sub>2</sub> (oxygen saturation)	%	i-STAT Alinity <sup>®</sup>
<b>Corticosteroids</b>		
1 $\alpha$ -OHB (1 $\alpha$ -hydroxycorticosterone)	ng.mL <sup>-1</sup>	Enzyme immunoassay (EIA)
<b>Other Blood Parameters</b>		
$\beta$ -HB (Beta-Hydroxybutyrate)	mg.dL <sup>-1</sup>	STAT-Site <sup>®</sup>
Glucose	mg.dL <sup>-1</sup>	Accu-Chek <sup>®</sup>
Lactate	mM	Lactate Scout <sup>®</sup>
Osmo (Osmolarity)	mmol.kg <sup>-1</sup>	Osmette <sup>®</sup> III
Hct (Hematocrit)	%	TRIACTM Clay Adams <sup>®</sup>
BC (Buffy Coat)	%	TRIACTM Clay Adams <sup>®</sup>

### *Statistical analysis*

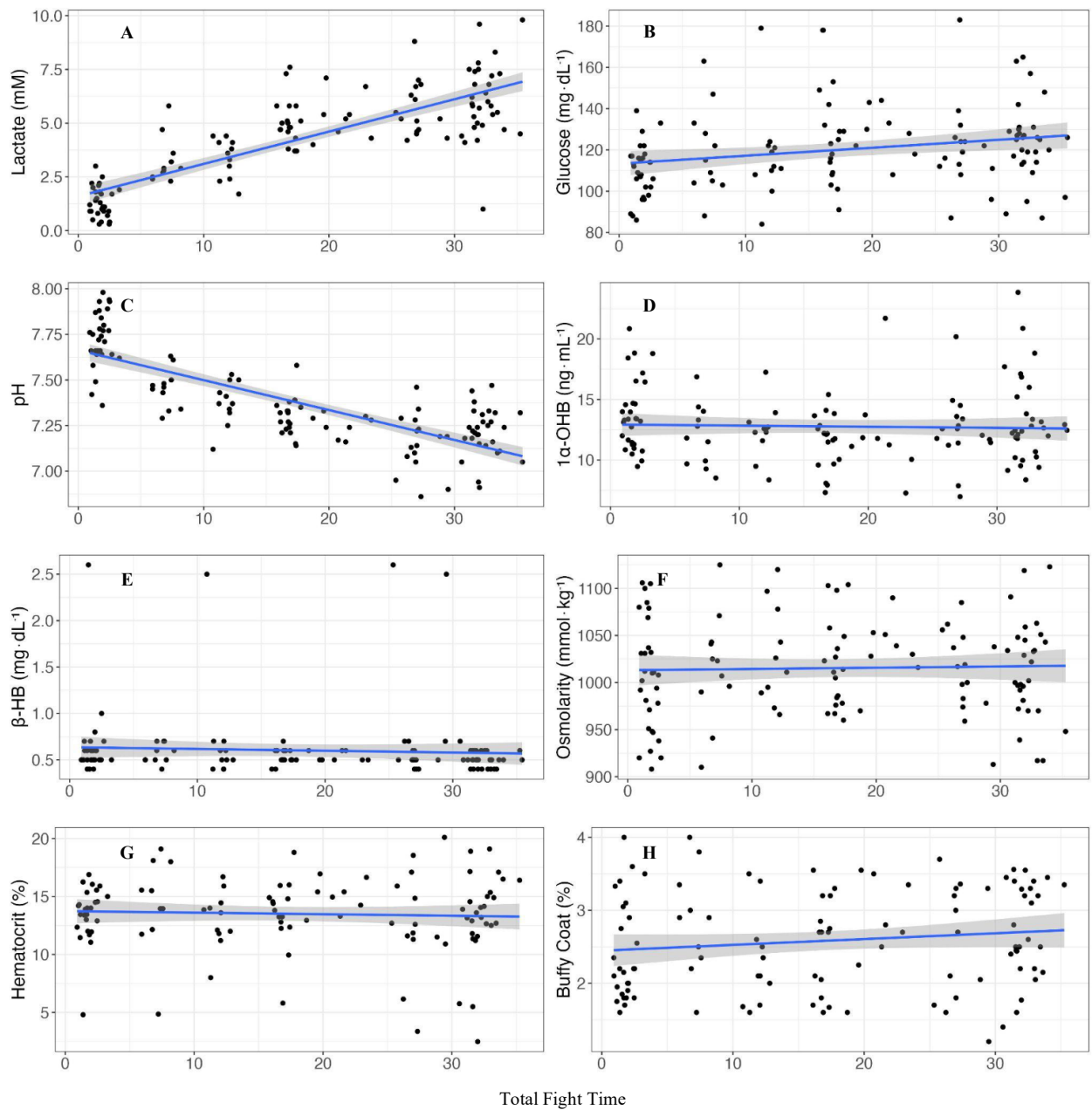
Linear mixed-effects models were developed for each outcome variable. These models aimed to estimate the values of physiological parameters based on a set of predictor variables, including total fight time, total length, temperature, and sex. Shark ID was included as a random effect. Outcome variables included for analysis were lactate, glucose, pH, 1 $\alpha$ -OHB,  $\beta$ -HB, and Osmo. Blood gasses did not have enough individuals represented to fit the model (n=10), so blood gasses were not statistically analyzed. Statistical analyses were conducted using R version 2023.12.1+402 using the “lmer” function in the “lme4” package (Bates et al., 2014).  $R^2c$  values are reported, which represents the conditional coefficient of determination and accounts for both fixed and random effects in the model.

## **Results**

Time was a significant predictor for the following blood variables: lactate ( $p < 2e-16$ ;  $R^2c = 0.7620$ ; Fig 1A), glucose ( $p = 0.000353$ ;  $R^2c = 0.5481$ ; Fig 1B), pH ( $p < 2e-16$ ;  $R^2c = 0.7302$ ; Fig 1C),  $\beta$ -HB ( $p = 0.000349$ ;  $R^2c = 0.6905$ ; Fig 1E), and BC ( $p = 0.0123$ ;  $R^2c = 0.7045$ ; Fig 1H; (Table 2).

Temperature was found to be a significant predictor for pH ( $p = 0.0000606$ ,  $R^2c = 0.7302$ ), 1 $\alpha$ -OHB ( $p = 0.0241$ ;  $R^2c = 0.1657$ ; Fig. 3), and Osmo ( $p = 0.0149$ ;  $R^2c = 0.4350$ ; Table 2).

Total length (TL) was found to be a significant predictor for 1 $\alpha$ -OHB only ( $p = 0.0343$ ,  $R^2c = 0.1657$ ; Table 2).



