# SPATIAL AND TROPHIC ECOLOGY OF THE BLUNTNOSE SIXGILL SHARK: ENVIRONMENTAL DRIVERS OF BEHAVIOR AND COMPARATIVE TROPHIC POSITION IN TWO DISTINCT HABITATS

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ABSTRACT

The bluntnose sixgill shark, *Hexanchus griseus*, is an apex predator and scavenger that has adapted to highly varied thermal structure, light penetration, productivity, and food web structure throughout its large geographic range. The behavioral adaptations to varied environmental conditions are poorly understood, and knowledge is particularly lacking in tropical latitudes where sixgill sharks inhabit deep water. This study aimed to investigate environmental drivers of movements of sixgill sharks in a tropical habitat, and to compare the trophic ecology of *H. griseus* in two distinct habitat types. Pop-up satellite archival tagging revealed diel vertical migrations between ~275-700m, and light and temperature were identified as important factors in determining depth habitat. Low oxygen concentrations did not appear to limit depth, and the animals spent about 50% of their time in hypoxic water (<60μmol). Home range size was expected to be small, but two mature males left the island slope where they had been tagged and traversed deep water. This long-distance pelagic movement could allow gene flow between distant populations. A preliminary investigation of prickly sharks (*Echinorhinus cookei*) revealed that they have an overlapping but shallower depth range, are much more sedentary, and are less light-averse than sixgill sharks. Amino acid compound-specific isotopic analysis of nitrogen indicated that sixgill sharks in Hawaii had a greater ontogenetic shift in trophic position than sharks from Puget Sound. This result suggests that scavenging may become important for large size sixgill sharks in deep oligotrophic habitats such as Hawaii, while adults in the more productive ecosystems are able to be selective predators. Alternatively, commercial fishing pressure in Puget Sound may have depleted stocks of sixgill shark prey items such as hake, forcing them to feed at a lower trophic level. Prickly sharks had a higher trophic position than most sixgill sharks, indicating that their feeding strategies may be different despite overlap in depth and geographic ranges. As a whole, this study provided insight on how a single species has adapted to be successful in very different habitats through isothermal submergence in low latitudes and a generalist feeding strategy, and has identified light and temperature as important parameters which determine sixgill shark habitat and behavior.

# TABLE OF CONTENTS

Acknowledgments ..... ii
Abstract ..... iv
Table of Contents ..... v
List of Figures ..... vii
List of Tables ..... ix
Introduction ..... 1
Literature Cited ..... 3
Chapter I ..... 4
Abstract ..... 4
Introduction ..... 5
1.1 Overview ..... 5
1.2 Study Site ..... 5
1.3 *Hexanchus griseus*, the bluntnose sixgill shark ..... 8
1.4 *Echinorhinus cookei*, the prickly shark or Cooke shark ..... 11
1.5 Hypotheses ..... 12
1.6 Objectives of this study ..... 14
Methods ..... 15
2.1 Capture and Tagging ..... 15
2.2 Data Recovery ..... 16
2.3 Anchored Tags ..... 16
2.4 Data analysis and statistics ..... 16
Results ..... 21
3.1 Fishing and animal captures ..... 21
3.2 Satellite tagging summary ..... 21
3.3 Tag data recovery ..... 21
3.4 Depth ..... 21
3.5 Horizontal movements ..... 22
3.6 Light ..... 23
3.7 Thermal habitat ..... 24
3.8 Hypoxic habitat ..... 25
3.9 Rate of movement and activity level ..... 25

3.10 Preliminary results: *Echinorhinus cookei* ..... 26
Discussion ..... 50
4.1 Horizontal movements ..... 50
4.2 Environmental drivers of depth ..... 52
4.3 Rate of vertical movements ..... 55
4.4 *Echinorhinus cookei* as a niche competitor in Hawaii ..... 56
Conclusions ..... 57
Literature Cited ..... 59
Chapter II ..... 65
Abstract ..... 65
Introduction ..... 65
1.1 Overview ..... 65
1.2 Stable isotope analysis as a tool in food web ecology ..... 68
1.3 Amino acid compound specific isotopic analysis of nitrogen ..... 69
Methods ..... 72
2.1 Bulk tissue isotopic analysis ..... 72
2.2 Compound specific isotopic analysis of individual amino acids ..... 73
2.3 Analysis ..... 74
2.4 Statistics ..... 75
Results ..... 77
3.1 Bulk stable isotope analysis ..... 77
3.2 Compound specific analysis of nitrogen isotopes in individual amino acids ..... 77
Discussion ..... 90
4.1 Bulk tissue ..... 90
4.2 Trophic position ..... 91
4.3 Calculation of trophic position versus expected values ..... 93
4.4 Implications and future directions ..... 94
Literature Cited ..... 96
Summary and Conclusions ..... 100
Literature Cited ..... 102
Appendix ..... 103

LIST OF FIGURES

Chapter I

1. Time-series data for sharks 1-5 .....28
2. Depth of Shark 1, a mature male *Hexanchus griseus* which left Hawaii during its time at liberty. .....30
3. Depth of Shark 2, an immature male *Hexanchus griseus* which remained near Oahu .....31
4. Depth of shark 3, an immature female *Hexanchus griseus* which remained near Oahu .....32
5. Depth of shark 4, a mature male *Hexanchus griseus* which traveled to Kauai. .....33
6. Depth of shark 5, an immature female *Hexanchus griseus* which traveled to Penguin Banks in the Kaiwi Channel .....34
7. Median depth versus time of day .....35
8. Median depth and IQR for all sixgill shark depth data pooled .....35
9. Map of horizontal travel of five *Hexanchus griseus* from tagging to pop-off location .....36
10. Two light records from constant depths plotted against a light record from Shark 5 during the same dates .....37
11. Box plot visualization of light levels experienced by all 4 sharks for which light records were available, and for the two constant-depth deployments .....37
12. Nautical dawn versus dive initiation. .....38
13. Median and maximum nightly depths over the time at liberty for each shark .....39
14. Duration of excursions above 16ºC .....40
15. Vertical rate of movement versus time of day for Shark 1 .....41
16. Vertical rate of movement versus time of day for Shark 2 .....41
17. Vertical rate of movement versus time of day for Shark 3 .....42
18. Vertical rate of movement versus time of day for Shark 4 .....42
19. Vertical rate of movement versus time of day for Shark 5 .....43
20. Vertical rate of movement versus time of day pooled across all five sixgill sharks .....43

21. Full archival record of shark 6, *Echinorhinus cookei*. .....46
22. Time at depth histogram for shark 6 .....46
23. Depth versus time of day for shark 6. .....47
24. Rate of movement versus time of day for shark 6.....48
25. Time of nautical dawn plotted against dive initiation times for shark 6 .....48

Chapter II

1. $\delta^{15}N$ values determined of bulk tissues of shark muscle, plotted against total length .....80
2. $\delta^{15}N$ and $\delta^{13}C$ values vs. total length, following a lipid and urea rinse.....81
3. $\delta^{13}C$ values versus molar C:N ratio and B) $\delta^{13}C$ values versus molar C:N after the lipid and urea rinse .....82
4. Total length versus molar C:N ratio and Total length versus molar C:N after the lipid and urea rinse .....83
5. A plot of rinsed $\delta^{13}C$ values versus rinsed $\delta^{15}N$ values.....84
6. Mean and standard deviations of the trophic and source amino acids plotted for each individual sixgill shark, alongside the bulk tissue $\delta^{15}N$ values.....84
7. $\delta^{15}N_{Phe}$ vs. $\delta^{15}N_{Source.}$ .....87
8. Calculation of trophic position based on glutamic acid and phenylalanine .....87
9. Calculation of trophic position based on three source amino acids (gly, phe, and ser) and three trophic amino acids (glu, pro, leu).....88

LIST OF TABLES

**Chapter I**

Table 1. Summary of all sharks tagged with pop-up archival tags .....27

Table 2. Non-parametric statistics for all variables measured by the MiniPAT tags.....29

Table 3. Rates of vertical movement for each shark individually, calculated for four diel periods .....44

Table 4. Median and maximum total vertical movements per day for each shark .....45

Table 5. Results of GEE analysis for five sixgill sharks .....45

Table 6. Vertical rates of movement observed in this study and others.....49

**Chapter II**

Table 1. Mean and standard deviation of nitrogen isotopic composition for all amino acids, separated by region .....85

Table 2. Trophic positions calculated by Method 1 (glu-phe) and Method 2 (trophic-source) for each individual shark .....86

Table 3. GAM results from a model explaining 94.7% of the variability observed .....89

# INTRODUCTION

The geographic range of a species may be bounded or influenced by multiple environmental and biological factors, including climate, productivity, geology, size and mobility of the organism, and method of reproduction (Brown et al., 1996). Geographic range sizes as small as $100m^2$ have been noted for certain fish and isopods inhabiting only a single spring, while a near-globally ranging organism such as the blue whale has a range size of about 300 million $km^2$. Species found in higher latitudes tend to have larger range sizes than species in the tropics (Stevens, 1989). Many cold-water shark species were originally noted as bi-temperate or bi-polar in their distribution (Hubbs, 1952) but were later found in deep water in tropical latitudes (Compagno, 1973, 1984; Crow et al., 1996), resulting in remarkably large latitudinal distributions for these ectothermic fishes.

Among those shark species with extensive geographic ranges are *Hexanchus griseus* and *Echinorhinus cookei*. Hubbs (1952) suggested that isothermic submergence allowed these animals to traverse latitudes in an ideal thermal environment. Catch records and previous research on these two species have largely supported the idea that they shift their depth habitat with latitude (Andrews et al., 2009; Carey and Clark, 1995; Crow et al., 1996; Dawson and Starr, 2009). In this wide depth and latitudinal range, these sharks are subject to large variations in pressure, oxygen concentration, light regime, productivity, and foraging opportunities. Animals inhabiting such divergent environmental conditions may have very robust adaptations in terms of tolerance of physical parameters such as pressure and light, and potentially have plastic behavioral strategies relating to ecological and biological parameters such as metabolism, oxygen availability, and foraging strategy. How can sharks spanning a wide latitudinal range be successful in diverse environments?

We can understand differences in the biology of a species across latitude by quantifying changes in habitat use and trophic level. In this study, the spatial and trophic ecology of the bluntnose sixgill shark *Hexanchus griseus* were investigated. Animals captured on the slope of the Hawaiian island of Oahu were tracked using satellite tags, and their vertical and horizontal behaviors were compared to a well-studied population of sixgill sharks in Puget Sound, WA. Potential environmental drivers of behavior were

assessed. Stable isotope analysis of muscle tissue was used to determine relative trophic positions for sharks in both ecosystems, and the results were examined for evidence of trophic shifts with size and location. Finally, the prickly shark *Echinorhinus cookei* was sampled opportunistically in Hawaii and studied as a potential niche competitor of the sixgill shark.

To effectively manage and conserve a species, especially in light of increasing anthropogenic pressures on deeper ecosystems such as overfishing, bycatch, and habitat damage (Simpfendorfer and Kyne, 2009), there is a need to better understand the habitat use and connectivity of organisms throughout their range (Jones et al., 2007). This study addresses the relationship of sixgill and prickly sharks to environmental variables, potential food sources, and discusses possibilities for long distance movements. Currently, the home range size, migratory behavior, and population connectivity of sixgill and prickly sharks is unknown, and their habitat use (particularly in deep habitats) is poorly understood. Overall, this study aims to better understand the habitat use patterns of sixgill sharks, and to contribute ecological knowledge which will be useful in managing deepwater elasmobranch populations globally.

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CHAPTER I

Satellite archival tagging of *Hexanchus griseus* in a deep oligotrophic habitat: Environmental drivers of behavior and evidence for long-distance movement


## ABSTRACT

Bluntnose sixgill sharks are apex predators and scavengers that are near-globally distributed in slope and shelf habitats, but many aspects of their behavior and ecology are poorly understood. Sixgill sharks exhibit isothermal submergence in lower latitudes, and are therefore subject to widely varying environmental parameters in different geographic locales, including pressure, oxygen concentration, primary productivity, and light regimes. In this study, archival depth, temperature, and light records for bluntnose sixgill sharks in the tropical oligotrophic habitat of Hawaii were analyzed and compared to records of sixgill shark behavior in other habitats, and to a prickly shark sharing the same slope habitat. Rates of vertical movement at various depths and times of day were compared to determine likely foraging habitat, and the potential influences of light, temperature, and oxygen concentration on the depth habitat and activity level of sixgill sharks in Hawaii were addressed. Light appears to be a primary factor influencing the diel diving cycle, and a combination of light and thermal limitations appears to exclude sixgill sharks from the tropical surface waters, though in Hawaii they ventured into warmer water than previously observed. Sixgill sharks are tolerant of both very cold temperatures (as low as 4.1°C) and low oxygen conditions (<60μmol). Horizontal movements were investigated based on tagging and pop-up locations, and revealed previously unobserved long-distance movement and pelagic swimming, which may allow population connectivity and gene flow between distant habitats. Fisheries are increasingly reaching into deep slope habitats, and increased knowledge of slope shark behavior is necessary to predict impacts and implement effective management. A better understanding of how oceanographic parameters can influence sixgill shark behavior may help predict their depths of occurrence and role in ecosystems throughout their geographic range.

# 1. INTRODUCTION

## 1.1 Overview

Apex predators play an important role in ecosystems through exerting top down controls on the food web (Ferretti et al., 2010), but rapid declines of deepwater shark populations have been observed with the deepening of commercial fisheries. Due to the long lifespan and presumed low fecundity of many deepwater elasmobranchs, they may be slow to recover from population declines (Simpfendorfer and Kyne, 2009). Assessing the impacts of anthropogenic pressures and implementing effective management for these species is difficult due to the challenges of studying these animals, such as low density in the wild (Ebert, 1986) and their occurrence below the photic zone. Even basic life history parameters such as age at maturity, fecundity, and breeding cycles are not well understood for many species.

In addition to the need to better understand the life history of deepwater elasmobranchs, the behavior, spatial ecology, and food web interactions of many deepwater species are often unknown, and there are few published studies of deepwater shark behavior and habitat use in locales where they inhabit depths greater than 250m (Carey and Clark, 1995). Environmental parameters such as light, temperature, oxygen, and bottom depth are potential drivers of behavior, and understanding the animal's relationship with these parameters will help define a global habitat envelope for wide-ranging species such as the bluntnose sixgill shark (*Hexanchus griseus*; hereafter referred to as the sixgill shark). In this study, the behavior of deepwater sharks on the island of Oahu, HI, was investigated using pop-up satellite archival tagging, with particular focus on sixgill sharks. Diel patterns and rates of movement were analyzed alongside oceanographic parameters of light, temperature, and oxygen, and the results were interpreted in an ecological and physiological context.

## 1.2 Study Site

Hawaii is located in the tropical north Pacific, with the island of Oahu located at about 21ºN and 157ºW. The islands are in the trade wind zone, so for most of the year, northeasterly winds drive cooler water temperatures to the east and north of the island, with warmer ocean temperatures on the south and west side of the island due to the lee effect. The islands are in the midst of the north Equatorial current, a westerly current that

hits the south-easternmost island of Hawaii and deflects around the island chain. In the canonical circulation, a countercurrent forms in the lee of the islands flowing east called the Hawaii Lee Countercurrent. When the countercurrent encounters the islands as it moves east, the Hawaii Lee Current forms and flows west just south of the islands (Lumpkin, 1998). Focusing in on Oahu, one notable physical oceanographic feature is the generation of large internal waves at the Kaena ridge, on the northwest corner of the island. These internal waves can heave the thermocline substantially and cause higher than normal variation in temperature at a particular depth. The effects are noted from the Waianae slope off western Oahu and from Mamala Bay on the southern shore of Oahu (Chavanne et al., 2010; Eich et al., 2004; McManus and Powell, 2011).

The following oceanographic parameters are presented to provide a contextual background for the discussion of deep shark habitat in this ecosystem. The Hawaii Ocean Time Series (HOT) has monitored both near-shore and open ocean oceanographic parameters near Hawaii for over 20 years, providing an excellent baseline describing the physical and biogeochemical properties and variability of the ocean environment (Karl and Lukas, 1996). The ocean near Hawaii has surface temperatures which range from 24-27ºC, with a thermocline between about 200-500m (HOT, 1989-2009). As an oligotrophic system, the surface nutrient concentrations are very low, and nitrogen limits primary production (Dore et al., 2008). The mixed layer ranges from about 40m in the lee of Oahu to about 100-120m in the nearby open ocean, and beyond that, nutrients increase deeper in the water column to a concentration maximum around 1000m.

The oxygen minimum zone (OMZ) is between about 650-800m depth, with concentrations as low as 20-30 μmol/kg (HOT, 1989-2009). Oxygen minimum zones vary globally, with the most extreme occurring in the eastern tropical Pacific and the Arabian Sea where oxygen content reaches lower than 4.5μmol (Karstensen et al., 2008; Morrison et al., 1999). OMZs in the Atlantic range from about 17μmol to 40μmol, and in the North Pacific, are generally less than 45μmol (Karstensen et al., 2008). Concentrations of oxygen less than 60μmol have negative effects on coastal fishes accustomed to well-ventilated water (Hofmann et al., 2011a) and pelagic fish such as tunas and billfish require oxygen concentrations above ~150μmol (Stramma et al., 2012). However, many fishes and invertebrates are well adapted to low oxygen (Childress and

Seibel, 1998) and some organisms can even thrive in suboxic conditions in the eastern tropical Pacific through the use of vertical migrations to repay oxygen debt and suppression of routine metabolic rate (Rosa and Seibel, 2010).

The Hawaiian Islands are volcanic in origin, and the geologic processes that formed the islands created steep island slopes. This feature makes Hawaii an ideal location to study deep ecosystems for several important reasons. First, the slope is much steeper than continental margins, where access to deep water can take hours of travel, so logistically it is ideal to study deep water organisms in Hawaii. Additionally, the slope habitat supports unique communities of organisms that are associated with the island and likely play an important role in the slope food web. For instance, the Hawaiian mesopelagic boundary community (MBC) is composed of slope-associated micronekton which undergo diel vertical and horizontal migrations following the contours of the island slope (Benoit-Bird et al., 2001; Reid et al., 1991). The MBC has daytime residence depths from 400m to greater than 600m near the seafloor, and at night, moves inshore and shallower to between 20-400m depth (Benoit-Bird and Au, 2006; Ebert, 1994; Reid et al., 1991). The animals comprising this community include shrimp, myctophids, hatchetfish, and squid, and the community is an essential component of the food web on the Hawaii slope.

The deep slope also houses a unique assemblage of megafaunal scavengers, which tend to subsist on animal carcasses from both shallow and deep depths (Yeh and Drazen, 2009). The island slopes and accompanying canyons support a hotspot of biomass in the oligotrophic tropical Pacific (Vetter et al., 2010), and additionally support larger organisms such as pelagic top predators and deep benthic sharks. Regulations ban commercial longline fishing within 50 nautical miles of the Main Hawaiian Islands (NOAA, 2006), so deep sharks are not often subject to by-catch impacts or targeted fishing in this habitat. The deep shark population in the Main Hawaiian Islands is likely unaffected by fishing compared to commercially fished habitats.

Deepwater sharks have previously been observed on Oahu via longline survey (Crow et al., 1996), baited camera (Yeh and Drazen, 2009) and the Hawaii Undersea Research Laboratory (HURL, www.soest.hawaii.edu/HURL). In a study of scavenging megafauna of the Hawaiian Islands, organisms identified to genus level or more included

15 teleosts, 6 elasmobranchs, 4 shrimps, and 11 crabs. *Hexanchus griseus, Somniosus pacificus, Pseudotriakis microdon,* and *Echinorhinus cookei* were the largest sharks observed, and depending on their depth ranges, they could be potential competitors. From shallow to deep, *E. cookei* was observed at 250m, *H. griseus* at 500, 508, and 1000m, *P. microdon* at 1000 and 1500m, and *S. pacificus* at 1000, 1500, and 2205m. Based on this work, the study species *H. griseus* overlapped in its depth habitat with *P. microdon* and *S. pacificus* in the deeper part of its observed range (Yeh and Drazen, 2009), but further investigation into species competition, food web interactions, and niche selection is needed.

### 1.3 *Hexanchus griseus*, the bluntnose sixgill shark

#### *1.3.1 Phylogeny*

*Hexanchus griseus* is a chondrichthyan fish of the Order Hexanchiformes, family Hexanchidae (Compagno, 1984). This family of elasmobranchs has six or seven gill slits instead of the common five, and includes four species: *Notorynchus cepedianus, Heptranchias perlo, Hexanchus vitulus*, and *Hexanchus griseus*. Sometimes, *H. perlo* has been grouped into a separate family, Heptranchidae. *H. griseus* is distinguished from *N. cepedianus* and *H. perlo* by the occurrence of six, rather than seven, gill slits, and is distinguished from its congener by its blunter snout, larger size, and a shorter distance between the start of the dorsal fin and start of the caudal fin (Compagno, 1984).

#### *1.3.2 Size, maturity, and reproduction*

Female sixgill sharks grow larger than males, and the largest female reported reached a total length of 482cm (Bigelow and Schroeder, 1948). Size at birth ranges from 61-93cm total length based on length measurements of neonates (individuals with an open umbilical scar) captured off Namibia, and term embryos from a pregnant female captured off California were 61-64cm total length. Size at maturity is estimated at about 420cm for females and 310cm for males (Ebert, 2002), although few female sixgills between 320-405cm were included in the study. The smallest mature female sixgills have been observed with total lengths of 405 and 422cm (Ebert, 1986, 2002).

Sixgill sharks appear to be among the most fecund elasmobranchs, with observed litter sizes of 47-108 individuals from four different pregnant females (Desbrosses, 1938; Ebert, 1986). Ebert notes that pregnant female sixgill sharks tend to abort their embryos

upon capture, so these litter sizes could be artificially low. Pupping grounds and nursery areas are not well known, but juvenile sixgill sharks have been observed to enter nearshore bays and estuaries and also have been caught in deeper water (Desbrosses, 1938; Dunbrack and Zielinski, 2003; Ebert, 2002; Williams et al., 2010). Observations of juvenile and neonatal sixgill sharks on a shallow water reef near the Strait of Georgia, British Columbia, coupled with a seasonal pattern of occurrence during June-September, suggest that sixgill sharks may use shallow estuaries for periodic or seasonal breeding events (Dunbrack, 2008).

*1.3.3 Geographic and depth ranges*

Sixgill sharks have an extensive latitudinal range, among the largest of elasmobranchs. Scientific studies of sixgill sharks have included specimens from Bermuda, California, Baja California, Puget Sound, British Columbia, and South Africa (Andrews et al., 2007; Carey and Clark, 1995; Dunbrack and Zielinski, 2003; Ebert, 1986, 1994), and specimens of the species have also been observed on the Atlantic continental shelf and north Atlantic (Desbrosses, 1938), in the Bahamas (pers. comm. Dean Grubbs), in the Hawaiian Islands, and on Cross Seamount, south of Hawaii (Hawaii Undersea Research Laboratory, www.soest.hawaii.edu/HURL). They appear to inhabit almost all tropical and temperate continental shelves, and also non-continental habitats of suitable depth such as islands, seamounts, and the mid-ocean ridges.

Their depth ranges vary substantially in different locations, from near-surface to as deep as 1875m (Compagno, 1984). In higher latitude locations, the sharks tend to be found shallower; in the Strait of Georgia, sixgill sharks were observed at 40m, and occupied habitat at least from 40-300m (Dunbrack, 2008; Dunbrack and Zielinski, 2003). In the Puget Sound, observed depths of sixgill sharks ranged from about 14-250m (Andrews et al., 2009). In lower latitude habitats, the sharks are found to inhabit a deeper depth range. In Bermuda, active tracking of sixgill sharks revealed a depth range of 600-1500m (Carey and Clark, 1995). In Hawaii, sixgill sharks have been observed by submersible between 500 and 1400m, captured at 110m (n=1) 330-366m (n=8) in University of Hawaii's 1967-1969 longline surveys (Crow et al., 1996) and observed on baited camera at depths of 500 and 1000m (Yeh and Drazen, 2009).

*1.3.4 Diet and trophic ecology*

Stomach content studies of sixgill sharks have revealed a generalist diet consisting of cephalopods, teleosts, chondrichthyes, and marine mammals, and there is an observed ontogenetic shift in important prey items (Ebert, 1986, 1994). Off of South Africa, small sixgills less than 120cm TL prey heavily on cephalopods, with the most important prey item being *Todorodes anglolensis*, and also eat significant amounts of teleost fish (particularly hake) and elasmobranchs (particularly catshark). From 120-200cm TL, the sharks' diet shifted to favor teleost fish over cephalopods, and larger sharks >200cm TL had a diet with a high proportion of marine mammals and teleosts. The largest shark stomachs observed, from animals >400cm, commonly included swordfish and marlin as prey items (Ebert, 1994), which could indicate either pelagic foraging or feeding on sinking carcasses of these pelagic fishes. Meta-analyses of trophic position based on stomach contents by (Cortes, 1999) and FishBase (www.fishbase.org) agree on a trophic position of 4.3 for *H. griseus;* however, the ontogenetically changing diet suggests that trophic position may change with size. Additionally, food web structure is likely to vary significantly within the sixgill shark's large geographic range, so this trophic position assignment requires further examination.

*1.3.5 Behavior and Spatial Ecology*

Little is known of the sixgill shark's behavior and ecology, particularly in deeper habitats. They are found in multiple and varied habitat types including shallow estuarine systems and deep oligotrophic island slopes, and previous research suggests that their depth ranges vary significantly between these two habitat types (Andrews et al., 2009; Carey and Clark, 1995; Crow et al., 1996; Yeh and Drazen, 2009). To date, most studies of *Hexanchus griseus* behavior have been limited to productive estuarine systems where the sharks are present in shallow water up to 15m (Andrews et al., 2007; Andrews et al., 2009; Dunbrack, 2008; Dunbrack and Zielinski, 2003). Only one published study has focused on the behavior of specimens occurring in a deeper habitat (Carey and Clark, 1995).

Two adult female *H. griseus* specimens were tracked by active acoustics in a low-latitude, deep water habitat (Carey and Clark, 1995). The sharks showed no discernible patterns in vertical movement, and stayed close to the seafloor at about 600-700m with

deeper excursions up to 1500m. This study had a small sample size and only tracked the animals for a few days, so there remains a lack of knowledge about sixgill shark behavior in deeper habitats. In contrast, sixgill sharks in Puget Sound, a higher-latitude estuary, displayed regular and pronounced vertical oscillations ranging from 15-250m depth (Andrews et al., 2009). These sharks remained close to the seafloor most of the time, but were also observed swimming higher in the water column (Andrews et al., 2009). In the Strait of Georgia, sixgill sharks were observed moving upslope from depths of about 200-300m, and utilizing habitat less than 40m in depth, but this behavior was only observed in summer months (Dunbrack and Zielinski, 2003). Potentially, the seasonality of behavior could be due to thermal response or a reproductive cycle.

The home range size and horizontal movements of sixgill sharks are unknown throughout most of their range of occurrence. On short time scales, they appear to have a high degree of site fidelity. Two adult female sixgill sharks actively tracked in Bermuda travelled less than 10km from their tagging location (Carey and Clark, 1995), but the short time frame of the study does not allow robust conclusions about home range size. In a multi-year study in Puget Sound, sharks detected by passive acoustic monitors in Puget Sound were found at the same site as their previous detection 62% of the time, and in the same general region 72% of the time (Andrews et al., 2010). A consistent seasonal pattern was observed in shark distribution within Puget Sound, with the animals moving northward in the spring and farther south in the late summer to early fall. Larger females were more likely to leave the Sound, but once the sharks move onto continental shelf habitat, much less is known about their movement patterns. It is possible that sixgill sharks may return periodically to Puget Sound to mate or breed (Andrews et al., 2010). There is a lack of data concerning even short timescale site fidelity in tropical habitats, and globally, large scale and long term movements and migrations of the sharks (if any) are unknown. The degree of population heterogeneity, presence or absence of migratory behavior, and home range size are important gaps in knowledge that need to be addressed to design effective management practices for sixgill and other deepwater sharks.

### 1.4 *Echinorhinus cookei*, the prickly shark or Cooke shark

Though this study focuses on the sixgill shark, *Echinorhinus cookei* (hereafter "prickly shark") is another deepwater shark that inhabits the slope ecosystem of the

Hawaiian Islands and may be a niche competitor with the sixgill shark. The prickly shark is a squaliform shark of the family Echinorhinidae. Like the sixgill shark, the prickly shark is widely distributed on continental and insular slopes (Compagno, 1984), but details of its life history, population, behavior, and feeding ecology are poorly known. Stomach contents have included a wide range of benthic and nearshore fishes, crustaceans, and cephalopods (Compagno 1984a). One behavior study revealed diel periodicity in the movements of juvenile prickly sharks studied near Monterey Canyon, CA (Dawson and Starr, 2009), with the prickly sharks more active at night than day and with the highest activity levels occurring at dawn, as determined by active acoustic tracking. The depths inhabited ranged from near surface to 100m at night, and about 150-250m during the day (Dawson and Starr 2009). Like the sixgill shark, the prickly shark may exhibit changing depth ranges throughout its wide geographic range, but it has never been studied in Hawaii or other tropical environments.

Though deepwater sharks are found in a wide range of habitat types, there could be common environmental drivers of their movements. For example, movement patterns of fish have previously been explained by factors including light (Nakano et al., 2003), thermal habitats (Perry et al., 2005), turbulence (Bakun and Parrish, 1982), food availability (Barnett et al., 2010), and bioenergetic advantage (Sims et al., 2006). In this study, we aim to use environmental data and time-series records from tagged sixgill sharks to determine vertical movement patterns, depth, thermal, and light habitat, and address several hypotheses regarding environmental drivers of movements.

## 1.5 Hypotheses

### *1.5.1 Sixgill sharks will not cross open water or channels deeper than 2500m.*

Unlike bony fishes, chondrichthyes are essentially absent from the abyssal ocean. Elasmobranchs generally are not found deeper than 2500m in the ocean (Priede et al., 2006). *Hexanchus griseus* is generally thought to be bentho-pelagic; if the sharks are limited by association with the benthos and a maximum depth of 2500m, they cannot cross the open ocean. Whether or not the animals are limited by a depth barrier has implications for population connectivity.

*1.5.2 Sixgill sharks move to maintain a consistent light level.*

Many animals in the ocean move in a diel vertical migration proximately driven by changing light levels in the ocean (Boden and Kampa, 1967). For prey species, avoiding light helps avoid predation, and some predators, such as the basking shark, have adapted to undergo vertical migrations to follow their food source (Sims et al., 2005). The bigeye thresher shark, which possesses orbital *retia mirabilia* in its advanced eye structure, also has been show to move vertically in the water column in response to changing light levels (Weng and Block, 2004). Sixgill sharks, however, have been shown to exist in a wide variety of light environments, from the shallow, well-lit water in the Flora Islands, British Columbia (Dunbrack and Zielinski, 2003), to the near-total darkness at 600m depth off Bermuda, so it is unlikely that light is a singular driver of movements. Continued growth of the elasmobranch eye has been documented in *Squalus mitsikurii*, with an ontogenetic shift in focal ratio (Litherland et al., 2009a), so perhaps an ontogenetic shifts in light sensitivity is possible for sixgill sharks and could help explain the variation in light tolerance in different habitats. Examination of archival depth and light records will allow an assessment of the importance of changing light levels in daily movement patterns for sharks captured in Hawaii.

*1.5.3 Sixgill sharks exhibit a thermal tolerance ceiling at 16°C, the highest temperature observed in the literature (Dunbrack and Zielinski, 2003).*

Thermal range could be very important in explaining a latitudinal shift in depth. Furthermore, if a thermal ceiling or limit is discovered for *H. griseus*, implications for climate change become apparent. Warming oceans cause marine animals with thermal boundaries to change their distribution by moving to cooler water, either horizontally or vertically (Perry et al., 2005). If thermal boundaries are important, warming temperatures could potentially exclude sixgill sharks from important nursery or feeding grounds.

*1.5.4 Sixgill sharks avoid depths where the oxygen concentration is <60µmol.*

Most fishes become stressed in hypoxic conditions, which are defined in this study as oxygen concentrations below 60µmol/kg based on (Hofmann et al., 2011b). If sixgill sharks avoid hypoxic water in Hawaii, they would avoid spending time deeper than about 560m (based on HOT data). Alternatively, if they are tolerant of hypoxic

conditions, the maximum depth could be based on a minimum temperature or food availability.

*1.5.5 Rate of vertical movement will be greater in shallow, warm water than in deep, cold water.*

Total organism biomass typically decreases with depth, so successful foraging is more likely in shallow water where prey are more abundant. If the animals are foraging more in warm water, they will have a corresponding higher rate of vertical movement as they search for food (Nasby-Lucas et al., 2009; Sims et al., 2006). Changes in rate of movement with changing temperature may indicate metabolic regulation, and help predict where the animal is feeding and what food webs it can interact with.

**1.6 This study**

In this study, the movements and behavior of *Hexanchus griseus* are investigated to clarify temperature and depth habitat, determine their behavioral relationship with various physical oceanographic parameters such as temperature and light, and attempt to elucidate which physical parameters are most important in explaining variability in their behavior, and if those parameters appear to be consistent across multiple habitats. Another deep slope shark, *Echinorhinus cookei*, was studied opportunistically and compared to *Hexanchus griseus* as a possible niche competitor.

## 2. METHODS

### 2.1 Capture and Tagging

Sharks were captured using a bottom set longline, with 5-40 hooks per set. Gangions consisted of a longline clip with swivel, 2-6m of wire leader, and a 13/0 to 18/0 size circle hooks. Hooks were set approximately every 15m on the main line. Set depths ranged from 300-600m depth, and planned set times ranged from 8-14 hours, typically soaking overnight. There was one unintentional longer set of 20 hours, due to a tsunami closing the harbors. When captured, animals were brought to the side of the boat and immobilized using a tail rope and, when practical, were constrained in a sling suspended from the rail of the vessel. If lifted out of the water by the sling, the animals were irrigated with a seawater hose.

Each animal was measured from snout tip to precaudal notch, yielding the precaudal length (PCL), and were additionally measured for fork length (FL) and total length (TL) when possible. When PCL was the only measurement available, total length was calculated using a PCL-TL biometrics regression equation provided by Greg Williams of NOAA (pers. comm.), based on >100 measurements of PCL and TL on juvenile to adult sixgill sharks. The sex of each animal was recorded, and tissue samples of muscle and skin were taken for other analyses. The animals were also photographed and their condition was subjectively rated as poor, fair, good, or excellent. “Poor” sharks displayed very weak swimming or no tail beats, and may have had scar or entanglement marks from the longline. “Fair” animals were tired or slightly injured, and displayed some swimming. “Good” animals were able to swim downward on their own and looked relatively healthy, and “excellent” animals were feisty, energetic, and had not sustained visible injuries.

When possible, all sharks were tagged with conventional external tags manufactured by Roto-Tag (Ontario, Canada). Tags were positioned on the trailing edge of the first dorsal fin. Sharks in fair to excellent condition were tagged with Vemco V16 acoustic pingers (Vemco, Halifax, Nova Scotia) for an island-scale horizontal movement study, the results of which will be published in a later work. These tags were surgically implanted in the ventral body cavity, and when necessary, the wound was closed with 2-3 sutures. Selected “good” and “excellent” *Hexanchus griseus* specimens were tagged with

pop-up satellite archival tags manufactured by Wildlife Computers (MK-10 PAT and MiniPAT models, Seattle, WA). The tags were attached externally on the dorsal surface of the animal with a plastic dart, which was attached to the tag with a wire leader. Tags recorded time-series data for depth and temperature at intervals of 5 or 7.5 minutes, and recorded archival data at 3 or 5 second intervals, depending on the length of deployment. Days at liberty ranged from 55-93 days. On the date of release, the tag's internal clock signaled the start of corrosion of the link between the leader and the tag, allowing the tag to release and float to the surface.

**2.2 Data recover**y

Data were recovered via Argos satellite communications when the tag detached from the animal and reached the surface. The tags transmitted time-series data, which were then available for download from the Argos website. The tags transmit every 60 seconds, and the transmissions can be detected with a radio direction finder (RDF). The use of the RDF facilitated the physical recovery of tags that popped up within range for recovery (Doppler Systems, AZ; Communications Specialists, Orange, CA).

**2.3 Anchored tags**

Two MiniPAT tags were deployed on the southern slope of Oahu at constant depths of 410m and 210m to assess ambient light levels and any fluctuations in temperature and depth. The tags were affixed to a buoy and attached to an acoustic release for recovery (Sonardyne, Yateley, UK). The tags recorded depth, temperature, and light data at one second intervals for 7 days.

**2.4 Data analysis and statistics**

Time series data were analyzed by computing the median and interquartile range (IQR) for each variable - depth, temperature, and light - at each of four predefined diel periods of dawn, day, dusk, and night. Sunrise, sunset, nautical dawn and dusk, and moon illumination data were obtained through astronomical tables available from the Astronomical Applications department of the U.S. Navy (aa.usno.navy.mil). Dawn and dusk were defined as encompassing one half-hour before and after the time of sunrise/sunset as listed in the Navy tables. All analyses were performed in Matlab (Natick, MA, 2011).

The first 3-5 days of each depth record were omitted from analysis, since the behavior of the shark was inconsistent with the remainder of the record and indicated a recovery period after the stress of capture and handling. Recovery periods have been noted for other shark species (Campana et al., 2009; Frick et al., 2010) and are often a period of rest, extended time at a particular depth inconsistent with later behavior patterns, or other unusual behavior. The recovery period in sixgill sharks was characterized by a departure from the normal depth regimes, and in some sharks, seemingly random vertical movement instead of the diel vertical migration pattern.

Addressing the proposed hypotheses

*2.4.1. Sixgill sharks will not cross open water or channels deeper than 2500m.*

This hypothesis was addressed by mapping tagging and pop-up location to determine if there was unequivocal evidence that the shark left the Oahu slope and traversed water deeper than 2500m. A tag could end up transmitting over deep water or across a deep channel by several pathways: for instance, the tag could move offshore after the pop-up due to surface currents before it transmits a location; the tag could be ingested by another animal which then carries it elsewhere before rejecting it; the shark could be caught, the tag removed, and the tag could be carried by a boat before transmitting a location; or the tagged shark could actually be swimming over deep water.

Pop-up locations over very deep water that were close to a slope were subjected to further analysis, since a tag popping off a shark on the slope could potentially drift over open water as it floated several hundred meters to the surface. The time between release and first transmission was calculated, and, based on typical current speeds for the Hawaii region, was used to calculate a radius of origin to investigate whether the shark was likely associated with a slope at the time of pop-up. The last several days of the depth record will indicate whether the tag remained on a sixgill shark; if there are any major discrepancies in the depth pattern and habitat compared to earlier in the record and other sixgill sharks, the tag could have been ingested by another animal. Good evidence for pelagic swimming behavior is a tag which transmits away from the Oahu slope over deep water or across a channel without behavior change. The depth record may also indicate pelagic swimming if the depth traversed is much deeper than the depths recorded by the tag.

*2.4.2. Sixgill sharks move to maintain a consistent light level*

If sixgill sharks initiate diving behavior as light levels begin to increase at dawn, dive to daytime light levels equivalent to the observed nighttime light level, and/or change their median night depth in response to changing light levels based on moon phase, light could be an important factor driving movements. The variability of light levels recorded by the anchored MiniPAT tags versus tags on moving sharks was compared using an F-test for unequal variances. The median and minimum nighttime depth were compared to moon illumination data obtained from the U.S. Naval Observatory (aa.usno.navy.mil/data) to determine if changing moon brightness had any effect on nighttime depth.

Light levels throughout the diel cycle on a tagged shark were compared to records from the anchored tags and used to describe the daily light regime experienced by a sixgill shark as it moves throughout the water column. Potential effects of temperature on light level reading were explored in the lab by exposing two used MiniPAT tags to indoor fluorescent light, sunlight, and darkness in a room temperature bath and ice bath. The tags were positioned in the water bath with one light sensor facing directly upwards. The purpose of this test was to determine the direction of bias in light readings based on temperature, which would help interpret light data from a tagged shark moving through a wide thermal range.

The daily dive was defined as the transition from the shallow nighttime habitat to the deep daytime habitat. The initiation of the dive was easily visible in the graphed time-series, but challenging to pinpoint algorithmically due to oscillations at a variety of frequencies throughout the record. Smoothing functions and low-pass filter signal analyses were attempted; however, both approaches degraded the inflection point where the shark began to dive. Therefore, the initiation of this dive was defined for each day by finding the time at which the shark departed a normal nighttime depth (between the median and 3$^{rd}$ quartile of nighttime depth observations) and reached a typical daytime depth before returning. Quality control was accomplished through visual inspections of the depth record and generated dive times. These dive initiation points were compared to the time of nautical dawn for that day, and a linear regression was used to examine the relationship between dive initiation and nautical dawn for each shark.

*2.4.3. Sixgill sharks exhibit a thermal tolerance ceiling at 16°C, the highest temperature observed in the literature*

The temperature time series data were examined for evidence of a thermal ceiling. The highest temperature observed after the recovery period was determined for each shark and compared to literature values. The median and maximum nighttime temperatures were determined for each day and compared to the highest previously observed temperature of 16ºC (Dunbrack and Zielinski, 2008). The length of time of exposure to water warmer than 16ºC was calculated for each warm event. When blue sharks move into warmer water, muscle tissue warms following a half time constant of about 30 minutes (Carey and Scharold, 1990). Assuming similar warming in sixgill muscle tissue, any exposures of two hours or more would likely warm the muscle tissue above 16ºC and represent a true expansion of the known thermal tolerance of the species.

*2.4.4. Sixgill sharks avoid a hypoxic environment.*

Depth data were compared to nearby measured dissolved oxygen concentrations to determine if the animals spent significant time in the oxygen minimum zone. Oxygen data were derived from the Hawaii Ocean Time Series database at Station 1 (Kahe Point, Oahu), focusing on cruises and measurements that coincided with the dates (or when unavailable, the season) the animals were being tracked. The oxygen minimum zone on the Hawaiian slope was defined as the depth range in which the dissolved oxygen concentration was less than 60μmol (Hofmann et al., 2011a).

*2.4.5. Rate of vertical movement will be greater in warmer temperature water*

Rate of movement (ROM) was determined by finding the difference between two successive depth measurements. For statistical analysis, high-frequency archival depth data were sub-sampled in five minute intervals to be consistent with the slowest sampling interval in the transmitted record, and ROM was calculated from the resulting five-minute archival record. The absolute value of rate of movement was used as a crude proxy for shark activity level, although no direct measure of horizontal speed was available. For analyses and graphs of individual sharks, the highest sampling frequency available was used initially. Activity levels in meters per minute were calculated for each recovered archival record by summation of the depth difference between all consecutive points, but noise in the depth data biased results. Tags were observed to record

fluctuations of ±1m in successive observations (frequency of 1s) on anchored tags at constant depth. Therefore, data were sub-sampled at 1-minute intervals to reduce the effect of noise and provide better estimates of activity level.

Records of animal movements are often strongly autocorrelated from one time point to the next across multiple lag intervals, which does not allow the use of any statistical analysis assuming independence of observations (Dray et al., 2010). Sixgill shark depth data was analyzed for autocorrelation at lags of one observation (3 seconds, 5 seconds, or five minutes, depending on the tag), one hour, and one day. Generalized estimating equations (GEE) were then used to assess the strength of temperature, depth, moon phase, and time of day as predictors of shark vertical activity level.

GEE was chosen as an analytical tool because it is an extension of general linear modeling which accounts for non-independence (autocorrelation) in time series records by treating the autocorrelation as a "nuisance" term (Liang and Zeger, 1986). This statistical technique has been previously used in a bluefin tuna satellite tagging study for similar analyses (Wilson et al., 2005). The "GEEQBOX" software written for Matlab was used for the statistical analysis in this study (Ratcliffe and Shults, 2008). Due to computational constraints, the entire archival records could not be analyzed in GEEQbox. Therefore, the median depth and temperature from each diel period of each day were calculated, and this abbreviated dataset was used for statistical analysis. This dataset included median values of depth and temperature for dawn, day, dusk, and night for each day at liberty.

## 3. RESULTS

### 3.1 Fishing and animal captures

In 11 deep longline sets over 14 months, 221 hooks were set and 25 animals of 8 species were captured. Of these 25 fish, 24 were elasmobranchs and one was a teleost, *Lepidocybium flavobrunneum.* The most commonly captured animal was *Hexanchus griseus* (n=13), followed by *Galeocerdo cuvier* (n=4) and *Echinorhinus cookei* (n=3). Catch per unit effort (CPUE) overall was 0.113 and CPUE for the study species *Hexanchus griseus* was 0.058.

### 3.2 Satellite tagging summary

Seven satellite tags were deployed between March 2011 and March 2012. Six *Hexanchus griseus* and one *Echinorhinus cookei* were tagged (Table 1). The tagged animals were chosen due to their good condition upon capture. Focusing on the main study species *H. griseus*, sharks ranged in size from 266-333cm. 3 immature females, 1 immature male, and 2 mature males were tagged. The time at liberty ranged from 53-90 days, and 5 out of 6 tags reported data (83.3%). Retention time on the tags that reported was excellent: the tags remained attached to the animals and continued recording data for 100% of their programmed cycle.

### 3.3 Tag data recovery

Of the six tagged sixgill sharks, three tags were physically recovered near Oahu, one tag was recovered near Kauai, one tag transmitted time-series data from a distant pop-up location, and one tag never reported. The archival records resulted in 4 high-resolution data sets of depth, temperature, and light level sampled at 3-5 second intervals. The transmitted record consisted of depth and temperature readings sampled at 5 minute intervals, and 95.4% of the dataset successfully transmitted. Time-series plots were constructed for the depth record of each individual shark (Figure 1a-e). These time series plots revealed highly repetitive behavior in a diel frequency, a post-release recovery time of 3-5 days before assuming “normal” repeated behavior patterns, and occasional deep dives below 800m observed for sharks 1 and 2 only.

### 3.4 Depth

The non-parametric descriptive statistics for depth, temperature, and light level for each shark during four diel periods are shown in Table 2. All sharks displayed similar

strong diel vertical migrations, moving shallower at night (median depth for each shark ranged from 260.5m to 324.5m ) and deeper during the day (medians from 610m to 673.5m) (Figures 2-6). Plots of depth versus time of day show a steep dive at dawn, and usually a similar steep ascent at dusk (Figure 2-6), or in the case of shark 2 more gradual depth changes throughout the day (Figure 3; see section 3.9). Sharks dove before the first observed light on a ship-based light sensor (See section 3.6). The observations of the light sensor matched data from multiple sources including the United States Naval Observatory (www.usno.navy.mil) and the Kalaeloa SOLARMAP facility maintained by National Renewable Energy Labs (http://www.nrel.gov/midc/kalaeloa_oahu/). Median depths throughout the day for all sharks are shown in Figure 7, highlighting the similarities and differences in their individual movement patterns. Shark 6, an *Echinorhinus cookei* specimen that was tagged with a MiniPAT, is shown to emphasize the similarities of the sixgill sharks' movements compared to a different species. Median and IQR of all depth observations by time of day highlight likely sixgill shark depth habitat in the Hawaii region (Figure 8).

**3.5 Horizontal Movements**

The light field of sixgill sharks was never reliably detected due to their diel vertical migrations (Section 3.6) so light based geolocation was not possible. Therefore, the only two points available to address horizontal movement are the tagging location and the pop-up location of the tag. The distance between tagging and pop-up locations ranged from 29.8-969km (Table 1). Two of the five sixgill sharks, shark 2 and shark 3, remained on Oahu. Shark 1 traveled surprisingly far, almost 1000km southwest to near Johnston Atoll. Shark 4 crossed the Kauai Channel and was on the southern Kauai slope at pop-up, and shark 5 crossed the Kaiwi Channel separating Molokai and Oahu and appeared to be near the Penguin Bank slope when the tag released. The tagging and pop-up locations of each shark are mapped over multi-beam bathymetry provided by Hawaii Mapping Research Group at the University of Hawaii (Figure 9). Horizontal rates of movement calculated by total distance/days at liberty probably do not capture the true speeds of the sharks, with the possible exception of the Shark 1. The speeds calculated using this crude method ranged from 0.3km/day (Shark 5) to 17.2km/day (Shark 1).

Most tags popped up over depths <1000m, but the water depth at the pop-up location for shark 1 was approximately 5300m. The recorded depth at the time of pop-off was 297.5m, indicating that shark 1 was swimming in the pelagic. The lack of nearby land masses or shallow ridges or seamounts suggests that shark 1 swam in open ocean for an extended period of time. Additionally, Shark 4 crossed the deep channel separating Kauai and Oahu (>2000m) without any discernible change in behavior, and must have also been swimming in the pelagic during part of the record.