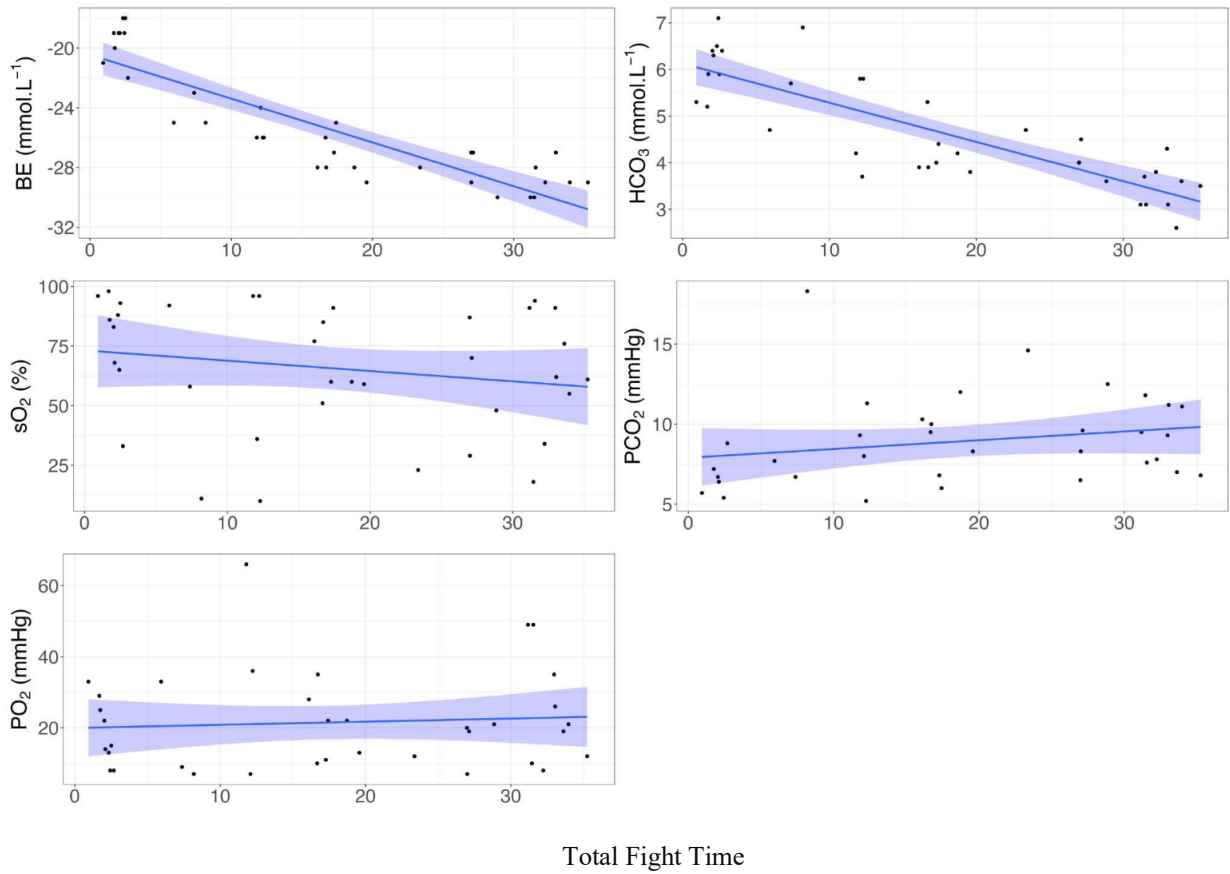
**Figure 1:** Response Variable over Total Fight Time. Scatter plots illustrating the relationship between fight time (Total Fight Time) and blood variables in sharks. Linear regression models were fitted to these variables, including lactate, glucose, pH, 1 $\alpha$ -hydroxycorticosterone (1 $\alpha$ -OHB),  $\beta$ -HB (Beta-Hydroxybutyrate), Osmolality, Packed Cell Volume (Hct), and Blood Cell Count (BC). Each point represents an individual observation.

**Table 2:** Results of linear regression analysis assessing the relationship between outcome variables (pH, Lactate, Glucose, 1a-OHB,  $\beta$ -HB, Osmo, Hct, BC) and predictor variables (Time, Total Length, Temperature, Sex) and blood variables, with associated p-values and coefficients. Notably, significant associations are observed between certain predictor variables and blood variables, indicating potential predictive power in blood parameter variation. Significant p-values are bolded.

Predictor Variable	Blood Variable p-value (coefficient)							
	pH	Lactate	Glucose	1a-OHB	$\beta$ -HB	Osmo	Hct	BC
R <sup>2</sup> c	0.7302	0.7620	0.5481	0.1657	0.6905	0.4350	0.7939	0.7045
Time	<b>&lt;2e-16</b> (-0.0162)	<b>&lt;2e-16</b> (0.1503)	<b>0.000353</b> (0.4040)	0.6716 (-0.0011)	<b>0.000349</b> (-0.001698)	0.5886 (0.09504)	0.6464 (-0.00321)	<b>0.0123</b> (0.0105)
Total Length	0.6931 (0.0002687)	0.8210 (-0.001752)	0.7638 (-0.04196)	<b>0.0343</b> (-0.0280)	0.9816 (0.0006736)	0.6819 (0.1155)	0.1504 (0.03833)	0.1217 (0.008411)
Temp	<b>0.000606</b> (-0.09227)	0.1320 (0.1325)	0.4368 (1.3293)	<b>0.0241</b> (0.3212)	0.3540 (-0.01027)	<b>0.0149</b> (8.7586)	0.4415 (-0.2258)	0.2788 (0.0777)
Sex	0.8632 (- 0.005522)	0.9600 (0.01806)	0.2765 (6.0780)	0.4435 (0.4738)	0.4682 (-0.02571)	0.5686 (7.1423)	0.3430 (1.091)	0.1097 (0.3342)

### *Blood gasses*

Linear regression analysis could not be conducted on the blood gasses due to low sample size ( $n = 10$ ; Fig. 2). Although there are no analytical results to evaluate blood gas relationships, a negative relationship can be seen with BE (Fig. 2B), a slight negative relationship can be seen with  $sO_2$  (Fig. 2C), and a slight positive relationship can be seen with  $PCO_2$  (Fig. 2D).



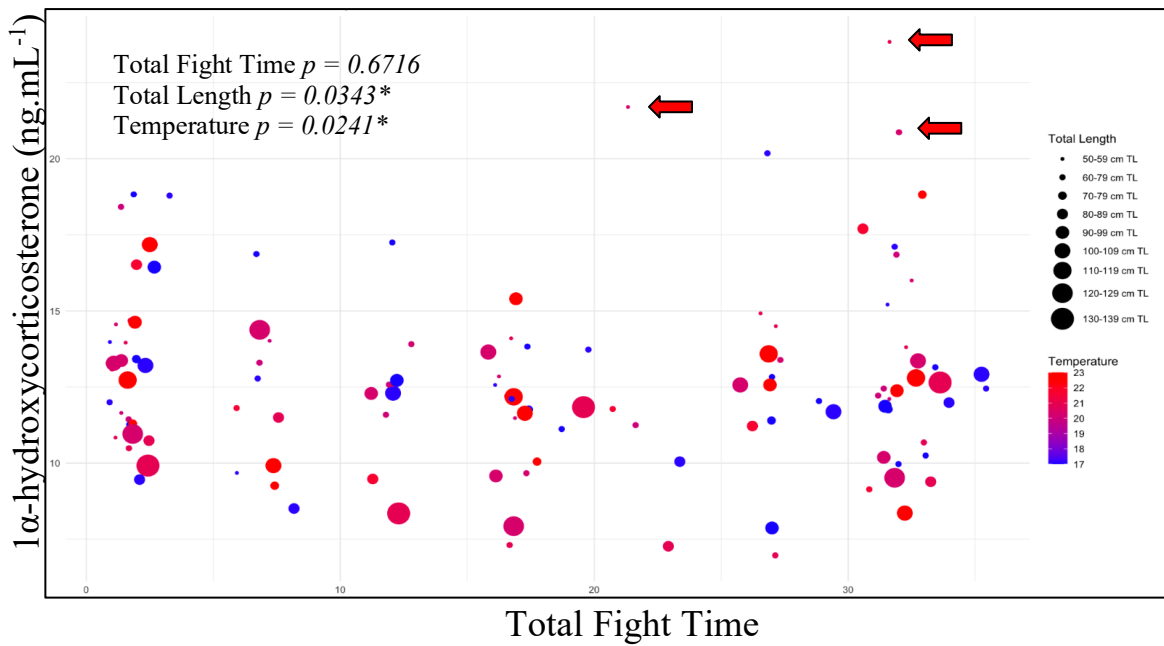
**Figure 2:** Blood Gasses over Total Fight Time. The scatter plots above illustrate the relationship between fight time (Total Fight Time) and various blood gas variables ( $\text{HCO}_3$ , BE,  $\text{sO}_2$ ,  $\text{PCO}_2$ ,  $\text{PO}_2$ ) in sharks. Each point represents an individual observation. While these variables were not included in the linear regression models due to data constraints, a basic trendline is added with the standard deviation shaded behind it.

**Table 3:** The number of individuals in binned size classes to visualize age- or size-related  $1\alpha$ -hydroxycorticosterone responses. Each animal had a total of 4 blood draws taken, for a total of 120 blood samples.

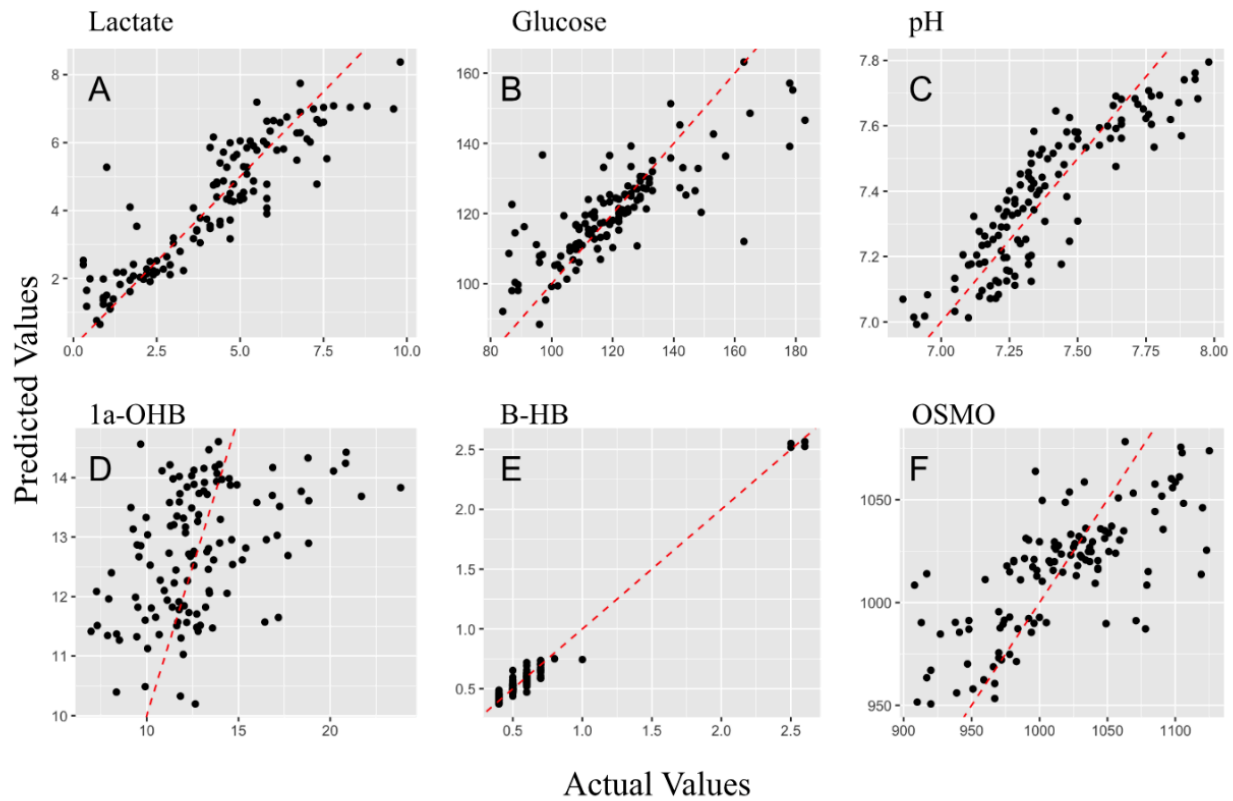
TL (cm)	50-59	60-69	70-79	80-89	90-99	100-109	110-119	120-129	130-139	140-149
# of blood samples	5	11	2	3	3	3	0	1	1	1



### 1 $\alpha$ -OHB Over Total Fight Time



**Fig 3:** 1 $\alpha$ -OHB over Total Fight Time with Temperature and TL. Scatter plot illustrating the relationship between 1 $\alpha$ -hydroxycorticosterone (1 $\alpha$ -OHB) and total fight time, color-coded by temperature (°C), and sized by the total length (TL). Total length is binned into 10 cm intervals, with each bin labeled to represent the range of total lengths it encompasses (Table 3). The linear regression model indicated that fight time was not a predictor of 1 $\alpha$ -OHB, but temperature and TL were.



**Fig 4:** Predicted Model Graphs for Outcome Variables. Scatterplots of actual versus predicted values for modeled outcome variables (lactate, Glucose, pH, 1 $\alpha$ -OHB,  $\beta$ -HB, and Osmo). The dashed red line represents the line of equality, where actual values equal predicted values.

### *1 $\alpha$ -hydroxycorticosterone*

Time did not emerge as a significant predictor of 1 $\alpha$ -OHB in this study. Significant predictors that did emerge included TL ( $p = 0.0343$ ) and temperature ( $p = 0.0241$ ) with an  $R^2c = 0.1657$  (Table 2).

Actual versus predicted values for modeled blood outcome variables were plotted (Fig 4). The lactate model demonstrates accurate predictions across a range of lactate values, as evidenced by notable clustering around the line of equality (4A). The glucose model shows strong predictive performance, with the majority of data points aligning closely with the dashed

line (4B). For pH predictions, while many points cluster around the line of equality, there are instances of deviation from actual values, suggesting caution is needed when interpreting results (4C). Both 1a-OHB (4D) and  $\beta$ -HB (4E) predictions exhibit deviations from the dashed line, indicating potential challenges in consistent prediction across the dataset. The model's performance for predicting Osmo (4F) appears mixed, with some points aligning closely with the line of equality and others deviating noticeably, suggesting variability in capturing osmolality levels based on the provided predictors.

## Discussion

This study investigated the physiological responses of juvenile sevengill sharks to catch-and-release fishing in their nursery habitat of San Francisco Bay, California. Time and temperature emerged as significant factors influencing several blood parameters, indicating the impact of exhaustive anaerobic exercise on sevengill blood physiology.