The long-distance travel to near Johnston Atoll was unexpected based on previous observations of sixgill shark site fidelity in Hawaii (Dean Grubbs, pers. comm.). To be confident in the location reported by the tag, the Argos location quality was examined and found to be fairly accurate, with location qualities of predominantly 2 and 1, translating to accuracies of 500m to 1km. Therefore, the position itself is likely to be accurate within a 1km radius (Argos User's Manual, 2011). Second, the depth record was compared to the other sixgill shark records to rule out the possibility of the tag being ingested and carried to the location by another animal or on a boat. The depth record and depth throughout the day were consistent with sixgill shark behavior as observed in other records (Figure 1).

**3.6 Light**

The light meter on the MiniPAT tags is not calibrated to a specific standard, but generally, a reading of about 215 corresponds to full sunlight, twlight reads ~150, a full moon ~110, and an overcast night ~50 (Wildlife Computers, Seattle, WA). Sixgill sharks' light level rarely exceeded 30, so excluding a few outliers (potentially encounters with biolumiescent organisms) these sharks did not experience any significant illumination throughout the their time at liberty. Regression analysis of light level at a constant, shallow night depth (10m range, depth analyzed varied by individual shark) revealed no effect of percent moon illumination on light level reading ($r^2<0.05$ for all records).

Light readings were consistently higher at night than during the day, which resulted in an inverse light curve (Figure 10). This inverse light field was not due to thermal effects; in a lab test, the light sensor on MiniPAT tags recorded higher light readings in cold water than warm water under constant conditions of fluorescent indoor

lighting, sunlight, and darkness (mean difference of 7.1 ± 2.7 light units). The temperature difference between the warm and cold water treatment was 12.9 ± 1.4ºC.

Two tags anchored tag at constant depths of 410m and 210m each recorded daytime light levels greater than 30, with expected (not inverse) light curves corresponding to dawn and dusk (Figure 10). These anchored tags had higher inter-quartile ranges than tags on a moving shark, even at the deep deployment. Tags on free-swimming sharks showed significantly lower light variability than tags anchored at 210m (one-tailed f-test for equal variances, p=0.00, fstat: 152.1204 df1: 698616 df2: 103678) and 410m (f-test; p=0.00. fstat :59.3491, df1: 700579, df2: 103678) (Figure 11). Light intensity measured by shark tags at night was similar to the nocturnal light measured by the tag anchored at 210m depth. The inverse light curve measured by shark tags was therefore caused by higher light intensity and shallow nocturnal habitats, vs. deeper diurnal habitats.

The time of dive initiation was positively correlated with nautical dawn for 3 sharks, but for two sharks there was a weak relationship or no relationship (Figure 12). Most dives occurred between nautical dawn and civil dawn, but sometimes dives occurred well before nautical dawn, particularly for sharks 2 and 5. Dive initiation in shark 3 was most closely related to the time of nautical dawn, with an $r^2$ of 0.82 based on Pearson's linear regression. Shark 2, on the other hand, was not linearly correlated with the time of dawn ($r^2$= 0.01), but still dove at or before civil dawn for 94% of dives. As Shark 1 moved away from the Oahu slope, it continued to initiate its dive following nautical dawn, assuming a constant westward movement.

**3.7 Thermal Habitat**

Observed temperatures ranged from 4.1-19.15°C, with sharks inhabiting cooler water during the day and warmer water at night (7.33 ± 1.33°C difference, mean ± standard deviation). The average maximum daily temperature for each shark was 15.00 ± 1.14°C to 17.09 ± 0.86° C (mean ± standard deviation). For all sharks pooled, the average maximum daily temperature was 16.50 ± 1.32°C. The highest temperatures individual sharks encountered ranged from 17.7-19.15°C. Preferred thermal habitat in the shallow regime exceeded the expected value of 16°C, and each animal individually had excursions above the 16°C mark, expanding the known thermal habitat of the sixgill

shark. Median nightly depths remained below 16°C, but the maximum nightly depths exceeded 16°C regularly for sharks 1, 3, and 4, and occasionally for sharks 2 and 5 (Figure 13). The durations of excursions into water warmer than 16°C were calculated. Most excursions were less than 5 minutes long, but excursions up to 20 minutes long were not uncommon especially for sharks 1, 3, and 4. The longest continuous warm water excursion was 115 minutes for both shark 1 and shark 4 (Figure 14), which should warm the tissue and represents a legitimate expansion of thermal habitat.

### 3.8 Hypoxic Habitat

The oxygen minimum zone near Hawaii occurs near 800m, but the water becomes hypoxic around 562m (HOT). The average depth of the start of hypoxic conditions was calculated based on the past 5 years of HOT data from Station Kahe, near Oahu. The sharks regularly inhabited depths greater than 562m, and therefore spent considerable time in hypoxic conditions. Percentage of time spent in water <60μmol/kg $O_2$ ranged from 42.79-52.94% of the record for all sharks.

### 3.9 Rate of Movement and Activity Level

Vertical rate of movement (ROM) was typically greater in magnitude during the crepuscular periods, which encompassed most of the large-scale diel dives and ascents. Predictably, ROM was most positive during dusk, as the sharks ascended, and most negative during the dawn as they dove. During both day and night, the rate of movement was centered around zero, indicating that the sharks oscillated vertically around a particular isobath. However, the larger spread of ROM and lower density of ROM=0 data points at night show that the sharks were more active during the night than during the day. Density plots of ROM for each shark are shown in meters per five minutes, to maintain consistency with the five-minute sampling rate of the transmitted record from Shark 1 (Figure 15-19). Data for all sharks pooled is shown in Figure 20, and highlights the trend of lower activity during the day.

For each archival record with high resolution data, the rates of vertical movement are reported in meters/minute (Table 3) and median and maximum kilometers/day (Table 4). The depth data were subsampled at 1-minute intervals, which yielded a more accurate portrayal of shark vertical movements and activity level, and only those data are reported here (Section 2.4). Generally, activity levels were lowest during the day for all sharks,

and higher activity levels were observed during the dawn and dusk depth regime transitions and during the night, when the sharks inhabited warmer, shallower water. Shark 6, the prickly shark specimen, was included for comparison, and had lower activity levels during all diel periods except for night, where activity level was comparable to the sixgill sharks.

The significance of depth, water temperature, time of day and moon illumination as predictors of the rate of movement was analyzed using generalized estimating equations (GEE). Results from the robust covariance matrix are shown in table 5. Depth, temperature, and time of day were found to be significant predictors of activity level when all four diel periods were included, but moon illumination was not. When excluding crepuscular periods to compare the deep and shallow regimes directly, temperature and time of day were significant predictors, but moon illumination was not.

**3.10 Preliminary results: Echinorhinus cookei**

One prickly shark (*Echinorhinus cookei*) was captured and tagged with a MiniPAT using the same methods as for the sixgill sharks. This shark was a mature male measuring 234cm total length. After 69 days at liberty, the shark moved only 3.6km from its tagging location, far less than the sixgill sharks. This *Echinorhinus cookei* specimen also displayed pronounced diel vertical migrations, but had a shallower daytime regime and displayed less vertical movement at depth than the sixgill sharks. This shark also appeared to have a particularly long recovery period of about 7-10 days (Figure 21).

The prickly shark was found shallower at night than during the day, similar to the sixgills, but did not move deep enough during the day to enter hypoxic habitat. It had a depth range of 167-442m and a thermal range of 7-21ºC (Figure 22, Figure 23). Very low rates of vertical movement during the day were evident, and the highest rates of movement were observed at dawn and dusk (Figure 24). Dive initiation often occurred before nautical dawn, but sometimes occurred at dawn or just after (Figure 25).

| Shark ID | Date | Species | Total Length | Sex | | Latitude | Longitude | Location | Distance (km) | Days at liberty |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Capture | 21.255 | -158.105 | Barber's Point | | |
| Shark 0 | 11-Mar-11 | *H. griseus* | 323 | F | Pop-up | N/A | | | N/A | N/A |
| | | | | | Capture | 21.32 | -158.15 | Kahe Point | | |
| Shark 1 | 21-Jul-11 | *H. griseus* | 325 | M | Pop-up | 17.986 | -167.291 | Near Johnston Atoll | 969 | 55 |
| | | | | | Capture | 21.347 | -158.158 | Kahe Point | | |
| Shark 2 | 23-Jul-11 | *H. griseus* | 266 | M | Pop-up | 21.575 | -158.367 | Kaena Point | 33 | 53 |
| | | | | | Capture | 21.276 | -157.933 | HNL Airport | | |
| Shark 3 | 27-Jan-12 | *H. griseus* | 281.5 | F | Pop-up | 21.317 | -157.515 | Makapuu | 44 | 90 |
| | | | | | Capture | 21.231 | -157.807 | Diamond Head | | |
| Shark 4 | 20-Mar-12 | *H. griseus* | 313 | M | Pop-up | 21.876 | -159.627 | South Kauai | 201 | 90 |
| | | | | | Capture | 21.231 | -157.807 | Diamond Head | | |
| Shark 5 | 20-Mar-12 | *H. griseus* | 333 | F | Pop-up | 21.183 | -157.524 | Kaiwi Channel | 30 | 97 |
| | | | | | Capture | 27.271 | -157.98 | HNL Airport | | |
| Shark 6 | 24-Feb-12 | *E. cookei* | 234 | M | Pop-up | 21.248 | -157.956 | HNL Airport | 4 | 69 |

Table 1. Summary of all sharks tagged with pop-up archival tags.

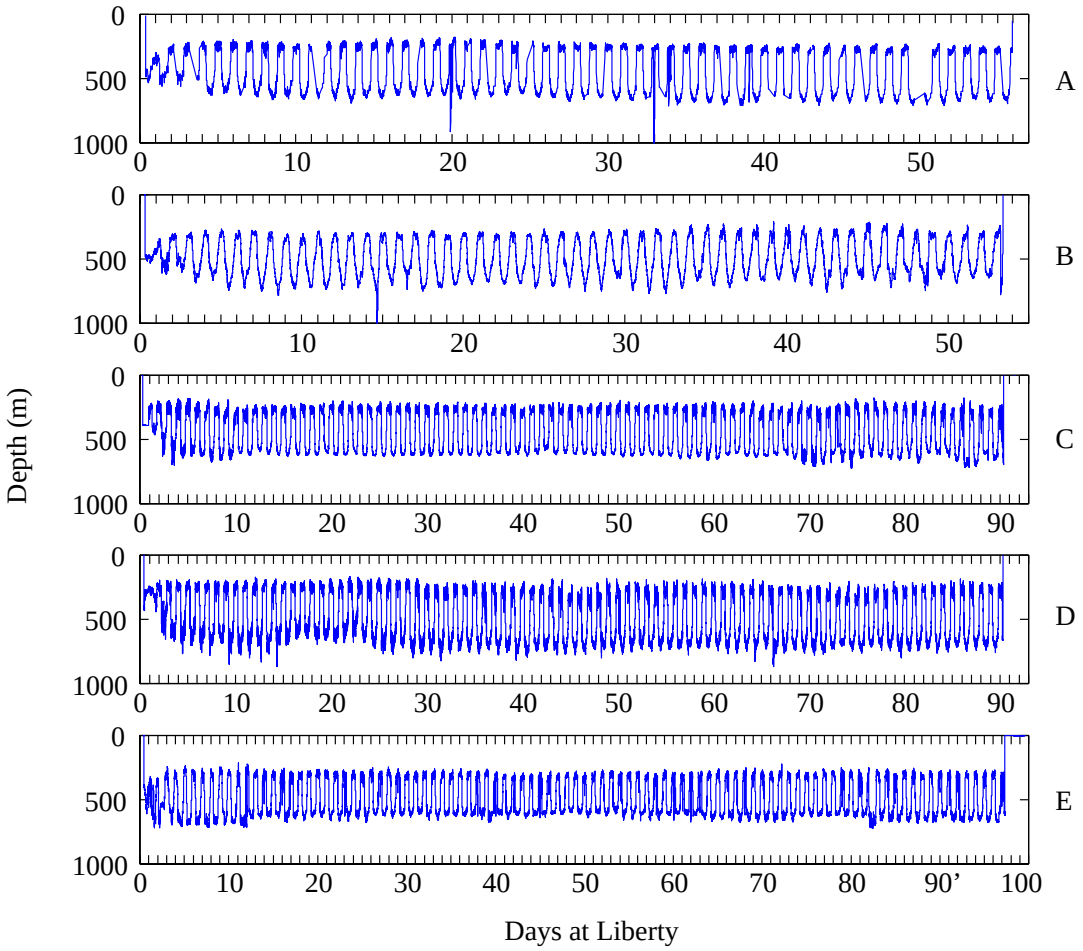



Figure 1. Time-series data for sharks 1-5 (A-E) showing pronounced diel vertical migrations. The first three days of each record were omitted to allow for the animal to recover from post-release stress evident in the depth record.

| | | Depth [m] | | | | Temperature [C] | | | | Light | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dawn | Day | Dusk | Night | Dawn | Day | Dusk | Night | Dawn | Day | Dusk | Night |
| | Q1 | 270.8 | 582.5 | 496.5 | 245.0 | 8.6 | 5.8 | 6.5 | 14.1 | | | | |
| Shark 1 | Median | 333.5 | 616.5 | 544.5 | 260.5 | 11.4 | 6.0 | 6.8 | 15.0 | | | | |
| | Q3 | 437.0 | 644.0 | 573.5 | 278.5 | 14.6 | 6.4 | 7.3 | 15.8 | | | | |
| | Q1 | 404.5 | 584.5 | 507.0 | 305.0 | 7.9 | 5.3 | 6.1 | 10.9 | 21 | 17 | 17 | 22 |
| Shark 2 | Median | 437.0 | 624.5 | 529.5 | 324.5 | 8.6 | 5.7 | 6.5 | 12.1 | 22 | 17 | 18 | 23 |
| | Q3 | 467.0 | 674.0 | 553.0 | 364.5 | 9.4 | 6.2 | 7.0 | 13.0 | 25 | 19 | 19 | 24 |
| | Q1 | 458.0 | 592.0 | 470.0 | 255.0 | 6.3 | 5.8 | 6.2 | 12.6 | 17 | 17 | 17 | 24 |
| Shark 3 | Median | 512.5 | 610.0 | 512.0 | 272.0 | 6.8 | 5.8 | 6.7 | 13.6 | 18 | 17 | 18 | 25 |
| | Q3 | 549.5 | 622.5 | 544.5 | 295.0 | 7.8 | 6.0 | 7.5 | 14.6 | 20 | 17 | 18 | 26 |
| | Q1 | 480.0 | 634.5 | 471.0 | 246.0 | 6.0 | 5.4 | 6.3 | 12.8 | 19 | 18 | 19 | 26 |
| Shark 4 | Median | 556.0 | 673.5 | 530.5 | 268.5 | 6.5 | 5.6 | 6.8 | 14.2 | 20 | 18 | 20 | 27 |
| | Q3 | 604.0 | 700.5 | 567.0 | 299.5 | 7.5 | 5.8 | 7.6 | 15.3 | 21 | 19 | 20 | 28 |
| | Q1 | 475.0 | 597.5 | 489.5 | 299.0 | 6.1 | 5.8 | 6.1 | 10.6 | 18 | 18 | 18 | 23 |
| Shark 5 | Median | 538.0 | 610.0 | 539.5 | 315.0 | 6.5 | 6.0 | 6.5 | 12.0 | 19 | 18 | 19 | 24 |
| | Q3 | 580.5 | 632.5 | 569.0 | 336.0 | 7.4 | 6.1 | 7.0 | 13.2 | 20 | 18 | 19 | 25 |
| | Q1 | 379.5 | 394.0 | 390.5 | 226.5 | 8.5 | 8.4 | 8.2 | 13.6 | 21 | 54 | 21 | 25 |
| Shark 6* | Median | 395.0 | 407.0 | 402.5 | 247.5 | 9.1 | 8.8 | 8.5 | 15.3 | 26 | 62 | 26 | 26 |
| | Q3 | 407.0 | 417.0 | 413.0 | 281.5 | 9.8 | 9.4 | 9.1 | 16.4 | 38 | 71 | 35 | 27 |

**Echinorhinus cookei*

Table 2. Non-parametric statistics for all variables measured by the MiniPAT tags. The record from shark 1 was transmitted via satellite and did not include light readings. Depth, thermal, and light preferences were similar for all sharks. Light readings were not calibrated to a specific unit, but a reading of about 50 corresponds to a moonless night.

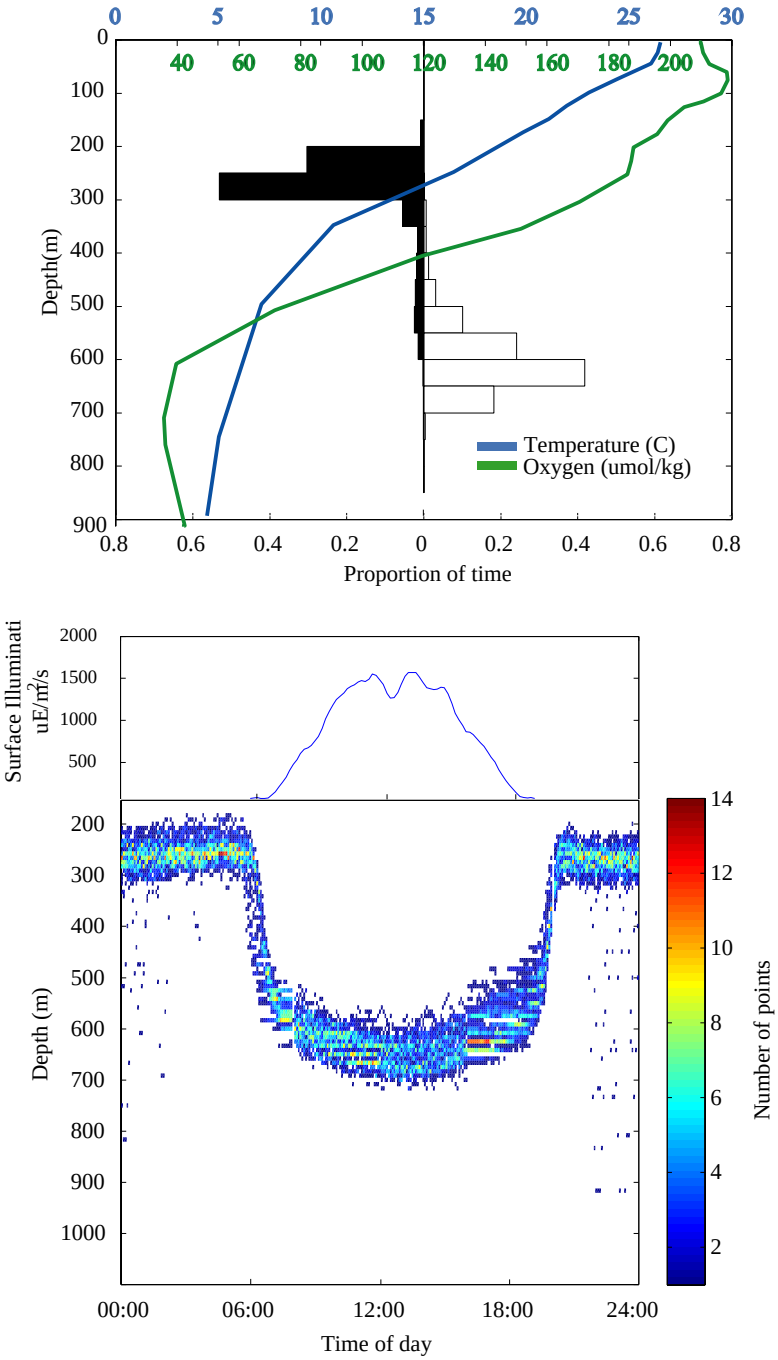



Figure 2. Depth of Shark 1, a mature male *Hexanchus griseus*, which left Hawaii during its time at liberty. (Top) Histogram of time at depth overlain with temperature and oxygen from HOTS Station Kahe. Black bars represent night, and white bars represent day. (Bottom) Depth versus time of day with surface illumination data from HOT. Color indicates density of observations in one location, with blue being low density and red being high.

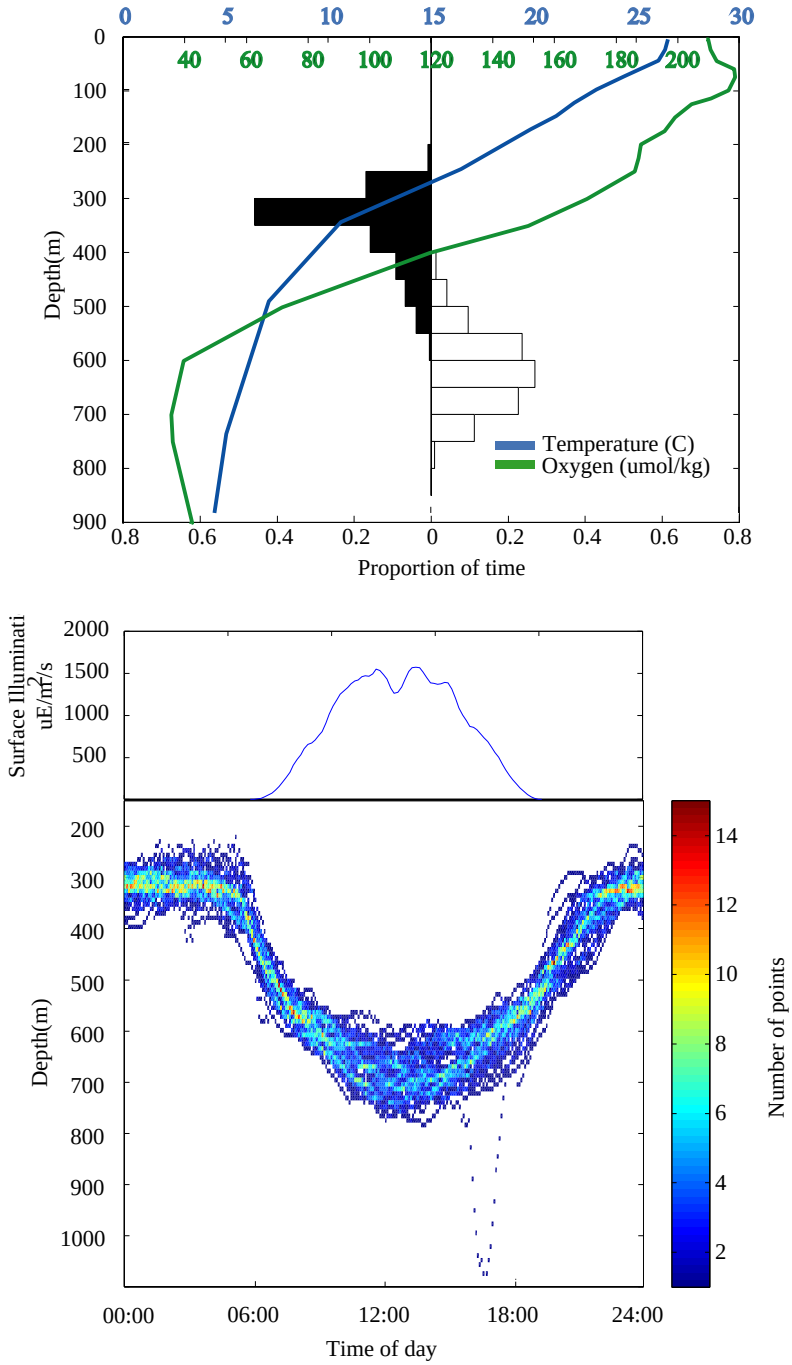



Figure 3. Depth of Shark 2, an immature male *Hexanchus griseus*, which remained near Oahu. (Top) Histogram of time at depth overlain with temperature and oxygen from HOTS Station Kahe. Black bars represent night, and white bars represent day. (Bottom) Depth versus time of day with surface illumination data from HOT. Color indicates density of observations in one location, with blue being low density and red being high.