### *Time as a predictor variable*

Time emerged as a significant factor influencing several, although not all, measured blood parameters. Among the blood parameters that were modeled, pH, lactate, glucose,  $\beta$ -HB, and BC were significantly correlated with time. The relationship of pH and lactate with time is consistent with other shark-related stress physiology studies and reflects onset of anaerobic exercise and increasing stress (ex: Brooks et al., 2011, 2012; Hoffmayer & Parsons, 2001; Hyatt et al., 2018; Kneebone et al., 2013; Schoen et al., 2021). glucose was significantly correlated with time, albeit with a moderate  $R^2$  value ( $R^2 = 0.5481$ ). Hyperglycemia is expected to occur as a result of exhaustive exercise in fish (Barton & Iwama, 1991; Hoffmayer & Parsons, 2001; Mazeaud et al., 1977; Wells & Davie, 1985). Catecholamines are released in the blood in

response to intense physical activity, which facilitate rapid glucose delivery to muscle tissues (Barton & Iwama, 1991; Hoffmayer & Parsons, 2001; Wendelaar Bonga, 1997). BC was significantly correlated with time ( $p = 0.0123$ ) with a relatively strong  $R^2c$  value ( $R^2c = 0.7045$ ) and may represent an immunological response to stress. Southern stingrays (*Dasyatis americana*) inhabiting high tourism areas are characterized by immune suppression (e.g., decreased and unresponsive lymphocytes and heterophils), as well as up-regulation (monocytes and thrombocytes) and down-regulation (eosinophils) of immune responses (Semeniuk et al. 2009). It is possible that the present study has observed an immunological response to acute handling stress in sevengill sharks, but future studies are needed to evaluate the extent of this response.

This study is consistent with other studies that have observed significant changes in lactate, glucose, and/or pH over time (see Skomal and Mandelman 2012 for a review). For example, an increase in glucose and lactate was observed in the Caribbean reef shark, *Carcharhinus perezi* (Poey 1876) that underwent longline capture, and noted physiological recovery as sharks neared the maximum fight time (250 minutes; Brooks et al., 2012). Other studies have used pSAT tags to evaluate post-release mortality and found a 90% survival rate with fight times up to 513 minutes on rod and reel (French et al. 2015). The duration of the fight did not affect mako shark survival but showed a significant correlation with levels of lactate and sodium ions. Hook injury was likely a stronger indicator of survival for this species than fight time, regardless of whether that fight time was 30 - 250 minutes. Given that several physiological indicators correlated with time in the present study over 30 minutes, sevengills may demonstrate a lower tolerance and stronger response to even short fishing interactions.

$\beta$ -HB also significantly correlated with time ( $p = .000349$ ;  $R^2c = 0.6905$ ), and may be demonstrated as a fuel source during exercise in sevengill sharks during this study. Interestingly,

the present study saw an inverse relationship between glucose and  $\beta$ -HB, which are both expected to provide rapid fuel sources to animals during extreme exercise. Other studies have found the same inverse relationship, although it is not always explicitly pointed out (A. N. Schoen, Bouyoucos, et al., 2021; A. N. Schoen, Treberg, et al., 2021). An inverse relationship between  $\beta$ -HB and glucose may suggest a similarity to the Randall cycle in mammals, which outlines that the metabolism of these metabolites both require conversion to acetyl-CoA. There may be negative feedback when both metabolites are consumed by extrahepatic tissues, although further research is needed to investigate this (Laffel, 1999; A. Schoen, 2023).

Hematocrit, Osmo, and  $1\alpha$ -OHB did not significantly change with time. Some shark species may increase their red blood cell count (hematocrit) to increase oxygen mobilization in the blood to tissues during exercise (Bouyoucos et al., 2020), but this was not seen in this species during this study. Osmolality was not affected, perhaps due to consistent salinity during 30-minute trial periods.  $1\alpha$ -OHB did not significantly change over time. If this hormone is indeed correlated with angling stress, I did not see that depicted in our results for this study. This could be due to the short nature of the stress event (30 minutes). Alternatively,  $1\alpha$ -OHB may not serve a role in physiological stress in elasmobranchs as previously suggested.

#### *Temperature as a predictor variable*

Temperature had a significant effect on pH,  $1\alpha$ -OHB, and osmolality. An increase in temperature can affect a shark's ability to transport and metabolize  $\text{CO}_2$  in the blood (Albers & Pleschka, 1967), which is correlated with decreasing blood pH over time (Albers & Pleschka, 1967; Hyatt et al., 2018). There was no osmotic response over time, which suggests that osmoregulation was not impacted in this study under these conditions.

### *1 $\alpha$ -hydroxycorticosterone*

As stated previously, 1 $\alpha$ -OHB was not correlated with time but was correlated with Temperature and Total Length (TL). Only one study has incorporated the impact of temperature on 1 $\alpha$ -OHB (A. N. Schoen, Bouyoucos, et al., 2021) which found a significant decrease in 1 $\alpha$ -OHB in blacktip reef sharks that were exposed to ambient temperature as compared to fish that experienced increased heat exposure. It is currently unknown why 1 $\alpha$ -OHB would increase under the thermal stress treatment. The present study demonstrated that smaller individuals produced significantly more 1 $\alpha$ -OHB than larger individuals ( $p = 0.0343$ ), whereas Schoen, Bouyoucos, et al. (2021) found that adults produced significantly more 1 $\alpha$ -OHB during handling stress at the time-0 baseline (t-test;  $p = 0.003$ ) and 5 min post-stressor (t-test;  $p = 0.038$ ). The differences could be a function of species or handling conditions or may be due to Schoen et al (2021) studying a tropical species compared to the temperate sevengills in the present study. Additionally, Schoen, Bouyoucos, et al. (2021) found a slight but significant increase in 1 $\alpha$ -OHB over a 5-minute time period (Schoen, Bouyoucos, et al., 2021), contradictory to our findings of 1 $\alpha$ -OHB over time in the current study. Interpretation of the significance of 1 $\alpha$ -OHB with temperature and TL should be taken with caution, as the  $R^2c$  values were low ( $R^2c = 0.1657$ ), and data points were rather spread over the predictive model graph (Fig 4). Further research, ideally spanning longer periods of time, would improve our understanding of the presence of 1 $\alpha$ -OHB in sevengill sharks.

### *Blood gasses*

Blood gasses showed a non-significant negative trend for Base Excess (BE) and positive trend for PCO<sub>2</sub>, reinforced by a notable decrease in pH observed over total fight time. The upward trend in PCO<sub>2</sub> also aligns with our understanding of metabolic processes. During

physical exertion, animals experience increased metabolic activity, leading to higher production of carbon dioxide as a byproduct of cellular respiration. This results in elevated levels of PCO<sub>2</sub> in the bloodstream, as the rate of carbon dioxide production can exceed the rate of elimination despite increased ventilation (Phillipson et al., 1981). Typically, bicarbonate buffers blood pH in response to such declines. Consequently, an adjustment in bicarbonate levels (Smatresk & Cameron, 1982), may have led to a concurrent alteration in BE.

### *Implications for management*

The investigation into the effects of time and temperature on various blood parameters in juvenile sharks within their shallow nursery habitat holds significant implications for understanding how climate change and anthropogenic fishing pressures may impact these critical environments. Juvenile sevengill sharks demonstrated a significant physiological response to a 30-minute fight time, which can act as a proxy for recreational angler interactions. That, combined with increased temperature emerging as a significant indicator for physiological responses, indicates compounding factors that demonstrate significant physiological changes that juvenile sevengill's work to overcome to return to homeostasis. Energy that is spent getting an animal back to homeostasis is not available for activities, such as hunting and growth (Bouyoucos et al. 2018). If these threats are continuous, they could have an impact on the individual's ability to survive long-term.