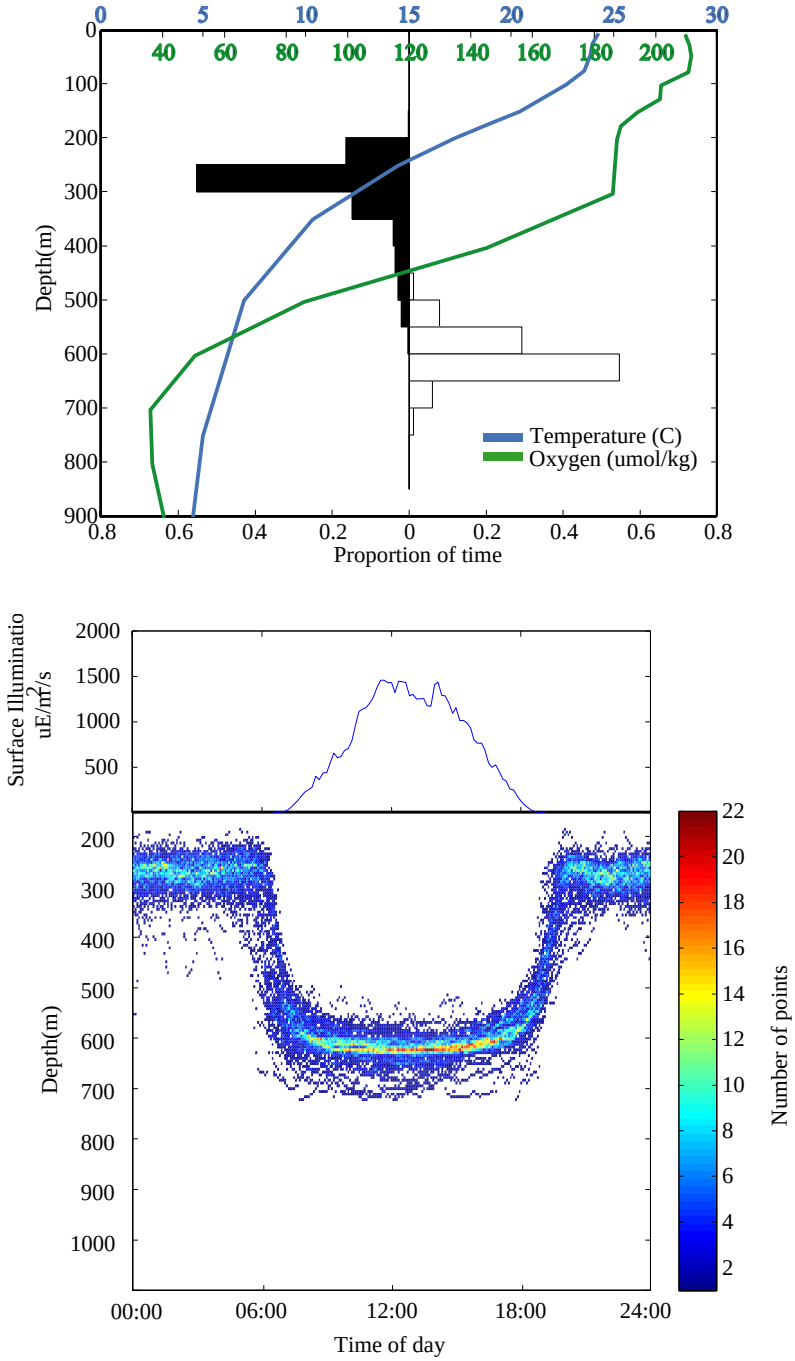



Figure 4. Depth of shark 3, an immature female *Hexanchus griseus*, which remained near Oahu. (Top) Histogram of time at depth overlain with temperature and oxygen from HOTS data: Station 1 January-April means, 2008-2010. Black bars represent night, and white bars represent day. (Bottom) Depth versus time of day with surface illumination data from HOT. Color indicates density of observations in one location, with blue being low density and red being high.

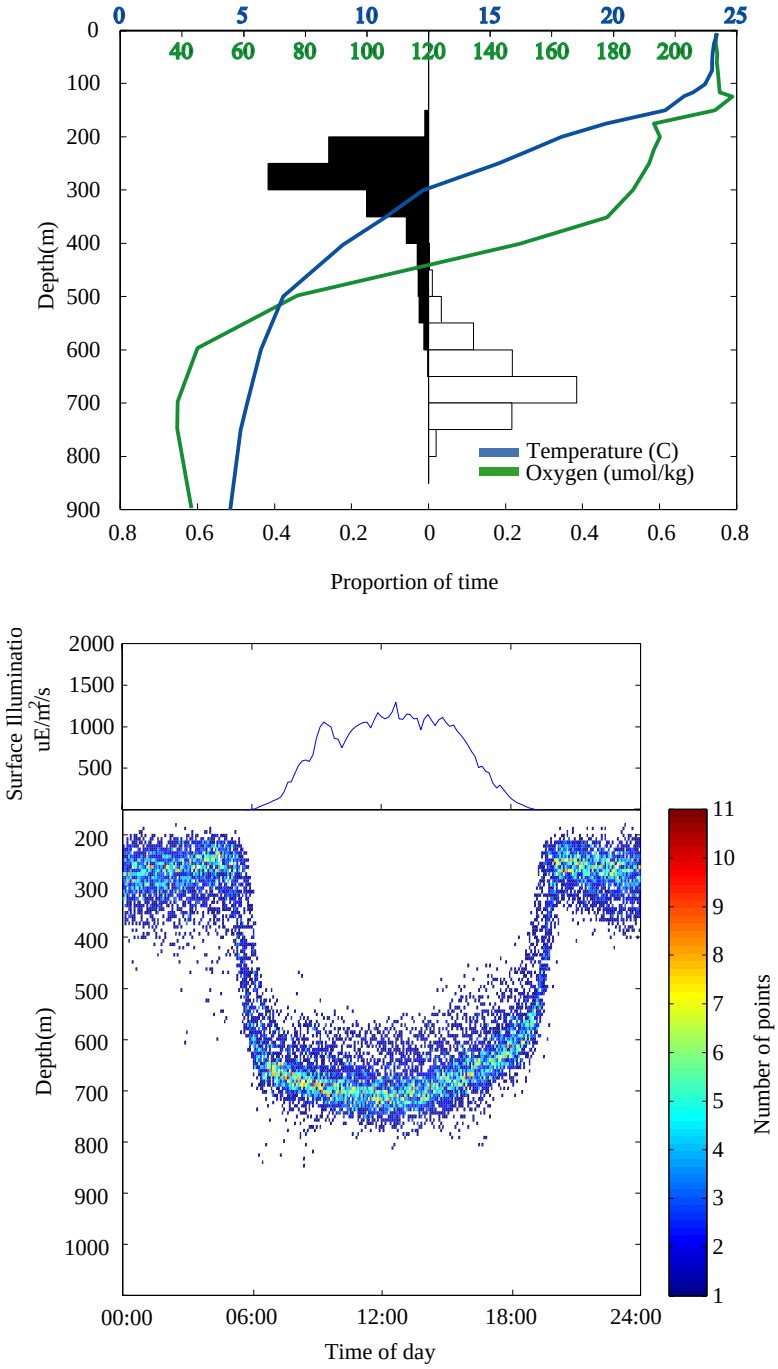



Figure 5. Depth of shark 4, a mature male *Hexanchus griseus*, which traveled to Kauai. (Top) Histogram of time at depth overlain with temperature and oxygen from HOT Station 1. Black bars represent night, and white bars represent day. (Bottom) Depth versus time of day with surface illumination data from HOT. Color indicates density of observations in one location, with blue being low density and red being high.

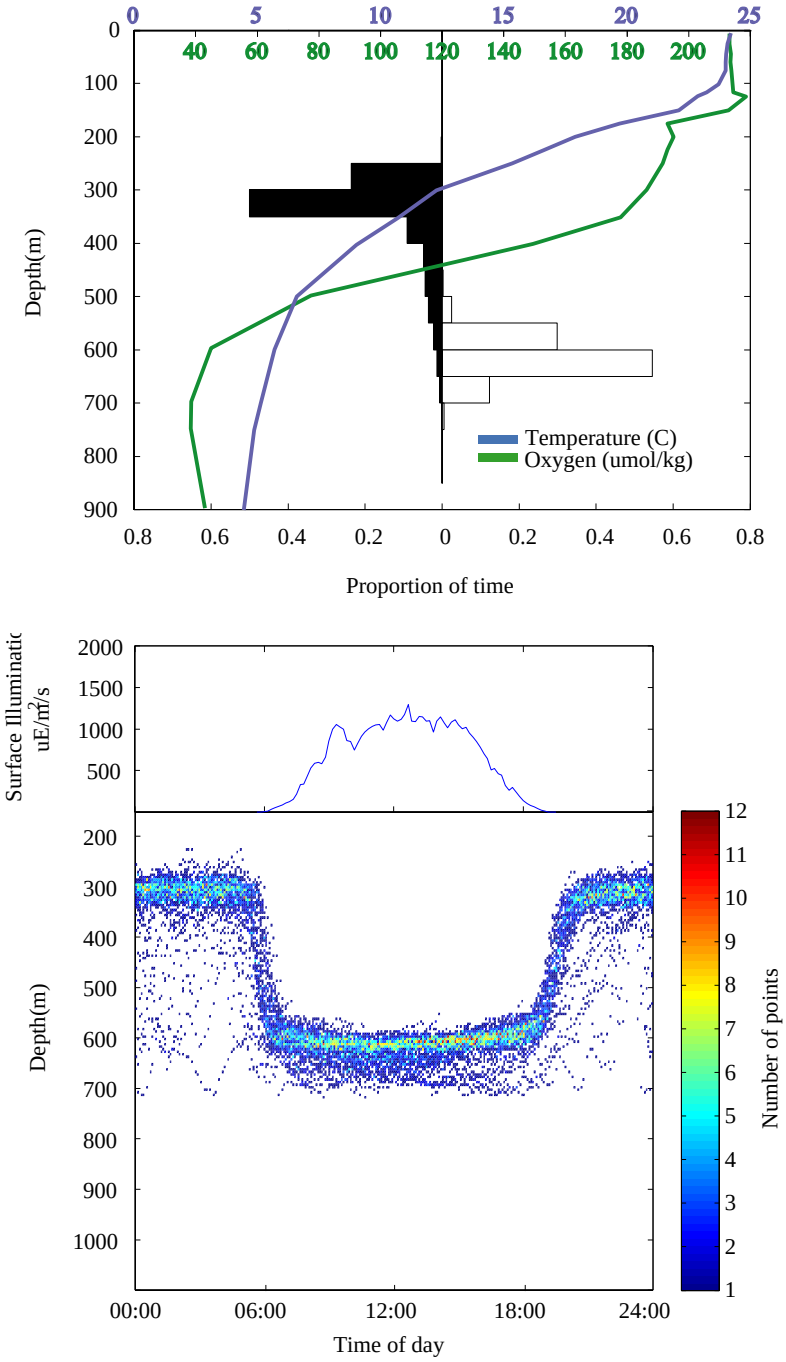



Figure 6. Depth of shark 5, an immature female *Hexanchus griseus*, which traveled to Penguin Banks in the Kaiwi Channel. (Top) Histogram of time at depth overlain with temperature and oxygen from HOT Station 1. Black bars represent night, and white bars represent day. (Bottom) Depth versus time of day with surface light illumination from HOT. Color indicates density of observations in one location, with blue being low density and red being high.

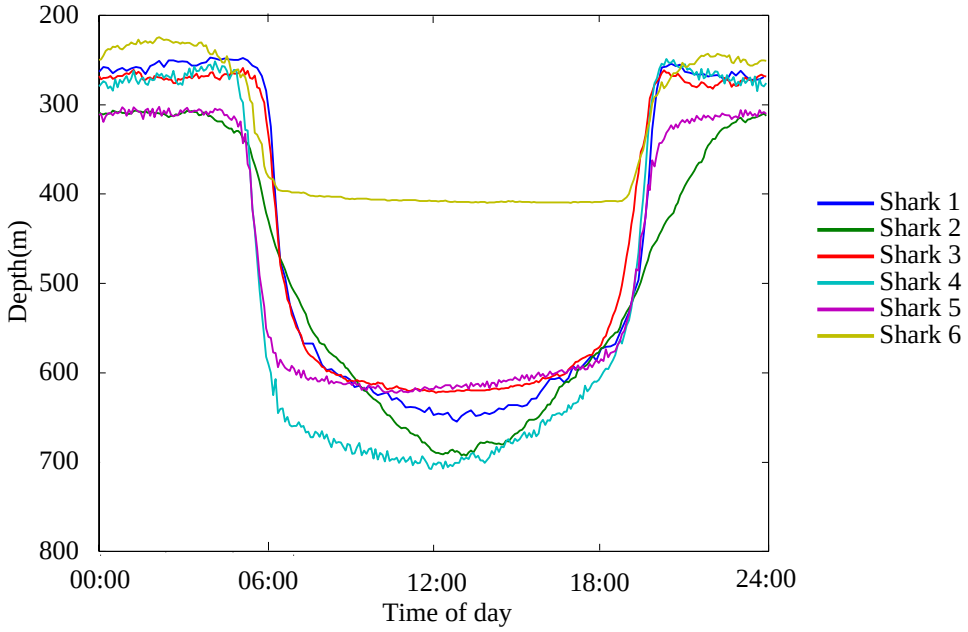



Figure 7. Median depth versus time of day. Sixgill sharks 1-5 had very similar depth curves, with the exception of the slower ascent and descents of shark 2. Shark 6, the prickly shark, had a nighttime depth habitat that was only slightly shallower than the sixgills, but the daytime habitat was much shallower, meaning the prickly shark did not enter the OMZ.

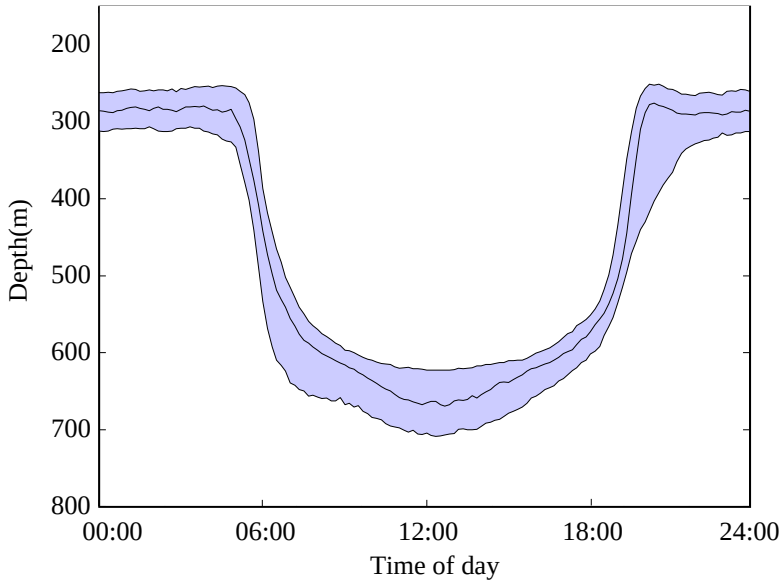



Figure 8. Median depth and IQR for all sixgill shark depth data pooled. Lowest variability occurred during the dawn transition to the deep regime.

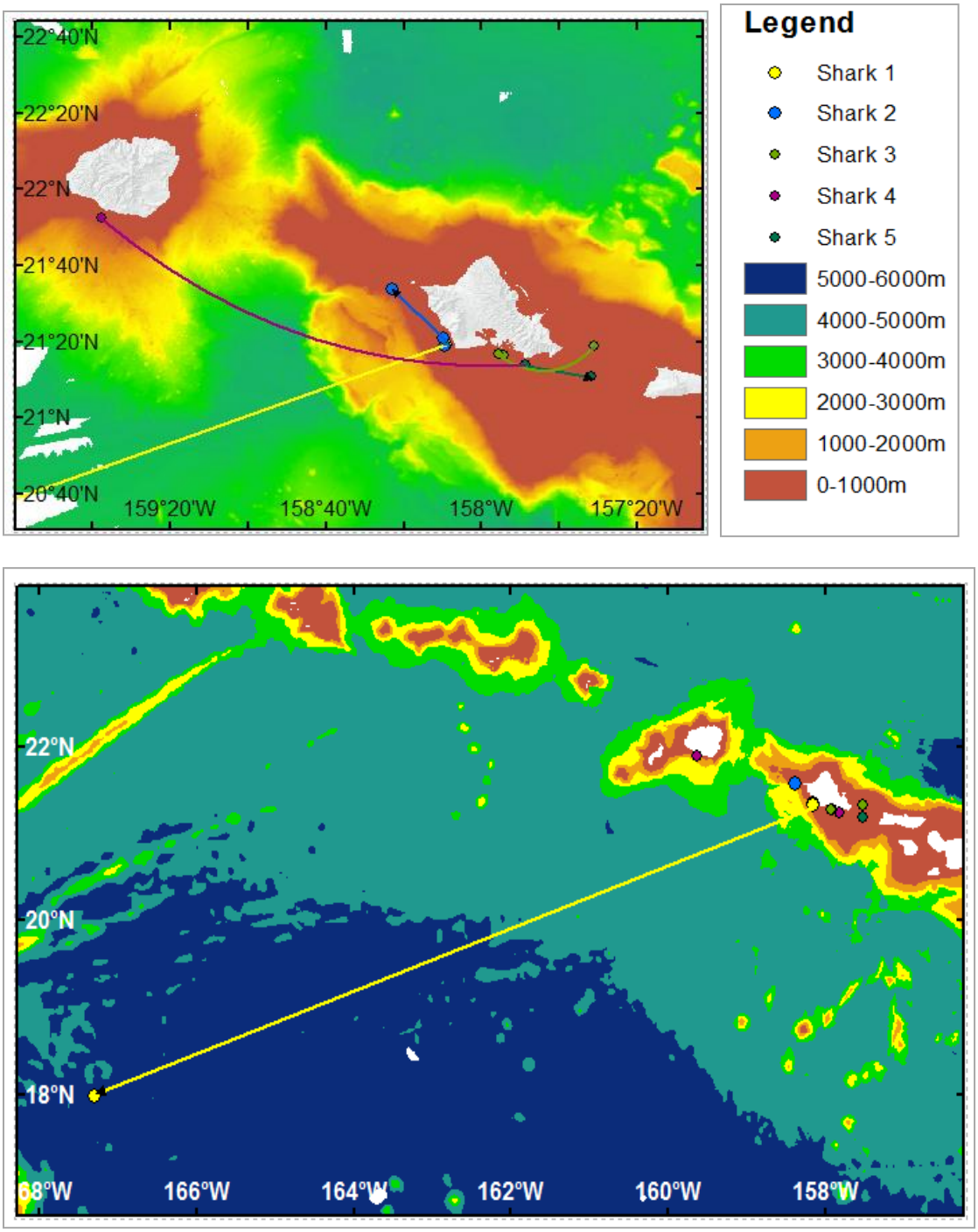



Figure 9. Map of horizontal travel of five *Hexanchus griseus* from tagging to pop-off location. Sharks 2 and 3 (blue and green) remained on the Oahu slope, but shark 1 (yellow) traveled almost 1000km towards Johnston Atoll. Shark 1 and shark 4 exhibited pelagic swimming, and shark 5 moved to Penguin Banks, a nearby slope habitat that is accessible with or without pelagic swimming.

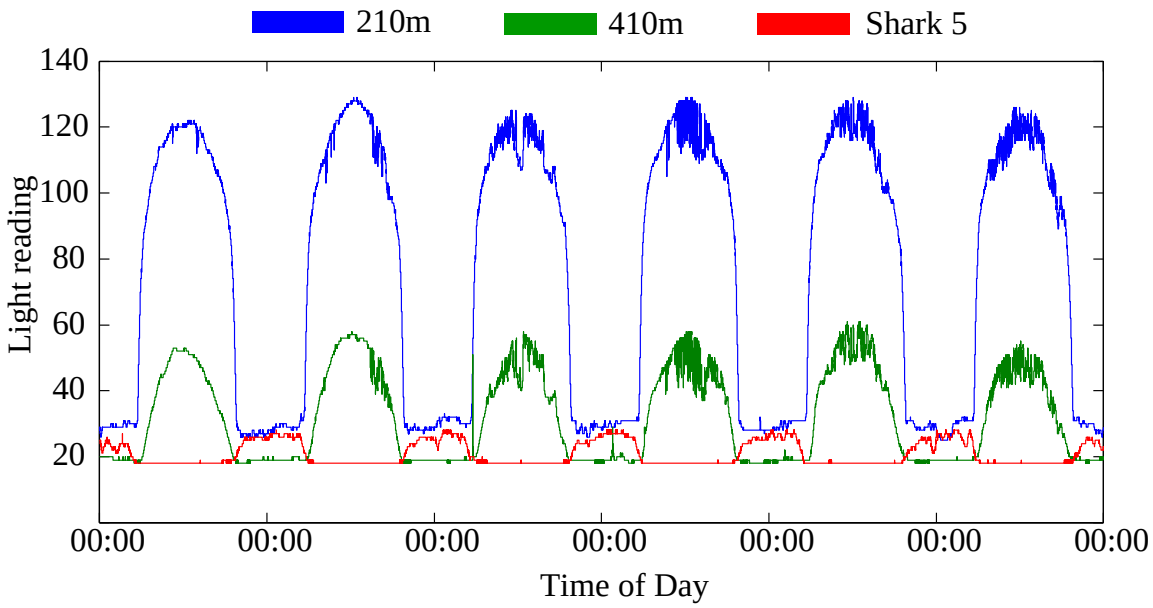



Figure 10. Two light records from constant depths plotted against a light record from Shark 5 during the same dates. The nighttime light level at 210m is just higher than the sixgill shark light curve. The daytime light curve at 410m is much higher than sixgill shark light habitat, which may be one factor explaining their deeper daytime depths. The shark (red) had a median nighttime depth of 315m (the nighttime light level at 210 m is only slightly higher) and a median daytime depth of 610m.

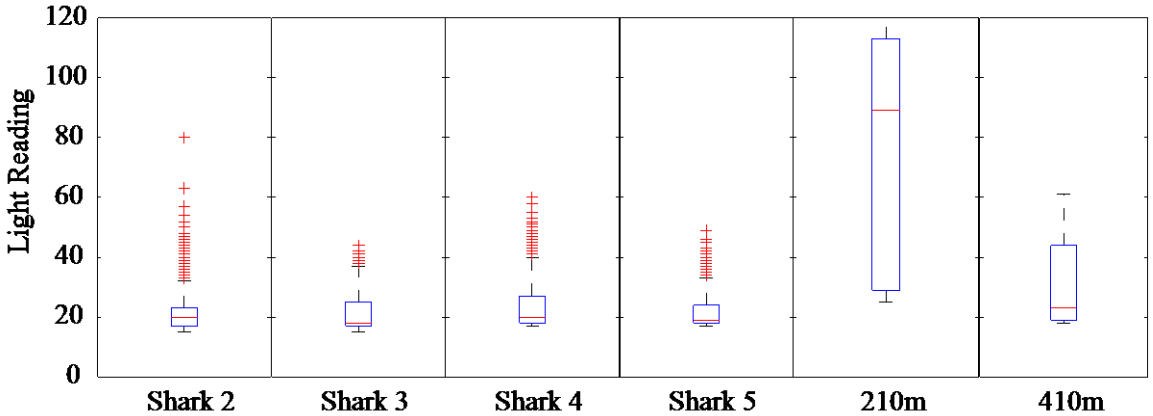
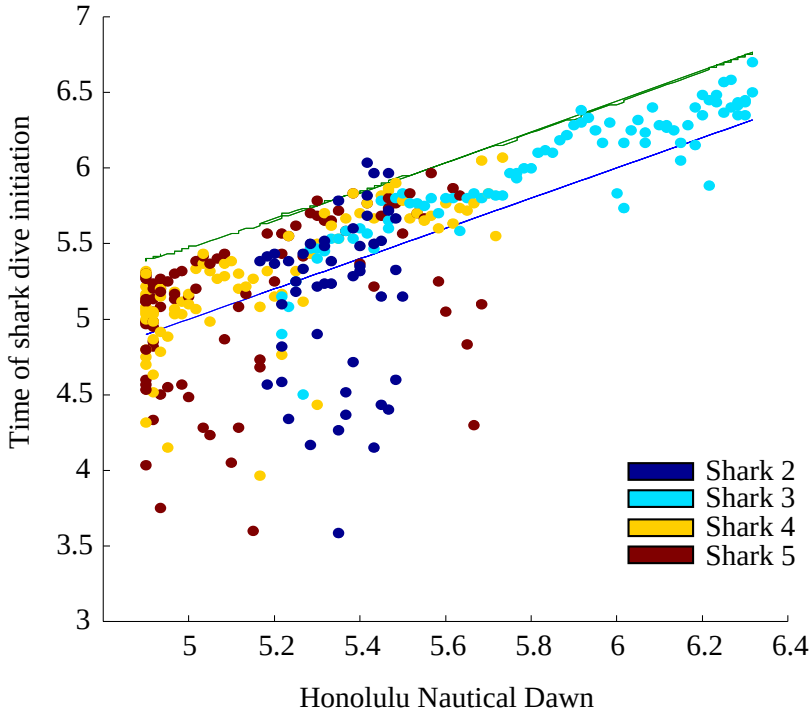



Figure 11. Box plots of light levels experienced by all 4 sharks for which light records were available, and for the two constant-depth deployments. Outliers are indicated by red crosses. The interquartile range of both constant-depth deployments is much higher than for tags attached to moving sharks, supporting the hypothesis that sixgill sharks move to maintain a consistent light level.



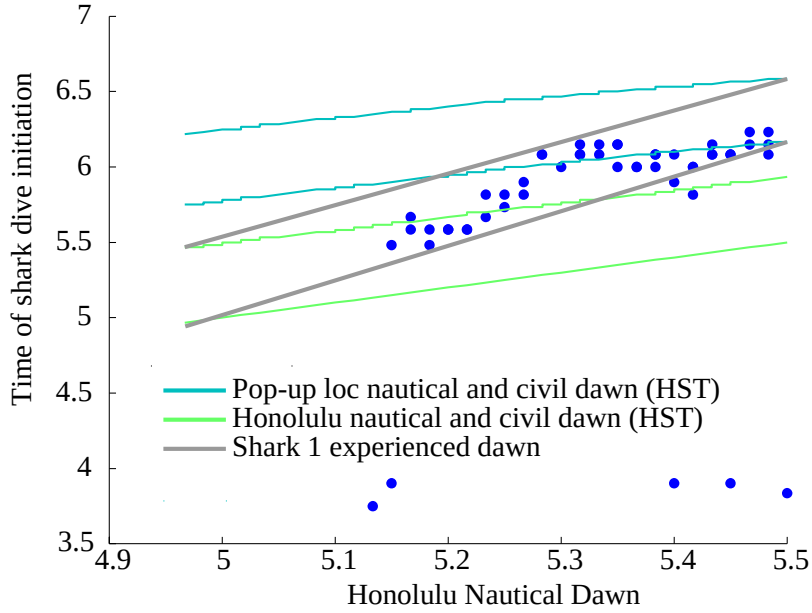




Figure 12. (Top) Nautical dawn versus dive initiation for animals that stayed near Hawaii. The lower (blue) line is the 1:1 line for nautical dawn, and the upper (green) line is civil dawn. Most dives were initiated between nautical and civil dawn. Multiple sharks dove much earlier than nautical dawn, but none dove after dawn. (Bottom) Nautical dawn versus dive Initiation for Shark 1. The grey lines are an estimate of the dawn that the shark experienced if it traveled in a straight line towards Johnston Atoll immediately following release. The animal's dive behavior is consistent with the expected change in time of dawn if it has moved westward as indicated by the popup position.

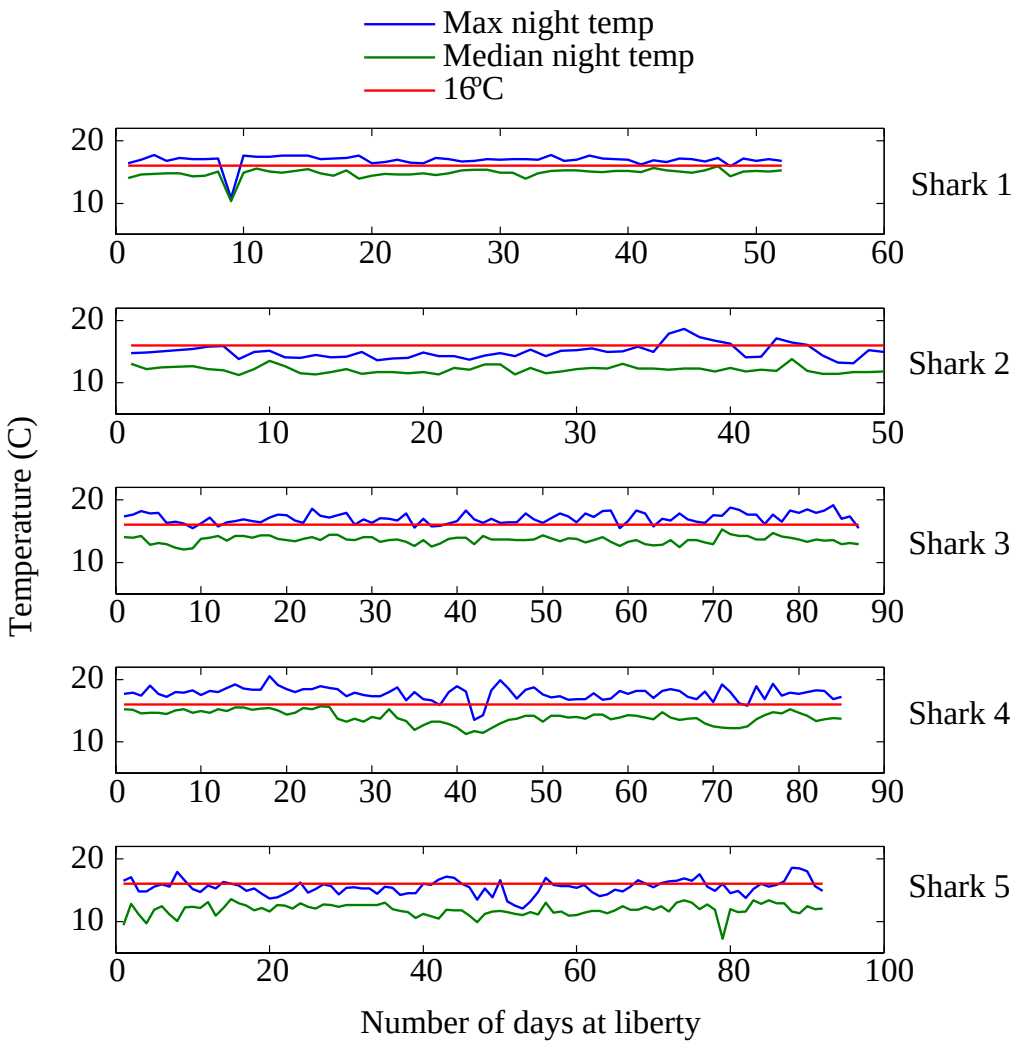



Figure 13. Median and maximum nightly depths over the time at liberty for each shark, plotted against the hypothesized 16ºC thermal ceiling. All sharks exceeded 16ºC at some point in the record, and Sharks 1, 3, and 4 regularly experienced warmer temperatures up to 19.15ºC.

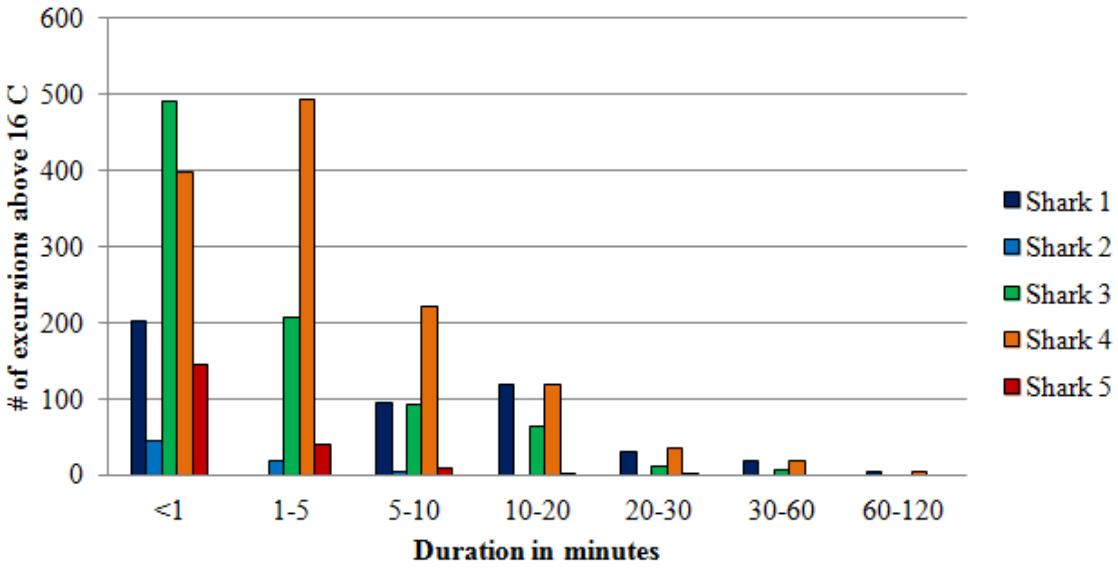



Figure 14. Duration of excursions above 16ºC. Sharks 1, 3, and 4 regularly experienced warm temperatures, but excursions above 16ºC were less common for sharks 2 and 5 and were nearly all <10 minutes in duration. These data indicate that, due to their large thermal inertia, the internal body temperature for sixgill sharks remains cool during these ascents into warm water. Sixgill sharks limit the duration of these forays into warm water to avoid thermal equilibration.

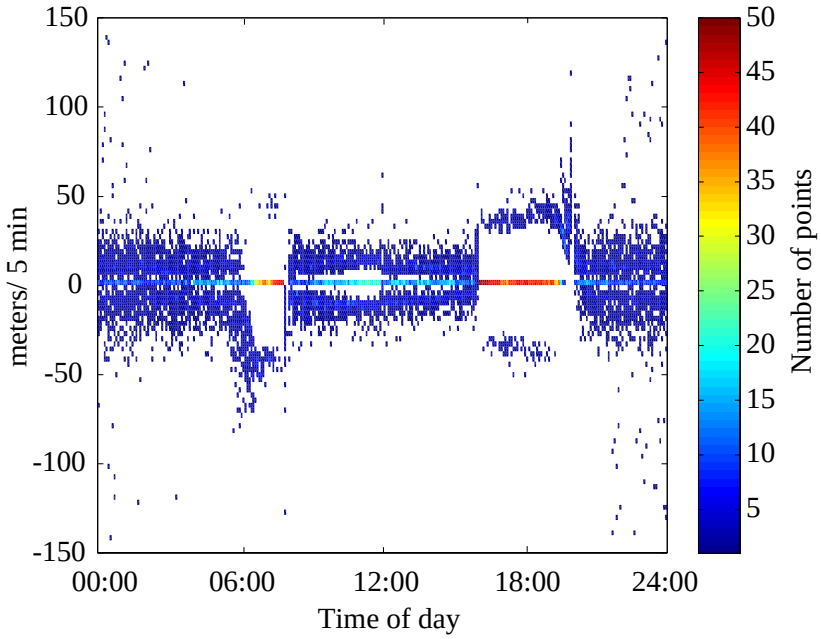



Figure 15. Vertical rate of movement versus time of day for Shark 1. The slower sampling rate (5 minute) for this shark appears to have influenced the plot of vertical ROM, but it still follows the same overall pattern as sharks 2-5.

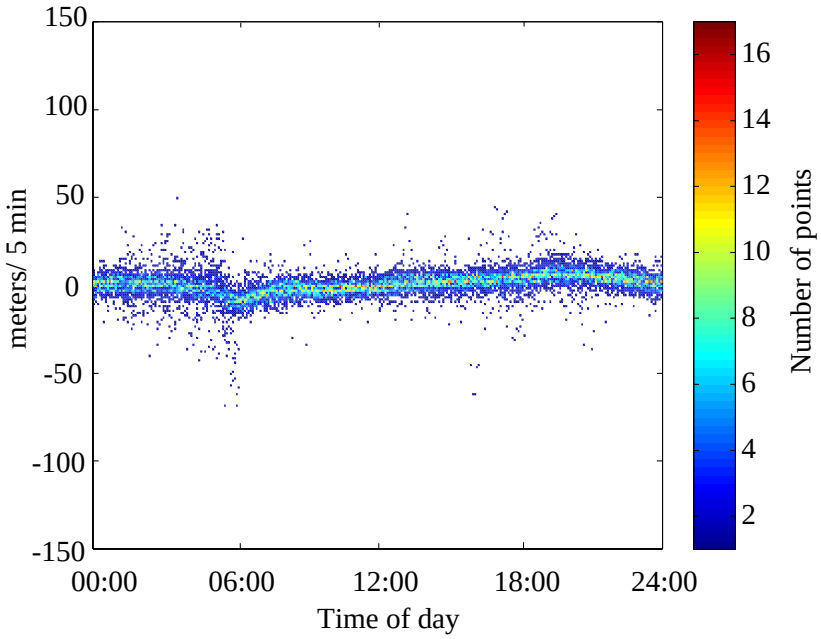



Figure 16. Vertical rate of movement versus time of day for Shark 2. This shark had lower rates of movement than other sharks, and less pronounced ascents and descents at dusk and dawn.

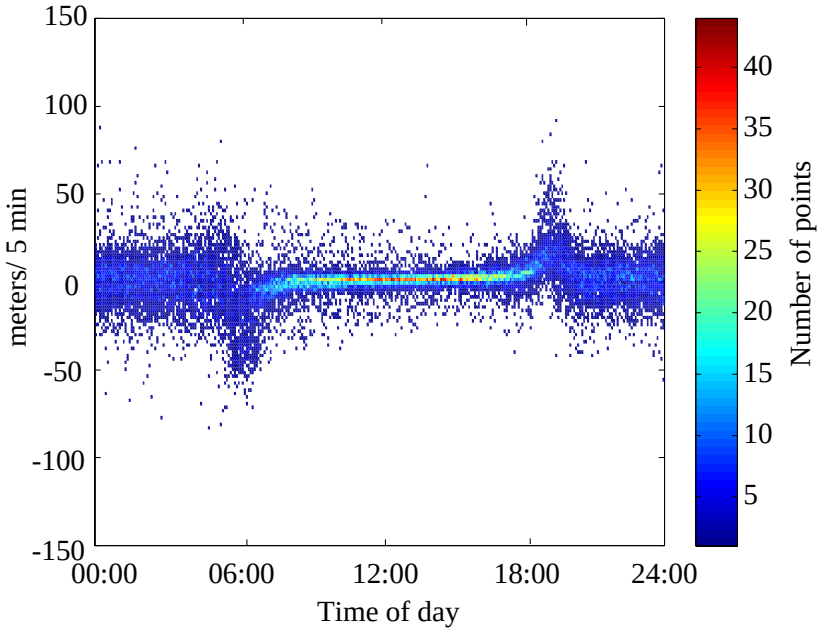



Figure 17. Vertical rate of movement versus time of day for Shark 3. This shark displayed a clear ascent and descent, and a greater rate of movement at night than during the day

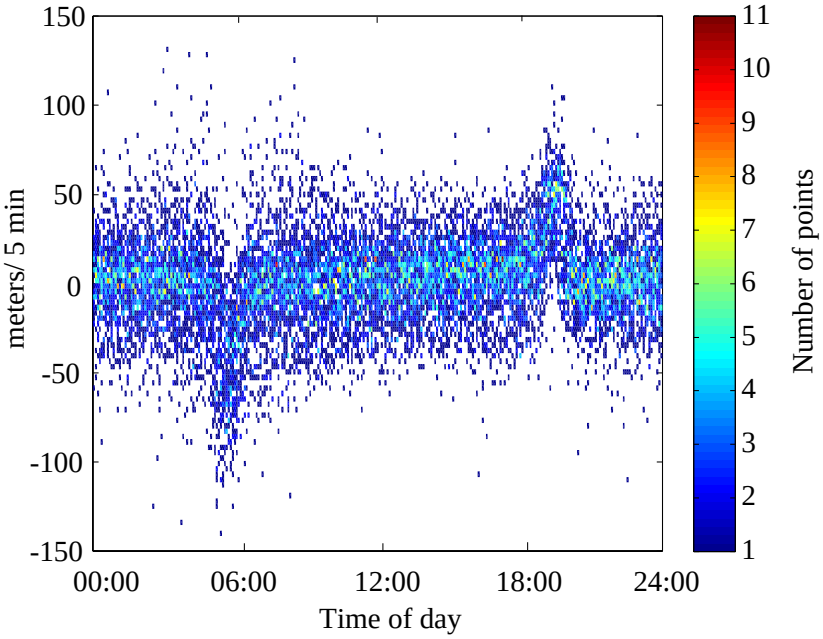



Figure 18. Vertical rate of movement versus time of day for Shark 4. This shark had a greater variability in rate of movement between sunrise and sunset compared to other sharks

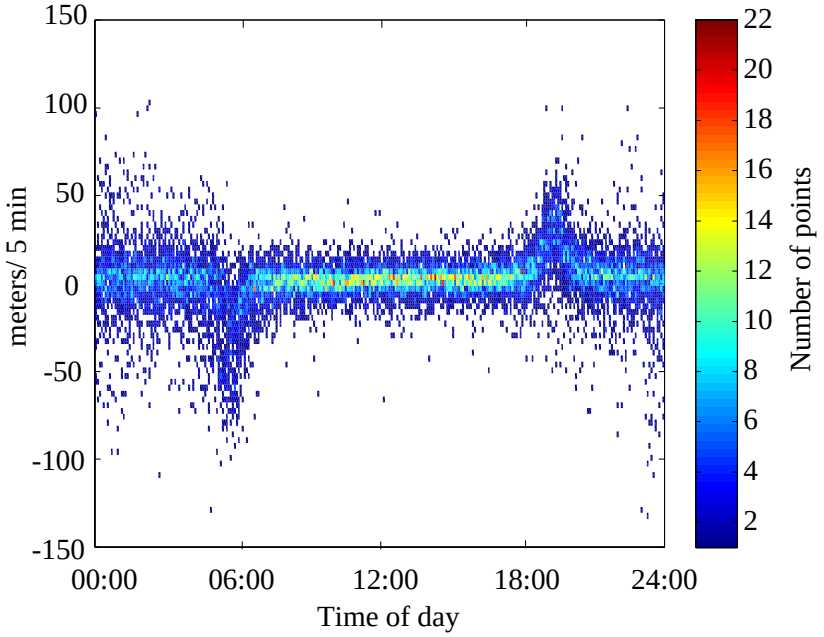



Figure 19. Vertical rate of movement versus time of day for Shark 5. This shark displayed very similar movement patterns to shark 3.

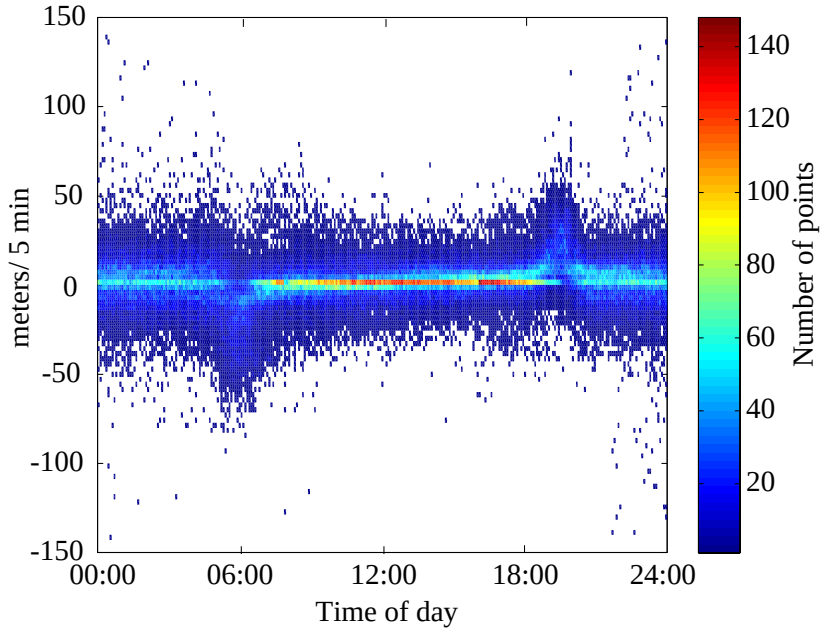



Figure 20. Vertical rate of movement versus time of day pooled across all five sixgill sharks. Deep dives were more common at night, and more rates of movement less than 0.5m/five minutes were observed during the day than at night.

| | Dawn | Day | Dusk | Night | | Dawn | Day | Dusk | Night |
|---|---|---|---|---|---|---|---|---|---|
| SHARK 2 | | | | | | | | | |
| Minimum | 0 | 0 | 0 | 0 | | | | | |
| 1st Quartile | 1 | 0.5 | 0.5 | 0.5 | | | | | |
| **Median** | **2** | **1** | **1.5** | **1** | **Mean** | **2.19** | **1.15** | **1.53** | **1.40** |
| 3rd Quartile | 3 | 1.5 | 2 | 2 | St. Dev | 1.89 | 1.12 | 1.30 | 1.40 |
| Maximum | 17 | 15 | 12.5 | 15 | | | | | |
| SHARK 3 | | | | | | | | | |
| Minimum | 0 | 0 | 0 | 0 | | | | | |
| 1st Quartile | 1.5 | 0.5 | 1 | 1 | | | | | |
| **Median** | **3** | **1** | **2** | **2.5** | **Mean** | **3.92** | **1.16** | **2.82** | **2.91** |
| 3rd Quartile | 6 | 1.5 | 3.5 | 4 | St. Dev | 3.12 | 1.29 | 2.56 | 2.36 |
| Maximum | 19 | 21 | 22.5 | 23 | | | | | |
| SHARK 4 | | | | | | | | | |
| Minimum | 0 | 0 | 0 | 0 | | | | | |
| 1st Quartile | 3 | 1.5 | 2 | 2 | | | | | |
| **Median** | **6** | **2.5** | **4** | **3.5** | **Mean** | **6.15** | **3.02** | **4.26** | **3.88** |
| 3rd Quartile | 9 | 4.5 | 6 | 5.5 | St. Dev | 3.69 | 2.28 | 2.73 | 2.74 |
| Maximum | 24.5 | 25.5 | 18.5 | 28.5 | | | | | |
| SHARK 5 | | | | | | | | | |
| Minimum | 0 | 0 | 0 | 0 | | | | | |
| 1st Quartile | 1.5 | 0.5 | 1 | 0.5 | | | | | |
| **Median** | **3** | **1** | **2.5** | **1.5** | **Mean** | **3.83** | **1.19** | **2.85** | **2.15** |
| 3rd Quartile | 6 | 1.5 | 4 | 3 | St. Dev | 3.03 | 1.19 | 2.12 | 2.15 |
| SHARK 6 | | | | | | | | | |
| Maximum | 18 | 21 | 16.5 | 25 | | | | | |
| Minimum | 0 | 0 | 0 | 0 | | | | | |
| 1st Quartile | 0 | 0 | 0 | 0.5 | | | | | |
| **Median** | **0.5** | **0.5** | **0.5** | **1.5** | **Mean** | **0.79** | **0.39** | **1.02** | **2.67** |
| 3rd Quartile | 1 | 0.5 | 1 | 3.5 | St. Dev | 1.45 | 0.45 | 1.65 | 3.26 |
| Maximum | 19 | 6.5 | 15 | 29.5 | | | | | |

Table 3. Rates of vertical movement for each shark individually, calculated for four diel periods. Archival records were subsampled to 1-minute intervals to reduce bias from noise in the depth record.

| | Median km/day | Maximum km/day |
|---|---|---|
| **SHARK 1*** | 2.665 | 5.135 |
| **SHARK 2** | 1.891 | 2.669 |
| **SHARK 3** | 2.872 | 6.257 |
| **SHARK 4** | 4.771 | 7.816 |
| **SHARK 5** | 2.51 | 4.175 |
| **SHARK 6** | 2.06 | 3.609 |

Table 4. Median and maximum total vertical movements per day for each shark. *Transmitted record based on units of meters per five minutes; all others based on units of meters/minute

Response variable: Rate of vertical movement (absolute value) for all diel periods

| | | | | | 95% confidence | |
|---|---|---|---|---|---|---|
| Variable | Beta | Standard Error | z-value | p-value | Lower | Upper |
| Depth | -0.0732 | 0.0136 | -5.3709 | **7.86E-08** | -0.1 | -0.0465 |
| Temperature | -2.3663 | 0.7819 | -3.0264 | **0.0025** | -3.8987 | -0.8338 |
| Time of Day | -3.157 | 0.6874 | -4.5926 | **4.38E-06** | -4.5042 | -1.8097 |
| Moon Illumination | -0.0553 | 0.0286 | -1.9419 | 0.0521 | -0.1111 | 5.13E-04 |

Response variable: Rate of vertical movement (absolute value) for day and night only

| | | | | | 95% confidence | |
|---|---|---|---|---|---|---|
| Variable | Beta | Standard Error | z-value | p-value | Lower | Upper |
| Depth | 9.45E-04 | 0.0022 | 0.4352 | 0.6634 | -0.0033 | 0.0052 |
| Temperature | -0.1612 | 0.0538 | -2.9963 | **0.0027** | -0.2666 | -0.0558 |
| Time of Day | 0.9321 | 0.3167 | 2.9435 | **0.0032** | 0.3115 | 1.5528 |
| Moon illumination | -0.0024 | 0.0033 | -0.7202 | 0.4714 | -0.0089 | 0.0041 |

Table 5. Results of GEE analysis for five sixgill sharks. Depth, time of day, and temperature were significant predictors of sixgill shark activity level, but moon illumination was not.