The result of this study highlights a possible need for consideration of management practices within San Francisco Bay for protecting the sevengill nursery area. More research is needed to understand the long-term tertiary impacts of fishing interactions and warming temperatures to understand the extent of these threats at the individual and population level of

sevengill sharks. This study was limited due to the lack of implementation of acoustic tracking of sharks post-release to measure post-release mortality. Implementing acoustic tracking of sharks post-release could help quantify post-release mortality rates and assess the impact of various stressors on shark fitness. These additional techniques would allow for the investigation of the hypothesis that temperature and length of fight time during fisheries interactions have a significant impact on an individual's ability to survive long-term.

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## Chapter 3

Title: Ecological knowledge of California stakeholders on population trends and conservation management of the broadnose sevengill shark, *Notorynchus cepedianus*

### Introduction

Interviewing fishers for their ecological knowledge is a valuable way to fill data gaps in poorly understood fisheries, but is frequently underutilized (Johannes et al. 2000, Brook and McLachlan 2005, Gilchrist et al. 2005, Sáenz-Arroyo et al. 2005, Silvano et al. 2006, Eddy et al. 2010, Tesfamichael et al. 2014, Bender et al. 2014, Mason et al. 2020, Almojil 2021). Stock assessments and management of fisheries are often driven by economically important species from wealthy countries (Hilborn et al. 2020, Pacoureau et al. 2023). This has led to underestimated total fish landings, with most fisheries unassessed (Pauly and Zeller 2016, Ovando et al. 2021, Pacoureau et al. 2023). Assessments are particularly difficult for migratory shark fisheries, which have been depleted globally (Davidson et al. 2016). It can be even more difficult to study deep-sea sharks due to the expense of ship time and access. Non-target deep-sea shark species are often caught as bycatch from commercial fisheries (Fauconnet et al. 2023), creating conservation challenges. Local ecological knowledge (LEK), often includes extensive, first-hand observations of species-fishers encounters (Wilson et al. 2006, Hill et al. 2010, Leite and Gasalla 2013, Serra-Pereira et al. 2014, Baker and Constant 2020), and can help inform conservation policy for species not captured in traditional stock assessment metrics

(Wedemeyer-Strombel et al. 2019, Leduc et al. 2021, Shephard et al. 2021, Ullah et al. 2023, Castagnino et al. 2023).

Effective incorporation of local ecological knowledge has provided insights informing species ecology and improved management for any elasmobranch species. Studies incorporating local ecological knowledge of elasmobranchs have ranged from identifying unrecorded extinct sawfish species' presence in Sri Lanka (Tanna et al. 2021), providing key information on elasmobranch-related product supply chains (Martins et al. 2018), status of Critically Endangered sawfishes (Leeney and Poncelet 2015), use of river habitats (Rasalato et al. 2010), and declines of shark species in data-poor regions (Almojil 2021).

Failing to incorporate local ecological knowledge can result in poor policy design and worse conservation outcomes (Johannes et al. 2008, Mason et al. 2020, Ullah et al. 2023). In Peru, for instance, policymakers responded to a declining population of smooth hammerhead sharks, *Sphyrna zygaena*, by implementing a seasonal ban on fishing (Mason et al. 2020). However, this seasonal ban only protected juvenile individuals, which contribute the least to the smooth hammerhead population, while failing to protect reproductive adults who visit seasonally, resulting in limited conservation benefits. Fishers were keenly aware of smooth hammerhead movements and knew the ban did not protect reproductive adults. Had fisheries managers engaged with fishers, policies could have incorporated timing, location, and gear restrictions that would have been more effective at achieving conservation and management goals (Johannes et al. 2008, Mason et al. 2020).

There is a substantial gap in knowledge of broadnose sevengill shark (*Notorynchus cepedianus*) ecology, particularly of the northeastern Pacific population. The IUCN Red List of Endangered Species classified sevengill sharks as decreasing globally (Finucci et al. 2020), but

evaluations of independent populations are lacking. Sevengill sharks typically inhabit the deep sea but visit select bays and estuaries for important parts of their life history. Repeated use by northeastern Pacific sevengill sharks in shallow waters is only reported in the literature for Willapa Bay, Washington, and Humboldt and San Francisco bays, California (Ebert 1986, 1989, 2001, Williams et al. 2012, Larson et al. 2015). Online searches reveal public reports of sevengills sharks aggregating in La Jolla, California (Scuba Diver Girls 2015, Blue Water Photo, In Focus 2024, San Diego Scuba Guide 2024). Of these shallow habitats, San Francisco and Humboldt bays, California have been reported as pupping and nursery areas for sevengill sharks (Ebert 1984, 1986, 1989, 2001). Published evidence of juvenile occurrence in San Francisco Bay date from as early as 1948 (Herald and Ripley 1951, Herald 1953). No published record reports juvenile size or density in Humboldt Bay, and sevengills do not show up in recent stock assessments (Chamberlain et al. 1993, Cole 2004). San Francisco Bay currently stands as the only habitat formally tested as a nursery area for sevengill sharks (Holst, 2024, Heupel et al. 2007), leaving the significance of Humboldt Bay for sevengill sharks unclear. Local ecological knowledge may provide more substantive information to evaluate the presence of juveniles in San Francisco and Humboldt Bays and provide a better understanding of fisheries interactions in these areas.

Recreational sevengill angling has been a popular, widespread activity in San Francisco Bay beginning with shark derbies in 1948 (Herald and Ripley 1951, Herald 1953). A single tournament landed as many as 301 sevengill sharks at Coyote Point in San Francisco Bay in 1952 (Herald 1953). Shark tournaments ceased in the 1950s, but individual recreational angling (including small-fisheries charter vessels) is still persistent within the San Francisco Bay. Sports anglers can have significant impacts on marine fish populations (Cooke and Cowx 2004,

Arlinghaus et al. 2007, Brownscombe et al. 2019), but data for small-scale fisheries, which contributes to the majority of elasmobranch catch in some countries, is lacking (Walker 1998, Jacquet and Pauly 2008, 2008, Cartamil et al. 2011, Worm et al. 2013, Mason et al. 2020). Fishing regulations are often set per individual angler without having overall limits on the fishery (Van Voorhees 2016). Shark species are especially vulnerable to very low fishing mortality rates (Dulvy et al. 2014), and therefore even small-scale and recreational fisheries should be evaluated for their impacts on sevengill shark recruitment. As of 2024, California has a 1-bag limit (i.e. 1 person can land 1 sevengill per day) that is not restricted by sex, size, season, or location. This currently has unknown impacts on sevengill sharks in their sole confirmed pupping and nursery ground for the northeastern Pacific population (Holst, Chapter 1, 2024).

In this study, I interview boat captains in California who charter sport fishing vessels and target sevengill sharks. Interviews measured the attitudes and opinions regarding conservation management to support effective conservation management. Based on preliminary discussions with charter captains over the years, I hypothesize that 1) charter captains have generally positive attitudes towards and support conservation management; 2) charter captains have an understanding of sevengill shark population changes and potential threats of sevengill sharks, and; 3) captains can substantiate claims that there is solid evidence of long-term presence of juvenile sevengill sharks in San Francisco Bay, and less evidence for Humboldt Bay (Chamberlain et al. 1993, Cole 2004). Investigating these perspectives may provide a critical understanding of small-scale fisheries impacts on sevengill sharks in California and highlight areas where more effective management may be necessary and supported by local anglers.