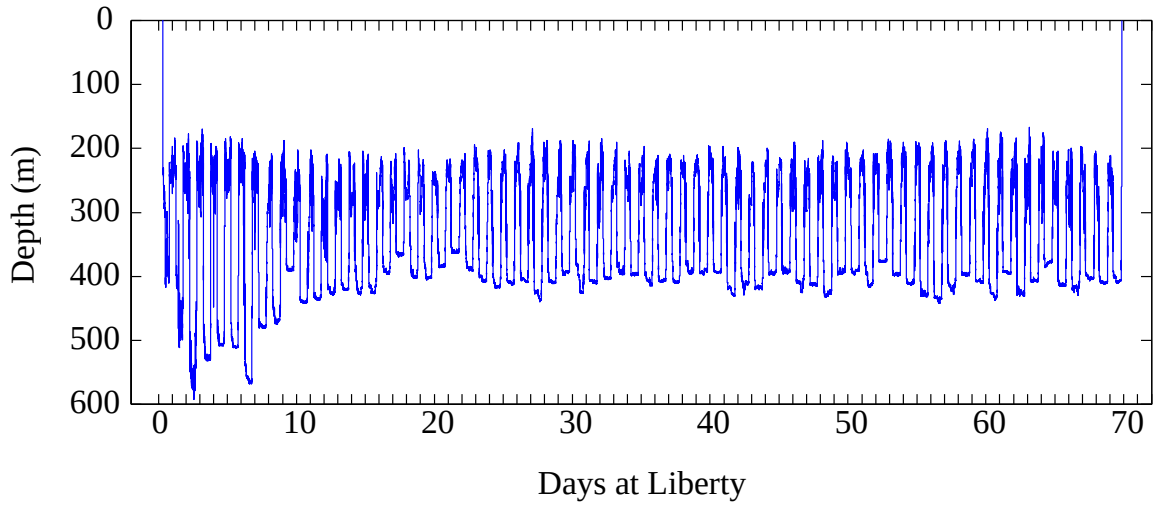



Figure 21. Full archival record of shark 6, *Echinorhinus cookei*. Note the longer recovery period, shallower daytime habitat, and little vertical movement during the day compared to sixgill shark archival records.

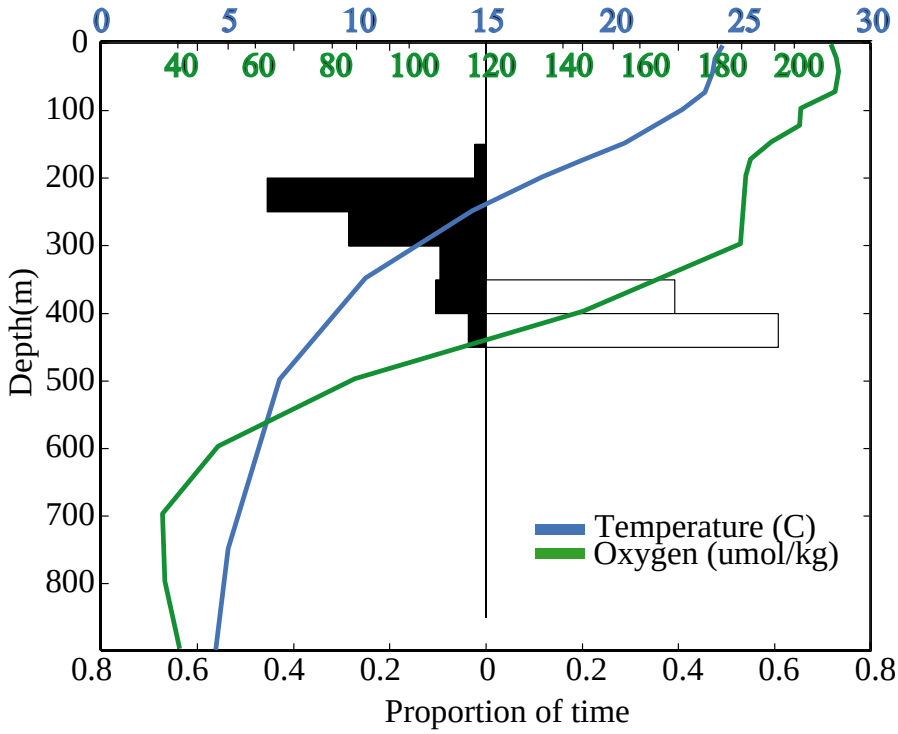



Figure 22. Time at depth histogram for shark 6. Unlike the sixgill sharks, the prickly shark did not spend time in the oxygen minimum zone of the Hawaii slope.

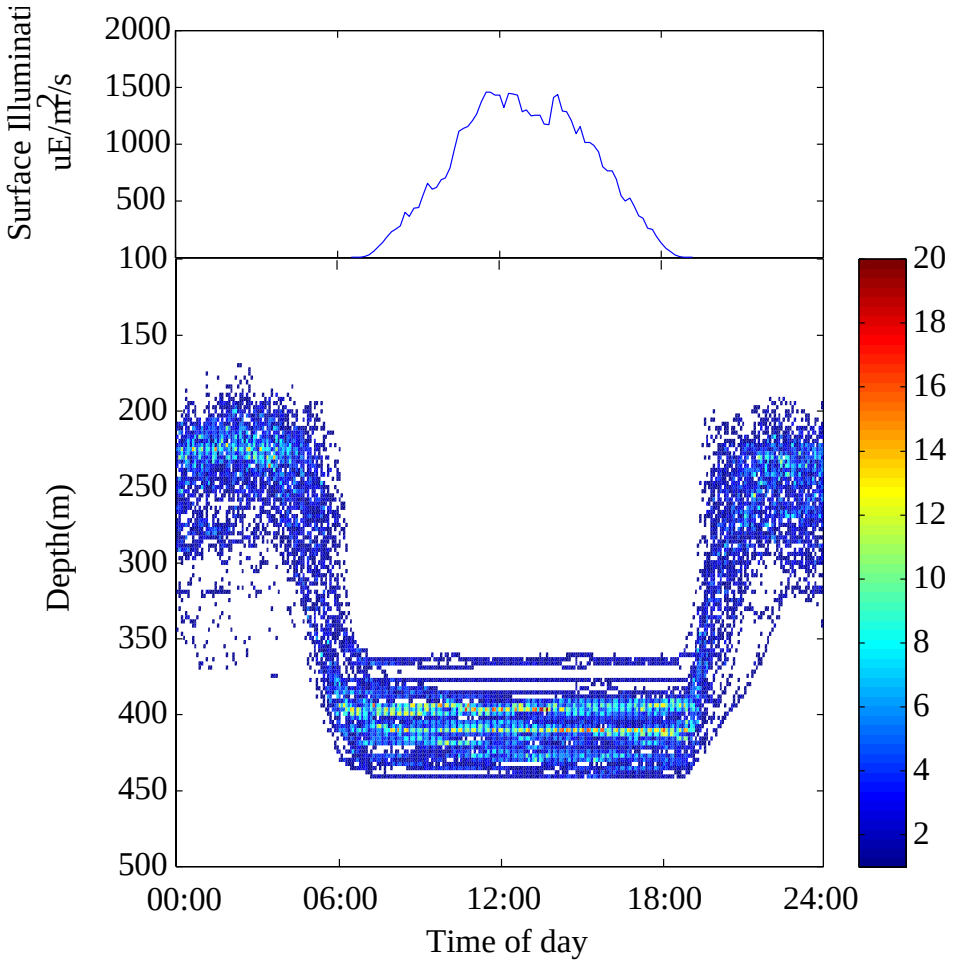



Figure 23. Depth versus time of day for shark 6. This prickly shark exhibited consistent diel vertical migrations with relatively consistent times of descent and ascent, roughly correlated with dawn and dusk.

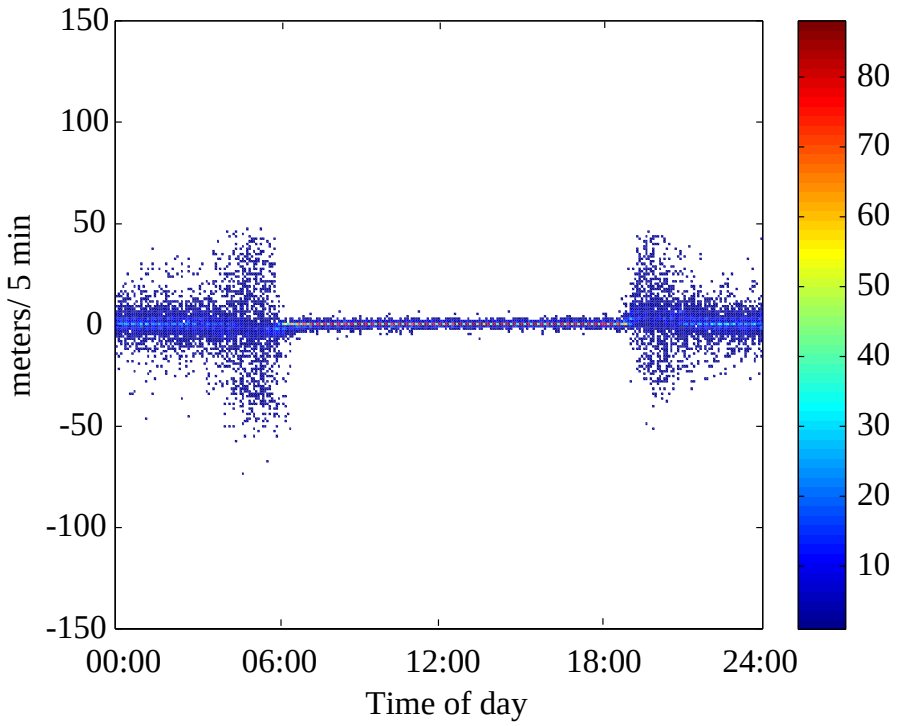



Figure 24. Rate of movement versus time of day for shark 6. This shark was very sedentary during the day, had highest rates of movement at dawn and dusk, and was fairly active at night.

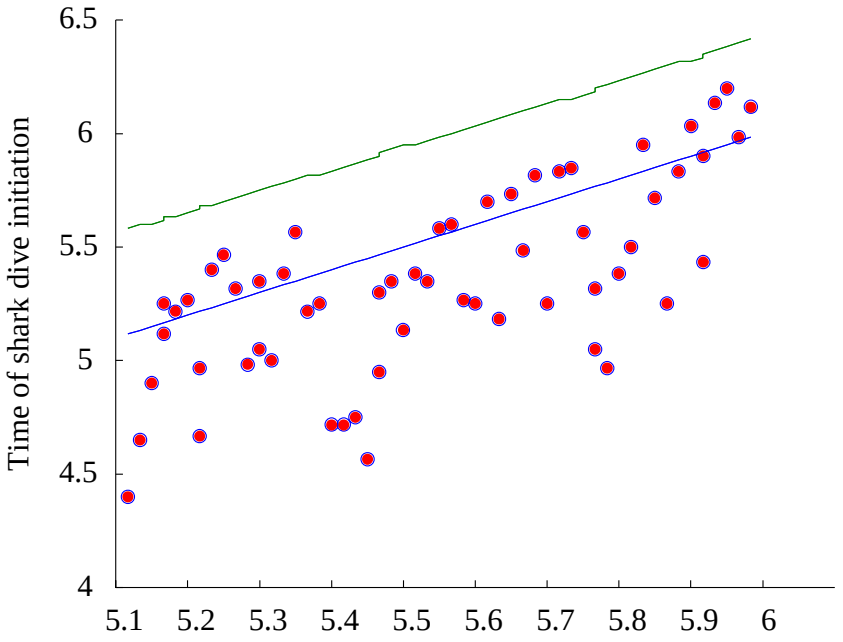



Figure 25. Time of nautical dawn plotted against dive initiation times for shark 6. The shark most often initiated the dive before nautical dawn, and always dove before civil dawn. The blue line represents nautical dawn and the green line represents civil dawn.

| Species | Study | Location | Division | Central tendency of ROM in m/min |
|---|---|---|---|---|
| *Hexanchus griseus* | This study | Oahu, Hawaii | Day | 1.15-3.02 |
| | | | Night | 1.40-3.88 |
| *Hexanchus griseus* | Andrews et al., 2009 | Bainbridge, Puget Sound, WA | Day | 1.25 |
| | | | Night | 1.57 |
| | | Elliot Bay, Puget Sound, WA | Day | 0.30 |
| | | | Night | 0.47 |
| *Scyliorhinus canicula* | Sims et al., 2006 | | Above thermocline | 0.60-1.40 |
| | | | Below thermocline | 0.00 |
| *Carcharadon carcharias* | Jorgensen et al., 2010 | Pacific; California to Hawaii | Coastal | 1.20 |
| | | | Offshore foraging area | 12.00 |
| *Somniosus pacificus* | Hulbert et al., 2006 | Gulf of Alaska | Throughout day | 4.03 |

Table 5. Vertical rates of movement observed in this study and others. Data from other publications was converted to units of meters/minute when necessary to enable comparison.

## 4. DISCUSSION

In this study, five sixgill sharks and one prickly shark were tracked for 53-97 days using pop-up satellite archival tags. This study is among the first to obtain archival data for sixgill sharks and and the first archival record from a prickly shark, enabling detailed analysis of vertical movements and depth/temperature habitat for these species. Using the detailed records obtained, several hypotheses regarding environmental drivers of the habitat and behavior of sixgill sharks in Hawaii were investigated.

### 4.1 Horizontal movements

Previous behavior studies, fishing records, and sightings by baited cameras and submersibles have supported the hypothesis that the sharks are closely associated with the benthos (Andrews et al., 2009; Carey and Clark, 1995; Crow et al., 1996; Yeh and Drazen, 2009). It appears common for sixgill sharks to stay near the benthos and associate with continental shelves and insular slopes, and all of the sharks captured in this study were caught on the bottom between 300 and 600m depth. However, the results of the pop-up satellite archival tagging positively demonstrate that sixgill sharks exhibit pelagic swimming behavior and move between island slopes and banks within the main Hawaiian archipelago, and occasionally move long distances. Notably, the vertical movement patterns of the sharks did not differ depending on whether the shark was swimming near island slopes or in the pelagic environment, so their movement patterns do not appear to be driven by proximity to the benthos.

The ability of sixgill sharks to swim in the pelagic and reach a new slope habitat has profound implications for population connectivity and management of the species. Two out of the five sharks moved across a channel to a new slope habitat in the Hawaiian Islands, with one of those sharks crossing deep water, and another shark swam in the pelagic for an extended period of time. At a minimum, the sixgill shark population throughout the Hawaiian Islands is likely to have some level of genetic flow between the islands. Larger scale movements could provide an avenue for genetic transfer between populations throughout the Pacific and beyond, including connections between continental slopes and mid-ocean ridges or seamounts.

Fish tagging alone is often insufficient to assess the degree of population connectivity between different geographic locations (von der Heyden, 2009) since the

length of time tracking a fish is usually much shorter than its lifespan, and even if the animal is observed moving to a new geographic location, there is no way to know if the animal reproduces successfully. A meta-analysis investigating gene flow between sharks captured in various regions would elucidate true rates of exchange of genetic information. Similar analyses have been conducted for whitetip reef sharks, which had surprisingly high levels of population connectivity considering their generally sedentary behavior (Whitney et al., 2012).

The long-range movement by shark 1 towards Johnston Atoll was surprising, and unfortunately the tag popped up while the shark was still swimming in the open ocean, so it is uncertain if the shark was heading towards a destination on a new slope habitat or if it accidentally left the Hawaiian Island chain with no directed movement. However, in the absence of light-based geolocation due to the depth of the sharks, the long distance travel provides the best estimate of a minimum speed per day. Assuming shark 1 left the Oahu slope soon after release, the horiztonal speed of shark 1 was calculated to be 17.6km/day, or 0.73km/hr. For comparison, the horizontal speed of white sharks (*Carcharadon carcharias)* has been reported ranging from 74km/day (Bruce et al., 2006) to 119km/day (Weng et al., 2007). For bluefin tuna, average speeds determined by archival tagging were 43.2-120.96km/day (Davis and Stanley, 2002). These endothermic species represent some of the fastest swimming fishes, and epipelagic ectothermic sharks appear to have a slightly lower average horizontal speed. For example, the speeds of two blue sharks in La Jolla Canyon, California, were calculated as 25.92 km/day and 51.84km/day (Klimley et al., 2002). The speed of the sixgill shark calculated here is much higher than previous measurements of 2-4.8km/day, which were based on active and passive tracking of sixgill sharks in Puget Sound (Andrews et al., 2007). Horizontal speeds for other deepwater sharks reported in the literature are 13.5k-21.024km/day reported for *Echinorhinus cookei* in the Monterey canyon (Dawson and Starr, 2009), and 18.72-21.312km/day for the gulper shark *Centrophorus sp.* (Yano, 1984) so the value calculated for shark 1 appears reasonable alongside other deepwater species. Since this speed was calculated using a straight-line assumption and aligns well with other deepwater shark speeds in the literature, this suggests directed movement away from the Hawaiian archipelago. If the shark was exhibiting a more “random walk” movement

pattern, it would have to travel much faster than other deepwater species, so a directed movement hypothesis is more appropriate.

**4.2 Environmental drivers of depth**

The sixgill sharks observed in this study typically inhabited depths ranging from about 250-700m, with a very distinct and repeated diel vertical migration. Their depth range overlapped with *Echinorhinus cookei,* which was observed between 150-500m (this study); however, the deepwater sharks *Psuedotraikis microdon* and *Somniosus pacificus* have a deeper range (>1000m) than the sixgills based on baited camera surveys (Yeh and Drazen 2009). The sixgill shark depth range also coincides with the dive depths of several pelagic sharks including tiger sharks *G. cuvier* (Holland et al., 1999), the white shark *Charcharadon carcharias* (Jorgensen et al., 2010; Weng et al., 2007), and the bigeye thresher shark *Alopias supercilious* (Weng and Block, 2004). Some epipelagic fish and mammals may occasionally encounter sixgill sharks; though yellowfin tuna usually inhabit the mixed layer, deep dives have been recorded to 1160m (Dagorn et al., 2006), and monk seals have been recorded diving to about 500m to forage on deep coral beds on the slopes of the Hawaiian Island chain (Parrish et al., 2002). The depth range of sixgills also coincides closely with the slope-associated mesopelagic boundary community primarily composed of myctophids, shrimp, and hatchetfish (Benoit-Bird et al 2006). While the organisms comprising this community are not typical sixgill shark prey items (i.e. Ebert 2002) they are likely to be a critical factor in the overall food web, and may attract larger fish on which sixgill sharks could predate.

*4.2.1 Shallow regime depth selection*

Investigation of temperature, light level, and oxygen shows that sixgill shark depth habitat is likely controlled primarily by light throughout the diel cycle, and secondarily by temperature in both the shallow and deep regime. Most often, the deep dive at dawn followed nautical dawn and occurred before civil dawn, and all dives occured before sunrise. While some dives occurred well before nautical dawn, this suggests that increasing light levels in the shallow regime are one proximate cause of the dive behavior. The dive initiation times followed changes in sunrise times based on time of year and also based on longitudinal changes for Shark 1.The variability in the light record experienced by a sixgill shark is significantly lower than that of a tag moored at

constant depth, and together with the dive initiation times, supports the hypothesis that sixgill sharks move to maintain a constant light level. Sixgill sharks dive before light registers on surface irradiance meters (Sections 3.4, 3.6), but the eyes of deep-sea fish have a wide range of specialized adaptations to life in very low light levels. Adaptations that may be particularly important in sixgill light sensitivity are those which expand the number of photons which are absorbed over wide spatial and temporal scale, including the summation of outputs of neighboring photoreceptors in the eye to increase the solid angle of space seen by each channel, and an increase in the "exposure time" of each photoreceptor similar to increasing the exposure time in a camera lens (Warrant and Locket, 2004). These anatomical adaptations can allow sixgills and other "twilight zone" organisms to see effectively in very low light conditions.

A light-driven change in depth at dawn is common among plankton and small fishes, since they avoid light to escape predation by visual predators (Boden and Kampa, 1967; Lampert, 1993). As a top predator itself, a sixgill shark does not need to avoid light to avoid visual predators; rather, they may have evolved to follow a migrating prey source, or may be extremely light-averse. For example, diel vertical migrations have been observed in large light-senstive pelagic fishes such as the bigeye thresher (Nakano et al., 2003; Weng and Block, 2004) and movements of sevengill sharks have been found to closely correlate with the movements of its main prey item (Barnett et al., 2010).

Despite evidence for light-averse behavior in this study, sixgill sharks in higher-latitude habitats have been observed to come close to the surface (Andrews et al 2009; Dunbrack 2003) and are likely exposed to higher light levels, even in turbid water. Sharks observed near the Flora Islets in the Strait of Georgia did not exhibit light-averse behavior and came into well-lit waters during the day at about 20-40m depth. Most of the sharks observed in very shallow water are juveniles, so it is possible that there is an ontogenetic shift in light sensitivity. It has been suggested that sensitivity of the elasmobranch shark to light is plastic, and could vary throughout the lifespan depending on the light regime of the animal in a particular habitat (Litherland et al., 2009b). The diel changes in depth habitat that were observed in this study were also observed in Puget Sound, with the sharks still avoiding the most brightly lit waters during the day

(Andrews, 2009). This behavior was not observed in Bermuda since the sharks remained at deep depths where light levels were likely minimal (Carey and Clark 1995).

Temperature may also play a role in defining the limits of sixgill shark depth habitat in Hawaii. Previously, sixgill sharks were observed shallower than 40m depth when surface water was about 16ºC (Dunbrack and Zielinski, 2003). This reported temperature corresponded to the maximum water temperature in the habitat, but the depth and temperature profiles of the sharks were not tracked at a fine scale, so it is unknown if they tolerated those surface temperatures or remained in slightly cooler water. In this study, the hypothesis that sixgill sharks will be limited to temperatures <16ºC was rejected. Maximum temperatures experienced by each shark exceeded 16ºC, but the sharks generally avoided temperatures greater than 19ºC. Excursions above 16ºC were usually short, but in multiple instances continuous exposure to temperatures >16ºC lasted over an hour. Temperature and light level are both greatest at the shallowest depths, so it is difficult to determine whether light avoidance or a thermal ceiling was the main factor driving the upper depth limit. Nighttime casts of a MiniPAT tag to about 300m would determine how quickly light level decreases with depth, and may help identify one of the two parameters as more important.

*4.2.2 Deep regime depth selection*

All sharks had similar daytime depth regimes and spent most of their time between 600-700m, regardless of whether they were swimming in a pelagic or benthic-associated habitat. Sixgill sharks in Hawaii were exposed to lower light levels in the deep daytime regime than at night, which suggests that other factors may drive the deep regime depth preference. Dunbrack (2003) suggested that the sixgill sharks observed in the Strait of Georgia may avoid water less than 7ºC; in this study, sixgill sharks' daytime temperatures centered around a median of about 6ºC, so they are not cold-limited at 5-6ºC, and one shark was observed at 4.1ºC during a brief deep dive. Sixgill sharks may be selecting a particular thermal niche in their deep regime to minimize energy costs (Sims et al., 2006), and the low temperatures combined with low oxygen concentrations at daytime depths may explain the slightly lower activity level observed during the day.

The prolonged time that sixgill sharks spent in the oxygen minimum zone suggests that they are well-adapted to low oxygen, and allows rejection of the hypothesis

that sixgill sharks avoid hypoxic environmental conditions. Over three years of HOTS data, the mean oxygen concentration at 600-650m was 52.8±9.1803 μmol/kg. For epipelagic and coastal fishes, oxygen concentrations below 80μmol typically result in reduced growth rates, altered behavior, or fish avoidance, and concentrations below 40-60μmol often result in fish mortality (Hofmann et al., 2011b). However, the coastal definition of hypoxia does not have the same ecological meaning in the context of a permanent oxygen minimum zone since organisms inhabiting those zones are not suddenly exposed to lower oxygen concentrations, but instead have adapted to become adept at extracting and utilizing oxygen for aerobic respiration even at low partial pressures (Childress and Seibel, 1998; Seibel, 2011). For example, on the slope habitat of Hawaii, the oxygen minimum zone is home to diversity of organisms including macro and megafauna (DeLeo et al. 2012, Yeh and Drazen 2009). Potential adaptations to low oxygen conditions include enhanced ventilatory capacity, enhanced gill surface area, enhanced % removal of oxygen from the ventilatory stream, and hemocyanin with high affinity for oxygen and a large Bohr effect (Childress and Seibel 1998). It is likely that sixgill sharks utilize one or more of these adaptations to tolerate low oxygen concentrations in Hawaii's oxygen minimum zone and maintain aerobic activity.

**4.3 Rate of vertical movements**

For most sharks, rate of movement was greater during the night than during the day, which could be controlled by a variety of factors. The colder temperatures at depth, combined with low oxygen concentrations, could cause lowering of the maximum metabolic rate and limit fast movements (Johnston et al., 1991). The sharks might also be resting in colder waters, following the "feed warm, rest cool" hypothesis (Sims, 2005). Feeding in more productive, warmer water is more likely to yield a meal, and digestion in cold water is slower and more efficient in terms of nutrient uptake (Sims et al., 2006). There may also be fewer foraging opportunities at deeper depths, and therefore oscillatory vertical movements searching for food and chases may be less common.

Rate of vertical movement for sixgills and other species is shown in Table 6. Comparing rates of vertical movement between studies can be problematic due to variable sampling rates between studies, with longer sampling intervals potentially missing fine-scale movements and very short intervals subject to the effects of noise in

the depth sensor as discussed in Methods, but here all speeds were converted to meters per minute for the sake of comparison. Sixgill sharks in this study had a greater difference in rates of movement between day and night than sharks in Puget Sound, but sixgills in both locations were more active at night. The observed rate of movement of 1-4 m/min (median) or 1.14-3.88m/min (mean) exceeded the rate of movement of the small-spotted catshark (*Scyliorhinus canicula*). This benthic catshark was more sedentary in its daytime habitat than the sixgill sharks (Sims et al., 2006). Vertical rate of movement data for white sharks (*Carcharadon carcharias*) has been reported off the coast of Hawaii and California, vertical movements were rare and the white sharks had lower activity levels than sixgills. Within the "white shark café" or "shared offshore foraging area," a locale between Hawaii and California which white sharks frequent, vertical rate of movement increased by an order of magnitude (Jorgensen et al., 2010) and far outpaced the sixgill sharks in this study, as could be expected for a comparison between activity in an endothermic carcharhinid shark and an ectothermic deep water shark. In a study in the Gulf of Alaska, the sleeper shark was observed to be more active but comparable to sixgill sharks, with median rates of movementat 4m/min (Hulbert et al., 2006). Overall, sixgill sharks appear to have intermediate activity levels and are probably fairly active foragers in both deep and shallow regimes, with slightly higher activity levels and potential for more foraging during the night in the shallow, warmer habitat.

### 4.4 *Echinorhinus cookei* as a niche competitor in Hawaii

The nighttime depths for *E.cookei* and *H. griseus* overlap, and since both species are considered generalist predators and scavengers (Compagno 1984), it seems likely that they feed on similar kinds of prey in Hawaii and potentially compete for prey. However, their daytime depths are very different, and based on the movement record, it is unlikely that *E. cookei* forages during the day. The changes in vertical movement rates are much less pronounced for *H.griseus*, and they may forage and scavenge during the day as well, during which they could gain access to food resources not accessible to *Echinorhinus cookei*. Stomach content analysis studies for prickly sharks are rare, and no stomach content studies for sixgills or prickly sharks have occured in the Hawaiian Islands ecosystem. As an alternative to stomach content analysis, stable isotope analysis of

sixgills and prickly sharks from Hawaii helps to better understand the degree of similarity in their feeding habits by comparing carbon sources and relative trophic position (Chapter II).

## 5. CONCLUSIONS

Overall, the results of this study show that *Hexanchus griseus* is farther-ranging and more tolerant of warm water temperatures than previously known, and displays light-averse behavior in both slope and pelagic habitats. This study also includes the first archival data from *Echinorhinus cookei*, a potential niche competitor of sixgill sharks on the Hawaiian slope, and expands the state of knowledge about both species of deepwater sharks in Hawaii. The information gathered in this study can be used to generate a theoretical "habitat envelope" for sixgill sharks throughout their latitudinal range, based on the thermal structure, light penetration, bottom depth, and oxygen concentrations of each potential habitat, which may ultimately aid managers in designing regulations and protected areas for sixgill sharks globally.

Further work is needed to better understand the degree of population connectivity between sixgills in various geographic locations, foraging habitat for sixgill sharks in Hawaii, adaptations to the oxygen minimum zone, and to identify important juvenile habitat. The evidence presented here of sixgill sharks swimming in the pelagic shows that there is a possibility of genetic transfer between both nearby slope habitats and distant populations, and analysis of genetic markers will help assess the degree of connectivity. Conversely, the prickly shark observed in this study moved very little over the 2 months at liberty and was very sedentary during the day. Confidence in data reported on the single prickly shark will be enhanced by additional archival records, and a larger sample size will help determine whether the extremely sedentary behavior exhibited by shark 6 is common for the species.

Regarding food webs and predation, sixgill sharks are slightly more active at night and may be foraging more in the shallow regime (between about 200-350m), but accelerometer data would allow the identification of specific feeding events and the comparison of feeding frequency in the shallow and deep regimes. Knowledge of where sixgill sharks feed will better define their role as predators, exerting top-down control on the slope ecosystem, and as scavengers, facilitating a rapid release of nutrients back into

the food web. Gaining a better understanding of their food web ecology will also help define essential habitat and focus management plans on areas of greatest importance for foraging. Finally, combining knowledge of environmental drivers of movements and worldwide depth habitat with research to determine important nursery habitats and foraging areas will allow managers to incorporate sixgill sharks into ecosystem-based management plans designed to support biodiversity and healthy fisheries.

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CHAPTER II

Stable isotope analysis of deepwater sharks:
Comparisons across latitude, body size, and species


## ABSTRACT

Apex predators influence ecosystem dynamics through top-down food web control, while scavenging organisms speed the recycling of nutrients in the system. Elasmobranchs can be both predators and scavengers, depending on the type of food available in their habitat, and their trophic position and food source may shift throughout their age and geographic range. In this study, muscle samples were collected from the extremely wide-ranging but poorly understood bluntnose sixgill shark in two distinct habitats, and were analyzed using bulk and compound-specific stable isotope techniques to investigate trophic position. Sharks from Puget Sound, a productive estuarine habitat, were found to have a slightly higher trophic position than equivalent size sharks in Hawaii, an oligotrophic deep slope environment. An overall trend of increasing trophic position with size was more pronounced for Hawaii sharks than Puget Sound sharks. Bulk analysis suggests that sharks in the oligotrophic habitat may be more reliant on scavenging than predation, and supports previous suggestions that small sharks feed primarily inside Puget Sound, while large sharks leave the Sound for other foraging habitat. Finally, samples from another deep slope shark, the prickly shark *Echinorhinus cookei*, were analyzed to investigate prey competition. The prickly sharks were found to have a higher trophic position than sixgill sharks of similar size in the same habitat, suggesting that they may scavenge more heavily or are more selective predators than the sixgill shark.


## 1. INTRODUCTION

### 1.1 Overview

Megafaunal predators and scavengers play an important role in ecosystems by exerting top-down control on populations and facilitating the release of nutrients back into the system. In shallow-water environments, scientists and fishermen have observed drastic ecosystem composition changes resulting from removal of top trophic level

organisms in the form of "trophic cascades" (Pace et al., 1999; Simpfendorfer and Kyne, 2009). Bycatch in commercial fisheries is one major contributor to the downward trend of many shark populations, and depleted fish stocks are causing fisheries to expand operations to deeper water, which allows fisheries to contact and impact deeper-dwelling species in the form of bycatch (Morato et al., 2006; Simpfendorfer and Kyne, 2009). Deepening fisheries may also alter the food web structure by depleting certain target species. However, little is known of the way large, deepwater sharks use their habitat and interact with food webs, which makes the consequences of population depletion for the ecosystem as a whole hard to predict and precludes adequate mitigation practices for deepwater sharks.

Bluntnose sixgill sharks (*Hexanchus griseus;* hereafter "sixgill sharks") are large-bodied Hexanchid sharks which are caught as bycatch in the dogfish and seamount fisheries (Canada, 2011; Graham and Wood, 1997). Their depth habitat varies substantially with latitude. In temperate climates, they can inhabit shallow surface waters, and in the tropics are found in deep, cool water i.e. (Andrews et al., 2009; Carey and Clark, 1995). Assumed to be a generalist predator and scavenger (Compagno, 1984a; Ebert, 1994), the specifics of sixgill shark feeding ecology are limited to a few site-specific studies e.g. (Ebert, 1994) and any variation in food web interactions across their remarkable geographic range remains poorly understood. The squaliform shark *Echinorhinus cookei*, or prickly shark, is an even less understood deepwater shark but also thought to be vulnerable to anthropogenic impact (Dawson and Starr, 2009). Therefore, the consequences of overfishing a slope habitat or a seamount, which may directly take sharks as bycatch or alter the food web by shrinking the population of other target and by-catch species, are difficult to predict and manage. A better knowledge base of the feeding ecology and trophic interactions of these large carnivores will contribute to a better ability to manage the species *Hexanchus griseus* and other deepwater sharks, and will also clarify the sharks' role in the food web of various ecosystems in which they reside.

Trophic ecology studies of sixgill and prickly sharks to date have been exclusively by stomach content analysis (Compagno and Niem, 1998; Cortes, 1999; Ebert, 1986) although other deepwater shark species have been examined with stable

isotope techniques (Condon, 2011; Pethybridge, 2010). From the limited existing stomach content studies, sixgill sharks were found to feed on a variety of prey items, including hake, hagfish, marine mammal carcasses, and other elasmobranchs (Ebert, 1986, 1994). In a meta-analysis, *H. griseus* was found to occupy a trophic position of 4.3 which was among the highest trophic positions for all elasmobranchs samples, exceeded only by the sevengill shark *Notarynchus cepedianus* (4.7), the white shark *Carcharodon carcharias* (4.5) and the bramble shark *Echinorhinus brucus* (4.4) (Cortes, 1999). The congener of the bramble shark is the prickly shark *Echinorhinus cookei*, another little-known deep shark impacted in fisheries that was sampled opportunistically in this study, but was not examined in the stomach content analysis study by Cortes *et al*. Other trophic position estimates are available from www.fishbase.org: *E. cookei* has a reported trophic position of 4.4±0.76, and for *H. griseus* 4.3 ± 0.5, based on EcoPath modeling using diet composition or individual food items.

There is potential for variation in trophic position of deepwater sharks based on their latitude and geographic location, since at higher latitudes they interact with shallower, more productive food webs where prey is abundant, while in deep water live prey is scarce. Changes in resource abundance have been previously shown to significantly affect trophic positions within the same species, specifically by observing shifts in trophic position in response to enhanced resources (Shaner and Macko, 2011). In the deeper, oligotrophic habitat, scavenging may become the prominent feeding strategy. Scavengers tend to have a higher $\delta^{15}N$ values than predators of similar body size (Stowasser et al., 2009) since they are able to feed on higher trophic level organisms although $\delta^{15}N$ values can also reflect changes in nitrogen biogeochemistry of the environment (see below). Alternatively, abundances of very high trophic position prey, such as marine mammals and elasmobranchs, are variable with location and may have a stronger impact on trophic dynamics than scavenging versus predation. Higher concentrations of fish and other large organisms are supported by more productive ecosystems, such as the upwelling zone on the western coast of North America (Iverson, 1990). If the availability of high trophic level prey were most important in determining trophic position of deepwater sharks, higher trophic positions would be observed from mid to high-latitude sharks living in the shallow cold water of upwelling areas.

## 1.2 Stable isotope analysis as a tool in food web ecology

The use of stomach content analysis to determine trophic position is a valuable technique, since prey items can potentially be identified individually. However, it has some fundamental limitations. Stomach content analysis only gives a snapshot of what the organism ate recently, which may or may not be representative of its overall diet, and for many animals, empty stomachs are common and a large sampling effort is required. Additionally, the technique requires destructive sampling for most organisms. For both sixgill and prickly sharks, population data is almost entirely lacking but both species are suspected to be threatened by anthropogenic impacts, and therefore lethal sampling is not ideal. Alternatively, questions of food web interactions and trophic ecology can be investigated by analyzing the ratios of stable isotopes of carbon and nitrogen in the animal's tissue (Minagawa and Wada, 1984; Peterson and Fry, 1987). Isotopic analysis of predator tissues can integrate the isotopic compositions of the organisms' diet over time and requires only a small amount of muscle tissue, which can be sampled non-lethally from a large animal.

Nitrogen isotopic ratios are often used to infer trophic positions, since the ratio of $^{15}N/^{14}N$ changes predictably in a food web: with each trophic transfer, there is about a 3-5‰ increase in $\delta^{15}N$ values because $^{14}N$ is preferentially excreted from the predator in metabolism (Minagawa and Wada, 1984; Peterson and Fry, 1987; Post, 2002). This isotopic fractionation does not appear to be affected by habitat, and so can be used for organisms in various geographical locations and ecotypes (Minagawa and Wada, 1984). Carbon isotopic ratios ($^{13}C/^{12}C$) are less enriched with trophic transfer and instead can help trace the source of primary production in the food web (Post, 2002). Isotopic analysis of bulk tissue provides a measurement of the total isotopic composition for the tissue(s) analyzed, and can readily be compared to other organisms feeding within the same food web to investigate nutrient flow and trophic position (Post 2002). However, this method does not readily allow trophic comparisons between marine organisms feeding in different food webs, since the nitrogen isotopic ratio in plants and algae at the base of a food web is variable between locations and with the type of primary producers (Popp et al., 2007). When investigating the same organism in two or more distinct food webs, estimation of trophic position from bulk tissue $\delta^{15}N$ values can be complicated by

variable starting points unless the $\delta^{15}N$ values of primary production are known (Dale et al., 2011; McClelland and Montoya, 2002; Popp et al., 2007; Schmidt et al., 2004).

**1.3 Amino acid compound specific isotopic analysis of nitrogen**

Data describing the $\delta^{15}N$ values of primary producers that contribute to each food web are required to successfully compare trophic positions of organisms feeding in more than one food web, which has historically required a large sampling effort (McClelland and Montoya, 2002). Determining the primary production $\delta^{15}N$ value for a particular organism can also be difficult or impossible, especially for consumers where the ultimate source of primary production is mixed or unknown, such as migratory animals or deep sea fish. To compare food web interactions for animals in these categories, an alternative technique, amino acid compound specific isotopic analysis (AA-CSIA), can be used to determine relative trophic position. These analyses can elucidate the integrated primary production $\delta^{15}N$ values at the base of each organism's food web without additional sampling of photoautotrophs.

Amino acid compound specific isotopic analysis of nitrogen improves on traditional bulk isotopic analysis by allowing the quantification and comparison of trophic position of animals feeding in different food webs. Some amino acids ("trophic" amino acids) become enriched in $^{15}N$ in trophic transfers, while other amino acids ("source" amino acids) have little or no stepwise enrichment (McClelland and Montoya 2002). Trophic amino acids typically undergo nitrogen-carbon cleavages during metabolic processes while the source amino acids do so at a much reduced rate or not at all (McClelland and Montoya, 2002; Popp et al., 2007). The "source" amino acids include glycine (gly), serine (ser), phenylalanine (phe), tyrosine (tyr), and lysine (lys), while the "trophic" amino acids include alanine (ala), aspartic acid (asp), proline (pro), glutamic acid (glu), leucine (leu), isoleucine (ile), and valine (val) (Chikaraishi et al., 2009; McClelland and Montoya, 2002). In consumer tissue, the trophic transfers of nitrogen are recorded as $^{15}N$ enrichment in trophic amino acids, and the $\delta^{15}N$ values at the base of the food web are recorded in the source amino acids, which allows quantification of trophic position without having to measure the isotopic composition of photoautotrophs which support the food web (Chikaraishi et al., 2009). The enrichment in $^{15}N$ in each trophic transfer is the "trophic enrichment factor" or TEF, and the

difference between trophic and source amino acids in the primary producers is the β-value (Chikaraishi et al., 2009). These values may vary depending on the amino acids used. The trophic position is calculated using Equation 1:

$$TP_{Tr-Sr} = \frac{(\delta^{15}N_{Tr-AA} - \delta^{15}N_{Sr-AA} - \beta)}{TEF} + 1 \qquad (1)$$

Multiple methods have been used to derive a trophic position from trophic and source amino acids. The most widely used method is based on the difference in $\delta^{15}N$ values between Glu and Phe (Dale et al., 2011; McClelland and Montoya, 2002) which have low standard deviations and therefore may produce more precise estimations of trophic position (Chikaraishi et al., 2009). In the Chikaraishi study, Gly and Ser were avoided due to high variability. Other authors have noted potential complications with using Glu and Phe as a trophic-source pair. Sherwood et al. (2011) used Glu-Phe but also confirmed trophic positions estimates using a broad suite of trophic and source amino acids, which included (trophic) Asp, Glu, Ala, Ile, Leu, Val, and Pro, and (source) Gly, Lys, Ser, Phe, and Tyr. While results for both methods were consistent in their work, they caution that the use of just two amino acids may be more sensitive to a measurement error or other inconsistencies than a suite of amino acids (Sherwood et al., 2011). McCarthy et al. (2007) used amino acid techniques to investigate phytoplankton and sinking particulate organic matter in the tropical North Pacific; they chose to use the trophic amino acids Asp and Glu, and the source amino acids Val, Leu, and Pro. Amino acids that were commonly re-synthesized during bacterial heterotrophy were avoided (Ile, Ala, and Phe), and the authors noted that the $\delta^{15}N$ value of Gly may change with organic matter degradation (McCarthy et al., 2007).

An average TEF of 7.6±1.2‰ was determined by Chikaraishi et al. (2009) in an experiment examining fractionation between a primary producer (TP=1), herbivore (TP=2), omnivore (TP=2.5) and predator (TP=3), using the combination of glutamic acid as a trophic amino acid and phenylalanine as a source amino acid. This TEF is consistent for the initial trophic transfer from primary producer to primary consumer, but may not be adequate to quantify absolute trophic position for all animals with trophic position >2

(Wallsgrove, 2011; Dale, Wallsgrove et al. 2011). Recommended TEFs for higher trophic level transfers have been calculated for a handful of species based on integration with stomach content analysis and bulk isotopic analysis, and range from 3.9-5.9‰ (Wallsgrove 2011; Dale, Wallsgrove et al. 2011). The differing TEFs may be a result of taxa-specific metabolic pathways. For example, it has been suggested that urea production in sharks reduces the importance of glutamate catabolism, and results in lower than expected $\delta^{15}N$ values for glutamic acid (Dale et al., 2011). No species-specific TEFs have been quantified for sixgill or other deepwater sharks. Even without confidence in the accuracy of the TEF, *relative* differences in trophic position among conspecifics can be investigated using AA-CSIA independent of variation in $\delta^{15}N$ values at the base of an organism's food web.

In this study, compound specific isotopic analysis of nitrogen was used to compare trophic position of *Hexanchus griseus* specimens from two dramatically different habitats, and this technique also allowed the assessment of a possible competitive relationship between *H. griseus* and *Echinorhinus cookei* in Hawaii. The trophic position for all animals was calculated and the data were analyzed for patterns relating to geographic location, size, sex, and species. In particular, the following hypotheses were addressed:

$H_o$: There will be no significant difference in trophic position between sixgill sharks captured in the deep oligotrophic habitat of Hawaii and the shallow estuarine habitat of Puget Sound, WA.

$H_a$: The trophic position of sixgill sharks is higher in an oligotrophic habitat where scavenging may be a more important component of the feeding strategy.

$H_o$: There will be no difference in the trophic position of the sixgill shark *Hexanchus griseus* and a possible niche competitor, the smaller prickly shark *Echinorhinus cookei* on the Hawaii slope.

$H_a$: The prickly shark feeds at a higher trophic position than the sixgill shark, based on stomach content analysis data from FishBase.

## 2. METHODS

Sixgill and prickly sharks* were captured by bottom-set longline on the south and west slopes of Oahu, Hawaii, at depths ranging from 300-600m. Tissue samples were taken using a specialized 1cm x 5cm long biopsy punch manufactured by Ceta-Dart (Copenhagan, Denmark). The long length on the biopsy punch was necessary to reach the white muscle tissue through a thick layer of skin and subcutaneous fat. Sampling on live specimens was accomplished by using a scalpel to cut an incision through the skin and fatty layer, and then inserting the biopsy punch through this incision. Tissue samples were stored in liquid nitrogen or on dry ice for transport, and then subsequently stored at -80°C until drying and analysis. Similarly preserved and prepared dried and ground white muscle tissue samples (6) from sixgill sharks captured in Puget Sound were generously provided by Greg Williams (NOAA).

*These are not necessarily the same sharks analyzed in Chapter I. Sharks 3, 4, 5 and 6 from Chapter I were successfully sampled for stable isotope analysis. Additional sharks were sampled for isotope analysis but not tagged with pop-up satellite archival tags.

### 2.1 Bulk tissue isotopic analysis

Samples were freeze dried for 36-48 hours and ground to a fine powder. The $\delta^{15}N$ and $\delta^{13}C$ values as well as C:N (mol/mol) ratio of white muscle tissue were determined on an isotope ratio mass spectrometer coupled to a CN analyzer (ThermoFinnigan Delta Plus XP/Conflo IV/Costech 291210296) δ-values are reported in ‰ relative to Vienna PeeDee Belemnite as a standard for carbon, and air as a standard for nitrogen. Lipids and urea are often present in elasmobranch muscle tissue and have been shown to affect isotopic measurements (Hussey et al., 2012; Kim and Koch, 2012). Lipids can be removed with a non-polar solvent, and urea with rinses of de-ionized water (Kim and Koch, 2012). For this study, a subsample from each tissue sample was rinsed 3 times with hexanes to remove lipids, and 3 times with de-ionized water to remove urea prior to analysis.

## 2.2 Compound specific isotopic analysis of individual amino acids

Amino acids were extracted from muscle tissue following the methods of Dale et al. (2011) and Wallsgrove (2011) with slight modification. The sample was subjected to acid hydrolysis, esterification, and trifluoracetylation to free amino acids from protein and to derivitize the amino acids for gas chromatographic analysis.

13-16mg of tissue were placed in a culture tube, flushed with $N_2$, sealed with a Teflon-lined cap, and hydrolyzed in 0.5ml 6 N HCl at 150°C for 70 minutes. The hydrolyzed tissue was evaporated under a stream of $N_2$ at 55°C, and the residue was re-dissolved in 0.01 N HCl. The samples were purified through a 0.2µm hydrophilic filter (VWR), and the filter was washed with an additional 1ml of 0.01 N HCL. The amino acids were separated from other organic compounds by a cation exchange column (GracePure$^{TM}$ SPE Cation-X). The columns were first rinsed with 1ml 0.01 N HCl. The sample was then passed through the cation exchange column, with amino acids remaining bound to the cation exchange filter while organic acids and carbohydrates passed through as waste, still dissolved in 0.01 N HCl. The amino acids were then eluted using 4ml 2N ammonium hydroxide and collected. The eluate was dried under a stream of $N_2$ at 80°C. The dried residue was re-dissolved in 0.5ml 0.2 N HCl, flushed with $N_2$, and heated to 110°C for 5 minutes.

The carboxyl terminus of the amino acids was esterified in 2.5ml of 1:4 acetyl chloride: isopropanol, flushed with $N_2$ gas, capped, and heated at 110°C for 60 minutes. The solution was dried under a stream of $N_2$ at 60°C. For the trifluoroacetylation step, amino acids were re-dissolved in 600µl methylene chloride with 200µl trifluoroacetic anhydride, flushed with $N_2$, capped, and heated to 100°C for 15 minutes. Samples were further purified using solvent extraction with 2ml of P-buffer ($KH_2PO_4$ + $Na_2HPO_4$ in distilled water, pH 7) and chloroform (Ueda et al., 1989). The esterified and acetylated amino acids were partitioned into the chloroform by vigorous shaking followed by centrifuging for five minutes. The chloroform layer was collected, and to ensure full derivitization, the sample was evaporated and the trifluoroacetylation step was repeated. Samples were stored at -20ºC until analysis. Prior to analysis, the 3:1 methylene chloride: trifluoroacetic anhydride was evaporated under $N_2$ at room temperature and the sample re-dissolved in 300µl-1ml ethyl acetate.

## 2.3 Analysis

The nitrogen isotopic composition of the amino acids was determined using a Delta V plus mass spectrometer interfaced with a Trace GC gas chromatograph through a GC-C III combustion furnace (980°C), reduction furnace (650°C) and liquid nitrogen cold trap. The samples (0.6-2µl) were injected onto a BPx5 capillary column (60m x 0.32m x 1.0µm) at a temperature of 180°C with a helium flow rate of 1.4ml/min. The column oven was held at 50°C for 2 minutes, then was increased in stages to 120°C at 15°C/min, to 195°C at 4°C/min, to 255°C at 5°C/min. Lastly it was heated at 15°C/min to a final temperature of 300°C, where it was held for 8 minutes. Aminoadipic acid and norleucine with known $\delta^{15}N$ values were co-injected with each sample as internal reference compounds, and samples were injected in triplicate. A suite of naturally-occurring amino acids with known $\delta^{15}N$ values and the reference compounds were injected surrounding every triplicate set of sample analyses for additional quality assurance/quality control, and was used to normalize sample results if quantification of the isotopic composition of the aminoadipic acid and norleucine reference compounds co-injected with the samples had been compromised by co-eluation with the amino acid compounds of interest in the samples. The average accuracy of measurements was 0.3‰ (range 0.03-0.8‰), which was determined by regression based on the known norleucine and aminoadipic acid isotope values in the amino acid suite surrounding each triplicate set of runs. Precision was determined by calculating the standard deviation of each amino acid for each triplicate set (mean 0.4‰, range 0.00-1.57‰). Values with precision > 1‰ were not used in calculations of trophic position.