## Materials and Methods

Charter captains that target sevengill sharks in California were identified via internet search, research contacts in Oregon and Washington, and snowball sampling from initial angler contacts. Internet search terms included but were not limited to “shark fishing California”, “sports fishing charters”, “California shark fishing charter”, and “shark fishing”.

All first or second captains were eligible for this study. First captains are the pilots in charge of vessel operations, while second captains are second in command and work closely with first captains. Interviews included both closed and open-ended questions to evaluate angler perceptions of sevengill conservation, population trends, population response to angler behavior, and attitudes surrounding the regulation of the northeastern Pacific sevengill population (See Appendix for full survey). Section 1 of the survey collected background information, including age, years of fishing experience, boat and gear types, and number of fishing trips per year. Section 2 focused on fishers' sevengill shark fishing experience and their ecological knowledge of sevengills. I asked fishers about their personal experience with sevengill sharks and observations on sevengill abundance. I asked anglers if they have noticed sevengill abundance increasing, decreasing, or staying the same, as well as the average size of sevengills they typically see. In Section 3, I asked anglers express their opinion on fisheries management generally and to share their thoughts on the sevengill shark policy specifically, as well as their recommendations for improvement. In addition to charter captain interviews, I interviewed a California Fish and Wildlife (CDFW) manager to investigate their knowledge of sevengill sharks and evaluate their thoughts on current and future management. All interviews were conducted over the phone.



Protocols were approved by the University of California, Davis Institutional Review Board (IRB) protocol ID #1900382-2. A total of 6 semi-structured interviews of charter captains occurred in San Francisco ( $n=5$ ) and Humboldt ( $n=1$ ) Bays. To my knowledge, these are the primary boat captains in California that regularly target sevengill sharks. One charter captain in San Francisco Bay could not be reached despite several attempts. Attempts to contact charter captains in Southern California were not successful. The sole California Fish and Wildlife (CDFW) employee who was interviewed ( $n = 1$ ) managed the team that sets all shark catch limits for the state of California. On average, interviews with charter captains were 36.37 minutes (ranging from 19.73 to 74.2 minutes).

## Results

### *Charter captain demographics*

All interviewees were male. One charter captain was from Humboldt Bay, and five charter captains were interviewed in San Francisco Bay (San Francisco Bay). Five of the six captains interviewed were first captains, and one was a second captain in San Francisco Bay. Only charter captains in San Francisco Bay actively targeted sevengill sharks at the time of this study. All San Francisco Bay captains reported fishing for other fish species, describing fishing for sevengills as a relatively small aspect of their annual fishing effort.

Charter captains averaged 54 years of age, ranging from 43-72. These captains had been fishing for an average of 45 years of their life, with a range of 32-57 years of fishing. An average of 28 of those years were professional fishing experience ranging from 7-57 years of professional charter experience. All captains relied on fishing for their living with 67% stating that fishing was their sole income.

### *Fishing reports*

All charter captains ( $n = 6$ ) reported using hook-and-line as their method for fishing sevengill sharks. Two participants (33%) volunteered that they use circle hooks to reduce gut hooking. One participant (17%) voluntarily mentioned pinching the barb of the hook down for ease of release. Of San Francisco Bay charter captains, three (60%) specifically mentioned fishing in South Bay, with two individuals reporting fishing south to the San Mateo Bridge.

Four of the five San Francisco Bay charter captains reported an estimated average of 27 sevengill landings per year, with an estimated range of 8 to 50. One captain in San Francisco Bay didn't respond to this question. The Humboldt Bay charter captain reported taking up to 3 sevengills per fishing trip in previous years starting in 1974 to roughly 2010.

### *Charter Captain Insights*

All captains ( $n = 6$ ) reported fishing for sevengills between July and December. All San Francisco Bay charter captains ( $n = 5$ ) reported seeing sevengill pups in San Francisco Bay, with 80% ( $n = 4$ ) specifically reporting sightings in South San Francisco Bay near Coyote Point. The captain in Humboldt Bay reported that smaller sevengills were extremely rare in Humboldt Bay, with only 2 memories of single sevengills that may have been juveniles, but not pups.

Four (80%) San Francisco Bay captains reported landing pregnant females, with one captain reporting landing a pregnant female in the deep South San Francisco Bay channel. The Humboldt Bay captain stated that he had never landed a pregnant female or observed female sevengill that looked fecund.

Captains reported that sevengill catch rates have been consistent throughout their fishing careers. Half said the abundance of sevengill sharks has been the same throughout their career.

Two (33%) stated that the sevengill shark abundance has been the same for the last 20 years. One San Francisco Bay captain said they have become more abundant during their career, noting that a decrease in the population prior to his career was due to the 1940s and 50s due to shark tournaments. Only one captain said there are fewer large individuals, stating: I'm seeing fewer really large sharks. And I don't know whether that's because they're just the really large sharks aren't coming into San Francisco Bay because [there are] environmental or ecosystem changes, or whether it's the fact that they have [become a] more fished species because some of the other captains making it more popular. So, I've seen a reduction in the 72 inch plus [sevengills]. It should be noted that this captain was also the captain with the least experience specializing in this fishery, with 7 years of professional experience.

#### *Conservation attitudes and proactive management of charter captains*

All charter captains indicated support for fisheries regulations, often qualifying that they support “science-based” regulation. For example, one captain stated “[I support} accurate science-based regulation... It's a politically based system and I think that is a detriment to the user groups that rely on fishing for a living”. Four (67%) captains report restricting their landings beyond what is required of CDFW based on sex, often restricting landings of large females in an attempt to protect sevengills that contribute the most to the population. One captain for instance stated, “I practice a lot more catch and release than when, even when I first got into it, because you have to educate people that those fish are not like Stripe bass and salmon, where you get a new school every four years. It's gonna take 'em seven to 11 years to go and have babies. And then if you're out killing the big ones, that's an old fish. It's never coming back. You took that. And any future survivors out of the gene pop.” Another captain stated, “I don't keep females. You know, I will talk [clients] out of keeping a female unless it's been a really slow day, which is a rarity.

I'll talk fishermen out of keeping a female... I mean, I've caught multiple females where you can see their stomachs swollen and moving around. So I don't like to keep a pregnant fish because that takes another dozen or more babies out of the ecosystem. So I try to keep only males only over 48 inches and, if at all possible, just catch and release all day.” Of the other two captains, one did not know how to sex the sharks, and the other mentioned that he would restrict landings if clientele agreed, but clientele satisfaction was prioritized, stating “If I have to make a choice, it's typically a smaller male”, and the other stated “I prefer to keep just the males; I try not to take the big pregnant females”. All charter captains in San Francisco Bay reported that they restrict landing size, with varying criteria and reasoning, to support sevengill conservation or clientele satisfaction. Some captains (40%) described size restrictions by shark weight, with one captain limiting landings to sevengills over 30 lbs, and one captain limiting landings within a 20-80 lbs range. “I let their conscious be their guide... there's no size restrictions, but you don't really want to take a pup that you're not going to eat off of, so usually 20-30lbs is usually the smallest [I take].... Other captains (60%) described size restrictions by shark total length, with one captain restricting landings to sevengills over 4 feet, one captain restricting landings to sevengills over 2 feet, and one captain restricting landings under 6 feet, stating, “I don't take them over 6 feet anymore, it's strictly catch-and-release. If [clients] don't like it, they can find another boat”. The Humboldt Bay captain stated that there was no need to restrict landings based on size or sex, as the vast majority of landings involved males between 5.5 and 6 ft in total length.

### *California Fish and Wildlife demographics and perspectives*

California has a single person who manages a team to decide on regulations (including bag limits) for all shark species in the state ( $n = 1$ ). This interview was conducted for 24 minutes.

Overall, the CDFW is aware of San Francisco Bay as a potential pupping and nursery ground from historical documentation of juvenile presence, citing previous literature (Herald and Ripley 1951, Herald 1953, Ebert 1984, 1986, 1989, 2001). When asked about sevengill movement patterns, the CDFW manager did not have evidence of this population being migratory and noted that sevengill sharks have relatively resident subpopulations along their northeastern Pacific range, citing Ketchum et al. (2017) as evidence. He also mentioned the juvenile presence in Humboldt Bay, citing Ebert 1989 and related work. He noted aggregations occurred off La Jolla, California for unknown reasons. The CDFW representative noted that fishery reports put catch rates on par with their 10-year average, stating: “[Looking at] the landings data that I have, there's an assumption that there was a significant decline in the population in the 1940s to 1960s, and that there has been some apparent recovery. But again, that's not a formal assessment, that's based on ... fishing data.”

When I asked the CDFW manager about the current management of sevengill sharks, he indicated that regulation of this species is not a current priority. However, he also stated “If [San Francisco Bay] were the sole pupping ground for the entire West Coast population, I think that would be a major factor. But given there are other areas where these sharks occur that are part of the west coast population, and where they are likely pupping it, it makes it less of a management concern”. The CDFW manager indicated data needed to evaluate alternative management include abundance estimates, evidence of the potential impact of fisheries regulation, and a clear demonstration of regulatory need. He noted that increased management without an understanding of conservation effectiveness could be overprotective of the sevengill conservation needs, without a complete understanding of their dynamics along the northeastern Pacific coastline.

## Discussion

I aimed to explore key informant knowledge and attitudes towards current sevengill shark conservation management in California and highlight areas where local ecological knowledge can be incorporated to improve fisheries management of this species. My first hypotheses were confirmed, as all captains demonstrated support for informed regulation, and were voluntarily implementing restrictions based on sex and/or size criteria, albeit with varying regulatory preferences. The support for informed regulation expressed by charter captains suggests a willingness among stakeholders to collaborate with policymakers in implementing conservation measures that balance ecological sustainability with the socioeconomic interests of local communities. But short of regulations, the incentive of satisfying customers will likely be prioritized for charter captains, so some management or stakeholder engagement may be called for.

Angler incorporation is an effective way to improve shark conservation management in other areas, such as Argentina (Cuevas 2015). In Cuevas (2015), recreational shark anglers were trained to capture, tag, and release sharks for conservation purposes and facilitated several outlets of shark conservation outreach in the community. Stakeholder engagement was so effective that shark fishing tournaments in Argentina were changed from catch-and-retain fishing to completely catch-tag-release programs. Incorporating stakeholders into conservation efforts in Argentina has also proven to be highly cost-effective, offering a viable solution for communities with limited resources dedicated to conservation initiatives (Cuevas 2015). Initiatives to incorporate charter captains in California for conservation could have similar results, where agencies such as CDFW are often limited in their ability to research, monitor, and enforce due to limited allocated government resources.

I confirmed the second hypothesis of this study, that charter captains had perspectives on population persistence over their careers and could identify potential threats. Local ecological knowledge in the present study would indicate that sevengill populations have remained relatively consistent over charter captain careers, which would agree with CDFW's assessment that sevengill catch data has remained consistent over a 10-year average. Yet, with a lack of current and historical pre-shark derby stock assessments, accurately assessing whether sevengill numbers in recent decades align with historical population estimates before shark tournaments in the 1940s remains elusive. Nonetheless, charter captains acknowledge the ecological importance of San Francisco Bay and voluntarily restrict landings to support long-term sevengill persistence. The increase in human presence in coastal areas and associated habitat modification (Heithaus 2007), combined with climate threats on nursery habitats (Crear et al. 2020, Rummer et al. 2022) put San Francisco Bay at greater risk for negative anthropogenic impacts in the future. Sudden declines in large shark species have been demonstrated to have disproportionate impacts on marine ecosystems (Heithaus 2007, Myers et al. 2007), and shark nurseries are critical for population recruitment (Heithaus 2007), further indicating that proactive management is in the best interest of long-term ecosystem management.

The third hypothesis, that captains could substantiate the persistence of juvenile sevengills in San Francisco Bay, but not Humboldt Bay, was also confirmed in this study. Local ecological knowledge supports that San Francisco Bay is an active nursery ground, consistently abundant with juvenile sevengill sharks across charter captain careers, with several captains specifically identifying South San Francisco Bay as an area where pups are abundant. Our findings indicate that Humboldt Bay may no longer be as crucial for reproduction as has been previously noted. A charter captain interview indicated that juvenile occurrence is extremely rare

in Humboldt Bay and recalled that the majority of sevengill catch has been predominantly male in his experience since 1974. It should be noted that this was the only charter captain identified in Humboldt Bay that has directly targeted sevengill sharks. Conversely, insights from the CDFW representative indicated that while San Francisco Bay is recognized as a potential pupping ground, sevengill regulation currently does not rank as a priority for management decisions. They emphasized the need for further data collection and understanding of sevengill dynamics before implementing significant regulatory changes. CDFW summarized their understanding of sevengill dynamics as primarily resident subpopulations, with nursery grounds spread along their range. However, recent research (Holst, Chapter 1) may suggest that San Francisco Bay has connectivity along this population's entire range, and therefore reconsideration of the importance of San Francisco Bay as a critical habitat that needs unique management is likely needed.

Literature indicates there is connectivity of sevengills among habitats along the northeastern Pacific range. For example, a recent study (Holst, Chapter 1) demonstrated long-distance movements from San Francisco Bay to Humboldt Bay, and from San Francisco Bay to their southernmost known range in Mexico. Genetic research also demonstrates relatedness of individuals between San Francisco Bay and Willapa Bay in Washington (Larson et al. 2015). These mark-recapture and genetic studies would indicate there may be more sevengill connectivity along their northeastern Pacific range that is currently understood by CDFW. Discrepancies between charter captains and the CDFW regarding habitat suitability in Humboldt Bay indicate the need for further collaboration and research to inform more targeted conservation strategies. Given the data that currently exists to support that San Francisco Bay is an essential fish habitat for sevengill sharks, and stakeholders are supportive and enacting proactive



management independent of CDFW, increased management may not only be necessary in the future but well supported.

A shortcoming of this study is the relatively small number of charter captains in California who specifically target sevengill sharks as part of their livelihood. Despite efforts to identify and interview charter captains with expertise in sevengill fishing, the sample size remained limited, potentially limiting the generalizability of the findings. The scarcity of charter captains specializing in sevengill shark fishing reflects the niche nature of this fishery within California's broader recreational fishing industry. As a result, the perspectives and experiences captured in this study may not fully represent the diversity of viewpoints and practices among all charter captains engaged in sevengill shark fishing across the state. Future research could seek to expand the sample size by engaging with a broader range of stakeholders, including additional recreational anglers, to provide a more comprehensive understanding of sevengill shark ecology and management in California waters. Future studies should also investigate Humboldt Bay to determine if juvenile sevengill presence persists as previously noted.

In addition to valuable insights gained from charter captains regarding sevengill shark ecology and management practices, they are also voluntarily limiting sevengill landings beyond the requirements set by CDFW. As such, policymakers should consider engaging charter captains as key stakeholders in the development and implementation of conservation and management strategies for sevengill shark populations. This collaborative approach not only ensures that management decisions are informed by the most up-to-date and relevant information but also fosters a sense of ownership and stewardship among charter captains, increasing the likelihood of compliance, enhancing the effectiveness and legitimacy of conservation efforts (Jentoft and McCay 1995, Smith 1999, Mikalsen and Jentoft 2001, Kapoor 2001, Soma 2003,

Gray 2005, Delaney et al. 2007, Marshall 2007, Berghöfer et al. 2008, Pita et al. 2010).

Furthermore, integrating charter captains' recommendations and practices into policy frameworks can help bridge the gap between scientific research and on-the-ground conservation action (Mikalsen and Jentoft 2001, Soma 2003, Gray 2005, Marshall 2007, Berghöfer et al. 2008), promoting adaptive management practices that are responsive to the dynamic nature of marine ecosystems. By harnessing the expertise and insights of charter captains, policymakers can develop more robust and inclusive management strategies that prioritize the long-term sustainability of sevengill shark populations while also supporting the socioeconomic interests of coastal communities (Smith 1999, Gray 2005, Marshall 2007, Berghöfer et al. 2008). Ultimately, my study contributes to advancing understanding and conservation efforts for the broadnose sevengill shark, highlighting the potential of stakeholder engagement and interdisciplinary collaboration in marine conservation.

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# Appendix

## *Questions for Small Fisheries (local charters):*

### Background:

1. What state do you typically fish in?
2. How old are you?
3. What would you call these sharks? (show picture array of local shark species, including sevengill, soupfin shark, leopard shark, brown smoothhound)
4. How many years have you been fishing? How long professionally?
5. Is fishing the main way you earn a living?
  - a. Retired but fishing was the main way to earn a living
  - b. Retired but fishing was NOT the main way to earn a living
6. Is fishing the only way you earn(ed) a living?
  - a. If not, what is/are your other occupations?
7. Which months do you normally fish?
  - a. All year
  - b. Winter (12-2)
  - c. Spring (3-5)
  - d. Summer (6-8)
  - e. Fall (9-11)
8. How many days per year do you fish in San Francisco Bay?
  - a. \_\_\_\_\_ days (low season)
  - b. \_\_\_\_\_ days (peak season)
  - c. No specific season/# days fishing per month \_\_\_\_\_
  - d. Why do you go out during this time? (e.g. weather dependent?)

### Boat and gear characteristics:

9. Do you primarily fish from a boat, or from shore?
  - a. Boat
  - b. Shore
10. If you fish from a boat, what is your position on the boat? (If they fish from a boat)
  - a. Owner
  - b. Captain
  - c. Owner and captain
  - d. Family member
  - e. Crew member
  - f. No fixed position
11. How many fisherMEN, including yourself, work on the boat at any given time?
12. What is the fishing gear you use when you have caught sevengills?
  - a. Longline
  - b. Bottom longline
  - c. Hook and line

13. What is the average duration of each trip?
  - a. \_\_\_\_\_ hours
14. How long have you been fishing in San Francisco Bay?
15. How long have you been fishing for sevensgills?
16. What part of the Bay do you typically fish?
  - a. San Francisco Bay proper
  - b. San Pablo Bay
  - c. Suisun Bay
  - d. South Bay
17. If you fish from shore, do you typically fish from a certain Pier? If so, which one?
18. Have you caught or fished for sevensgills outside of San Francisco Bay?
  - a. If so, where? \_\_\_\_\_
19. Do you use different gear types in different areas?
  - a. If so, how? \_\_\_\_\_
20. Have you observed other fishers fishing for sevensgills? If so, what are their practices?
21. Are practices different between charter vessels and recreational anglers? If so, how?
22. What do you think best practices are for sevensgill fishing? Why?

#### Sevensgill specifics

23. What size sevensgills do you typically see?
24. Have you ever seen sevensgill pups (<60cm TL)?
  - a. If so, where? \_\_\_\_\_
  - b. If so, when? \_\_\_\_\_
- 25.
26. Have you ever landed a pregnant female?
  - a. If so, when (year, time of year if possible)? \_\_\_\_\_
27. If you have landed more than one pregnant female, approximately how many do you think you have landed?
  - a. \_\_\_\_\_
28. Do you restrict your landings to a specific sex? If so, which one?
  - a. Yes: \_\_\_\_\_
  - b. No
29. Do you restrict your landings to a specific size? If so, what size?
  - a. Yes: \_\_\_\_\_
  - b. No
30. Did you catch any sevensgills this year?
  - a. Yes, around \_\_\_\_\_
  - b. No
31. Was this a typical number to catch per year?
  - a. Yes
  - b. No, usually \_\_\_\_\_
32. Do you catch more sevensgills on a fishing trip than you keep? (1 bag limit per person)
  - a. Yes, I usually catch \_\_\_\_\_ per trip
  - b. No, I keep the only one I catch
33. If you fish with other anglers, how many sevensgills do you think you collectively catch each trip? How many do you keep?

- a. I catch \_\_\_\_\_ sevengills per trip as a group
  - b. I keep \_\_\_\_\_ sevengills per trip
34. At which time of the year do you catch most/least sevengills?
- a. Most \_\_\_\_\_
  - b. Least \_\_\_\_\_
  - c. No specific reason/Caught all year \_\_\_\_\_
35. Have you noticed any changes in the frequency of sevengills you land?
- a. Yes
  - b. No
  - c. I don't know
36. Do you think sevengills are **more abundant, less abundant, or the same now** compared to:
- a. 5 years ago: \_\_\_\_\_
  - b. 10 years ago: \_\_\_\_\_
  - c. 20 years ago: \_\_\_\_\_
37. Have you noticed any changes in the size of sevengills you land since you started fishing?
- a. Yes (have them explain how): \_\_\_\_\_
  - b. No
  - c. I don't know
38. If you've noticed a change, why do you think there has been a change in sevengill frequency or size?
39. Do you support environmental regulation generally?
40. How would you feel about potential regulation of sevengill fishing to support a long-term population and fishery in San Francisco Bay?
41. What recommendations would you make based on your experience to support sevengill conservation?
42. Anything else?
43. Anybody else?

*Questions for California Fish and Wildlife (CDFW):*

**Survey:**

1. How familiar are you with current sevengill regulations? Can you tell me your impressions of the current regulatory state of sevengills?
2. Is there information that CDFW would want to know from fishers about sevengill sharks?
3. Has CDFW considered more regulation on sevengill sharks? Why or why not?
4. What is CDFW's perception of charter vessels targeting sevengill sharks?
5. What is CDFW's perception of recreational fishers targeting sevengill sharks?
6. Here are some examples of what local fishers have described in their experience. What are your thoughts about this?
7. Does CDFW think sevengills are in need of protection? Why or why not?
8. Do you think the sevengill shark fishery is an emerging fishery issue? Why or why not?
9. What are barriers to further regulation?
10. What future opportunities are there for further regulation?