Trophic positions were calculated using two methods. First, the method of Chikaraishi et al. (2009) was used, with glutamic acid as the trophic amino acid and phenylalanine as the source amino acid, following Equation 2:

$$TP_{Glu-Phe} = \frac{(\delta^{15}N_{Glu} - \delta^{15}N_{Phe} - 3.4)}{7.6} + 1 \qquad (2)$$

A Pearson's linear regression analysis of phenylalanine versus other source amino acids revealed a weak correlation (r=0.3) (see Results). Therefore, a second method was

used that included multiple source and trophic amino acids that occurred in all samples and in which the $\delta^{15}N$ value could be reliably determined. For this method, the trophic amino acids included glutamic acid, proline, and leucine, and the source amino acids included glycine, serine, and phenylalanine. TEF and β values were estimated from Chikaraishi, Ogawa et al. (2010), which were based on results of controlled diet experiments including a primary and secondary consumer (Equation 3).

$$TP_{Tr-Sr} = \frac{(\delta^{15}N_{Glu,Pro,Leu} - \delta^{15}N_{Gly,Ser,Phe} - 3.3)}{6.3} + 1 \qquad (3)$$

Error in each trophic position calculation was estimated using a propagation of error calculation (see Dale et al. 2011), taking into account the error in TEF and β values from Chikaraishi et al. (2010) and the analytical error associated with the isotopic measurement of the triplicate analysis of each individual amino acid used in the calculation.

**2.4 Statistics**

Linear regression was used to determine the slope, strength, and significance of the relationship between isotopic composition and total length, between molar C:N ratio and $\delta^{13}C$ values, and between molar C:N ratio and total length. The total length of sample animals was tested for homogeneity of variance between the two locations using an f-test for equal variances. The same test was used to determine if the trophic positions calculated from $\delta^{15}N$ values from trophic and source amino acids had equal variances. A one-tailed student's t-test was used to examine the hypothesis of zero difference in trophic position between animals in the two locations.

A generalized additive model (GAM) was then constructed, since generalized additive models allow descriptions of relationships that are not necessarily linear (Zuur et al., 2009). The GAM model included sex, location, and maturity (yes or no), with total length as a covariate, as factors contributing to variance in trophic position. The validity of the model was verified using normal quantile-quantile plot, a plot of response versus fitted variables, and a plot of residuals versus linear predictor. P-values were then computed using ANOVA within the GAM program. The generalized additive model was

constructed in R using the package “mgcv” (Wood, 2011). The alpha value for significance was set at $\alpha=0.05$.

## 3. RESULTS

### 3.1 Bulk Stable Isotope Analysis

A total of 14 samples of white muscle tissue from sixgill sharks (n=12) and prickly sharks (n=2) were analyzed for bulk carbon and nitrogen isotopic composition. For similar-size sharks, nitrogen isotopic values tended to be higher in Puget Sound than in Hawaii, and $^{15}N$ enrichment appeared to generally decrease with increasing shark size (Figure 1a). Carbon isotopic values clustered into three main groups: a relatively $^{13}C$ enriched group of immature ~250cm sharks in Puget Sound, a $^{13}C$ depleted group of sharks ~300cm in Hawaii, and a group of large mature females (~450cm TL) from both regions that were relatively much more depleted in $^{13}C$ (Figure 1b).

Removal of urea by rinsing the samples in deionized water increased the $\delta^{15}N$ values by an average of 0.6‰, and the hexane rinse to remove lipids increased the $\delta^{13}C$ values by an average of 2.1‰. Nitrogen isotopic compositions for all sharks generally increased when rinsed, with the exception of two samples (Figure 2a). There was no significant relationship between size and $\delta^{15}N$ values of bulk tissue (Pearson's linear correlation; p=0.105). Removing lipids from the samples caused the clustering visible in figure 1b to be much less distinct, and still indicated a visible, though insignificant, trend of a decrease in $\delta^{13}C$ values in larger sharks (Pearson's linear correlation; p=0.172) and decreased the spread of $\delta^{13}C$ values.

The C:N ratio in deep shark muscle tissue averaged 3.8±0.1 (mol/mol) after rinsing with hexane. Plots of the C:N ratio before and after hexane rinsing confirm that high C:N ratios in the un-rinsed samples were a direct result of large amounts of lipids, which contain no N and are relatively depleted in $^{13}C$ compared to protein (Figure 3a, Pearson's linear correlation, p=0.000063; Figure 3b). Larger sharks had higher C:N ratios (Pearson's linear correlation, p=0.018) in the un-rinsed samples, indicating lipid-rich tissue in the larger animals (Figure 4). Plots of rinsed $\delta^{15}N$ values against $\delta^{13}C$ values showed no easily identified clustering or relationships (Figure 5). All bulk isotopic measurements from each shark specimen are reported in Appendix A.

### 3.2 Compound specific analysis of nitrogen isotopes in individual amino acids

Isotopic compositions of up to 15 amino acids were quantified for each sample. Of those 15, eight were quantified in all 12 sixgill shark samples and in both prickly

shark samples. Some amino acids did not have a high enough concentration to allow reliable isotopic measurements in one or more samples (often isoleucine, tyrosine, and valine). The set of eight amino acids included three source amino acids and three trophic amino acids, which were subsequently used for calculation of trophic position. Mean and standard deviation of $\delta^{15}N$ values of individual amino acids for sixgills from each location are shown in Table 1. Mean $\delta^{15}N$ values for source amino acids, trophic amino acids, and bulk tissue $\delta^{15}N$ value for each sixgill shark are shown in Figure 6. Amino acids in Puget Sound sharks are enriched in $^{15}N$ relative to Hawaii sharks (paired t-test, df=15, $t_{crit}$=1.761, p=0.00002) by an average of 2.3‰. Source amino acids were enriched in $^{15}N$ by an average of 3.1‰ in Puget Sound sharks relative to Hawaii sharks; trophic amino acids were enriched an average of 1.9‰. All amino acid $\delta^{15}N$ values are reported for each shark specimen in Appendix A.

Calculation of trophic position (TP) based on glutamic acid and phenylalanine (method 1) yielded a range of trophic positions of 3.01-3.91 for Hawaii sixgills, 3.4-3.9 for Puget Sound sixgills, and 3.67 and 3.69 for the Hawaii prickly sharks (Table 2). Plotted against total length, Hawaii sixgills, Puget Sound sixgills, and prickly sharks all were at about the same trophic position regardless of length, disregarding the outlier at TP=3.01 (Figure 8). Using only the nitrogen isotopic compositions of glutamic acid and phenylalanine, there was no evidence for a change in trophic position with size.

Data for glutamic acid and phenylalanine calculations are presented for consistency and comparability with the literature; however, phenylalanine was not tightly representative of the source amino acids as a whole (Figure 7, $r^2$=0.3). With this in mind, I believe that the calculation of TP based on the combination of glycine, serine, and phenylalanine as source amino acids and glutamic acid, leucine, and proline provided a more accurate depiction of relative trophic position. Neither the phenylalanine $\delta^{15}N$ values alone or the combined source amino acid $\delta^{15}N$ values were representative of the bulk tissue rinsed $\delta^{15}N$ values ($r^2$<0.06 for both), which is probably a result of different $\delta^{15}N$ values of primary producers in various feeding locations.

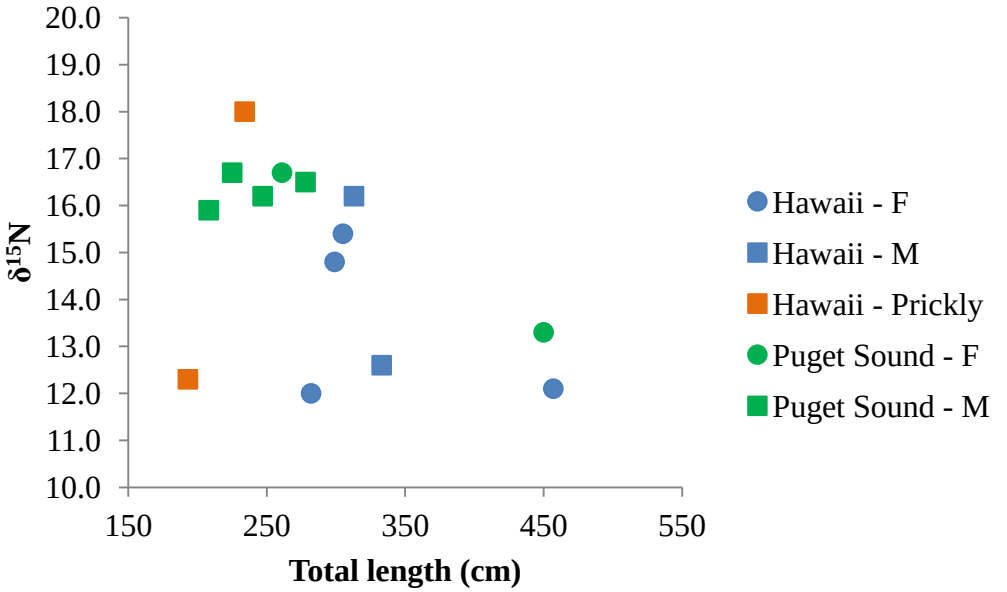
Calculation of TP based on the “trophic” minus the “source” $\delta^{15}N$ values of individual amino acids resulted in a range of TP=3.48-4.5 for Hawaii sixgills, TP=3.62-3.9 for Puget Sound sixgills, and TP=4.53 and 4.63 for Hawaii prickly sharks (Figure 9).

Plotted against total length, this calculation reveals relatively consistent trophic position for smaller sharks, and a trend of increasing TP with increasing size which is more pronounced for sixgill sharks from Hawaii than from Puget Sound, but present in both groups. Male sixgill sharks in Hawaii had a lower trophic position than females of similar size (Figure 8), but that was not observed in Puget Sound.

Despite a smaller body size, prickly sharks appeared to feed at a very high trophic position using the trophic position calculation based on multiple source and trophic amino acids. Also of interest, though these two prickly sharks were captured in the same location and were similar in terms of sex, maturity, and body size, the $\delta^{15}N$ values at the base of their respective food webs were quite different: 3.34% and -1.58%. The base of the food web for sixgill sharks also varied substantially in Hawaii (-0.12 to 5.65%).

An f-test for equal variances showed that the trophic positions in Hawaii were significantly more varied than trophic positions in Puget Sound ($p=0.0184$), but there was no significant difference in the variation of total length ($p=0.249$). A one-tailed t-test for equal means assuming unequal variance was insignificant and failed to reject the hypothesis that the difference between the means of $TP_{HI}$ and $TP_{PS}$ was zero. Linear regression analysis of total length versus trophic position was significant at $p=0.049$ ($r^2=0.33$).

A generalized additive model (GAM) was constructed using location, sex, and maturity as explanatory variables with total length as a covariate. The model explained 94.7% of the deviance observed, but no factor was significant at $\alpha=0.05$. Location and sex (with total length as covariate) were significant at $\alpha=0.10$. Sex:length explained 35.8% of the variance, followed by total length at 33.1%, location:length at 18.8%, and maturity:length at 7.0%. Results are shown in Table 3.



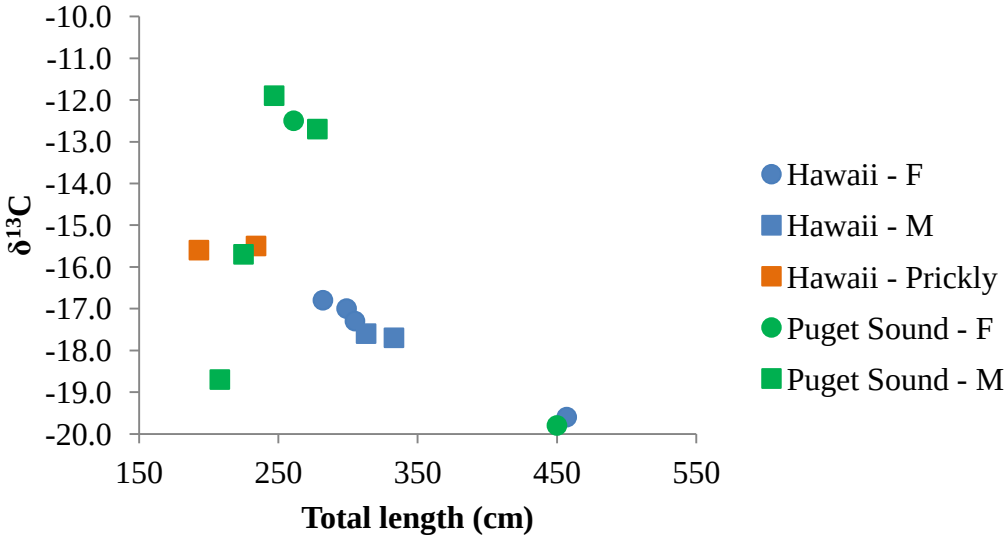
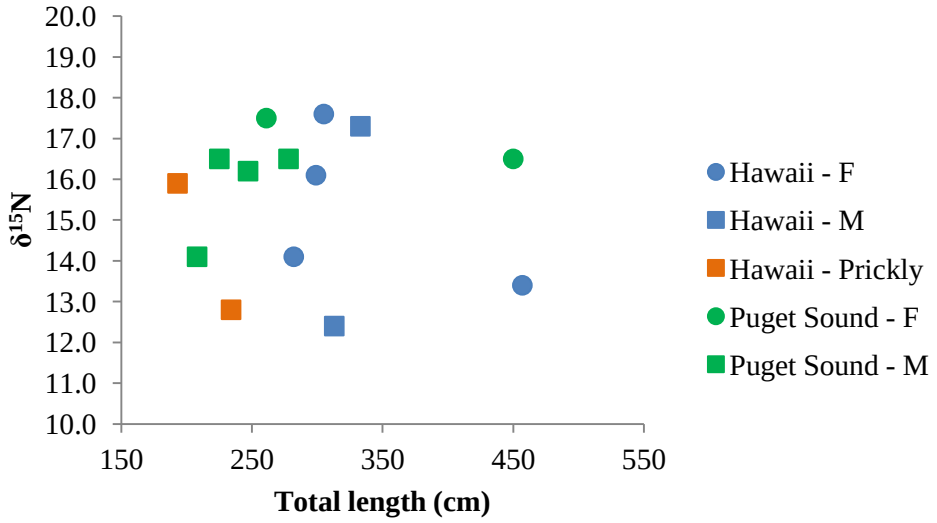




Figure 1. (Top) $\delta^{15}N$ values determined of bulk tissues of shark muscle, plotted against total length. The small sharks from Puget Sound cluster together and show slight enrichment in $^{15}N$ with increasing size. The clustering is likely a result of a shared foraging habitat and similar prey selection within the sound. (Bottom) $\delta^{13}C$ values for smaller Hawaii sharks cluster, as well as three of the mid-sized Puget Sound sharks. The two largest sharks were depleted in $^{13}C$ compared to the smaller animals.



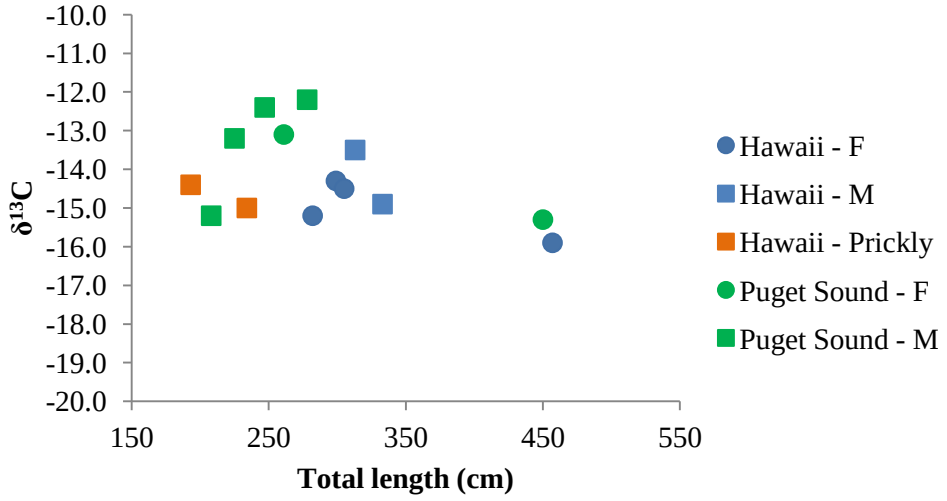




Figure 2. (Top) $\delta^{15}N$ and (Bottom) $\delta^{13}C$ values versus total length following a lipid and urea rinse. Both $\delta^{13}C$ and $\delta^{15}N$ values were slightly higher after rinsing, and the tight clustering previously observed in the carbon isotopic compositions became weaker. The largest animals still were the least enriched in $^{13}C$, but there was no clear trend in $^{15}N$ enrichment with size.

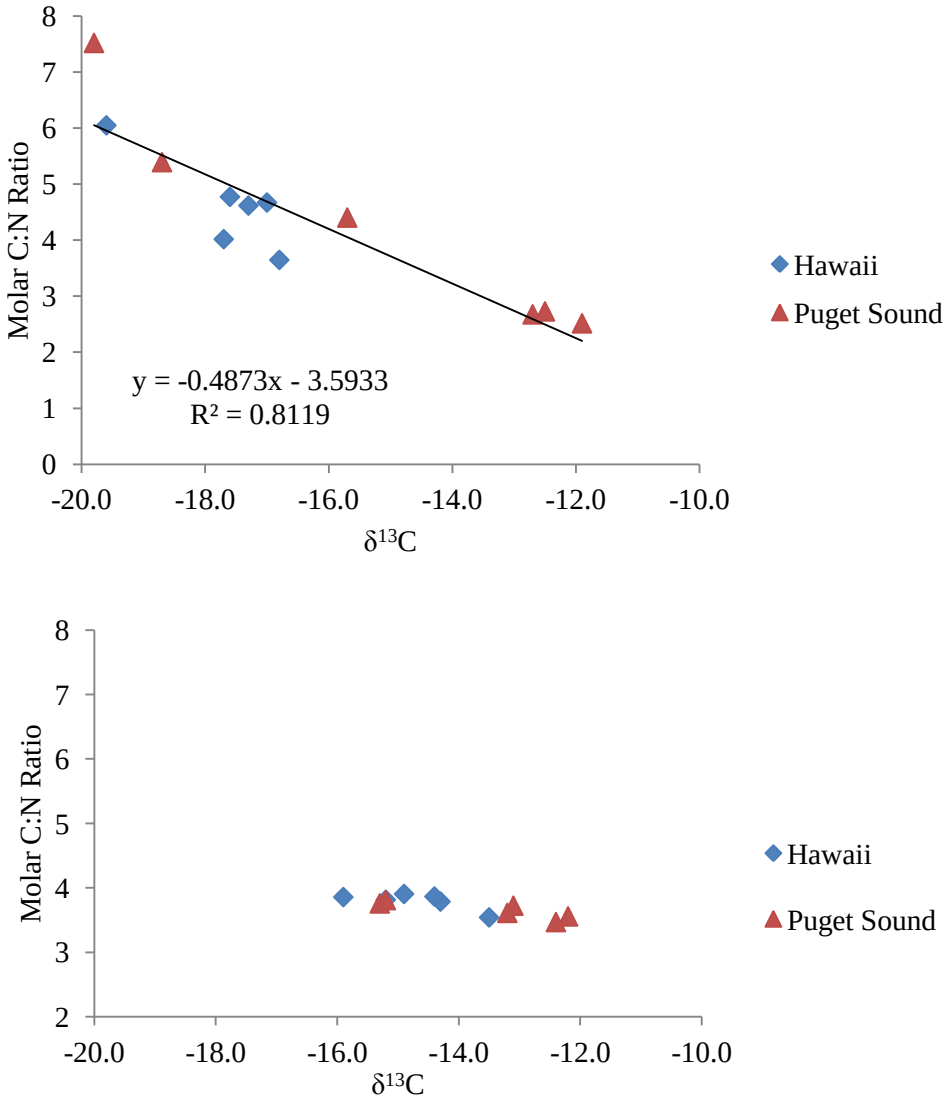



Figure 3. (Top) $\delta^{13}C$ values versus molar C:N ratio. The C:N ratio decreased as the tissue became more enriched. (Bottom) $\delta^{13}C$ values versus molar C:N after the lipid and urea rinse, which brought all the values in line with expected molar ratios of 3-4 C:N (mol:mol). This indicates that the high molar C:N ratios were due to high lipid content, which is expected.

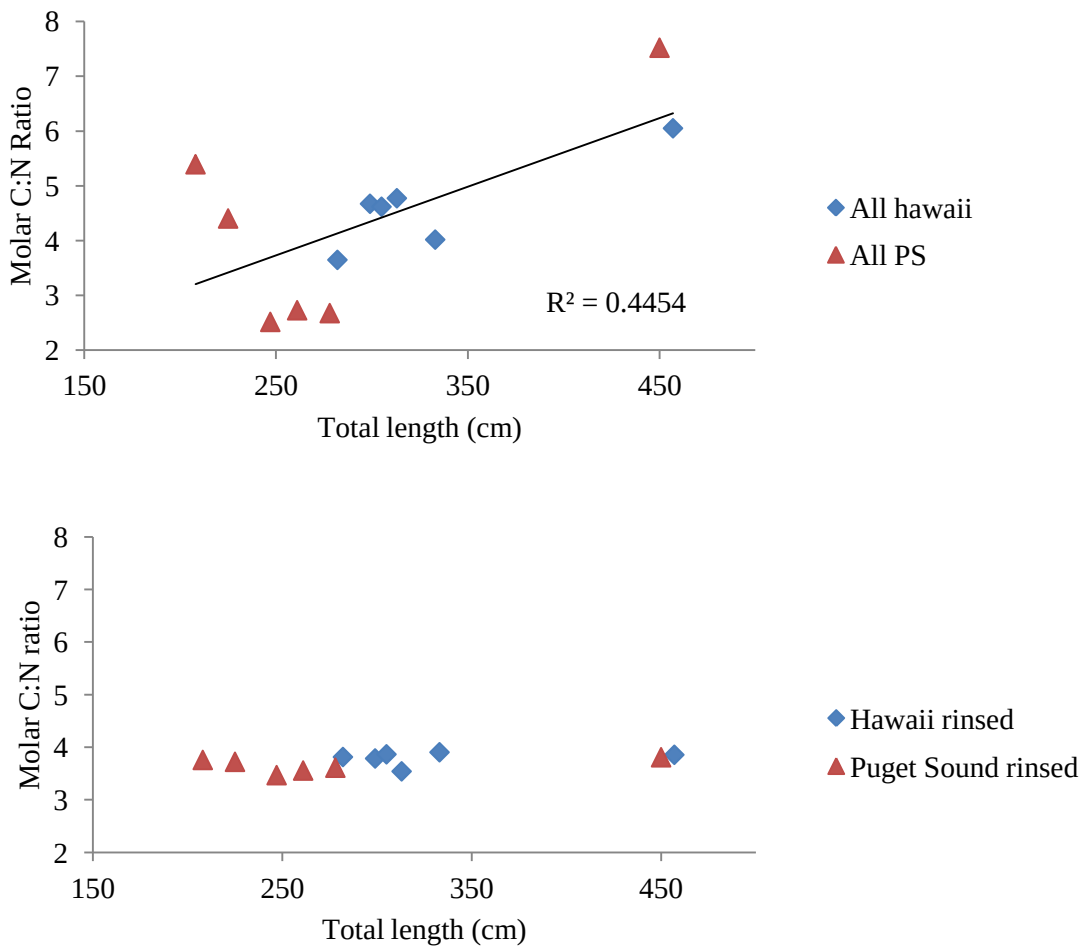



Figure 4. (Top) Total length versus molar C:N ratio. The C:N ratio increased with shark total length, indicating a higher lipid content in the muscle tissue as the animal grows and matures. (Bottom) Total length versus molar C:N after the lipid and urea rinse, which brought all the values in line with expected molar ratios of 3-4 C:N.

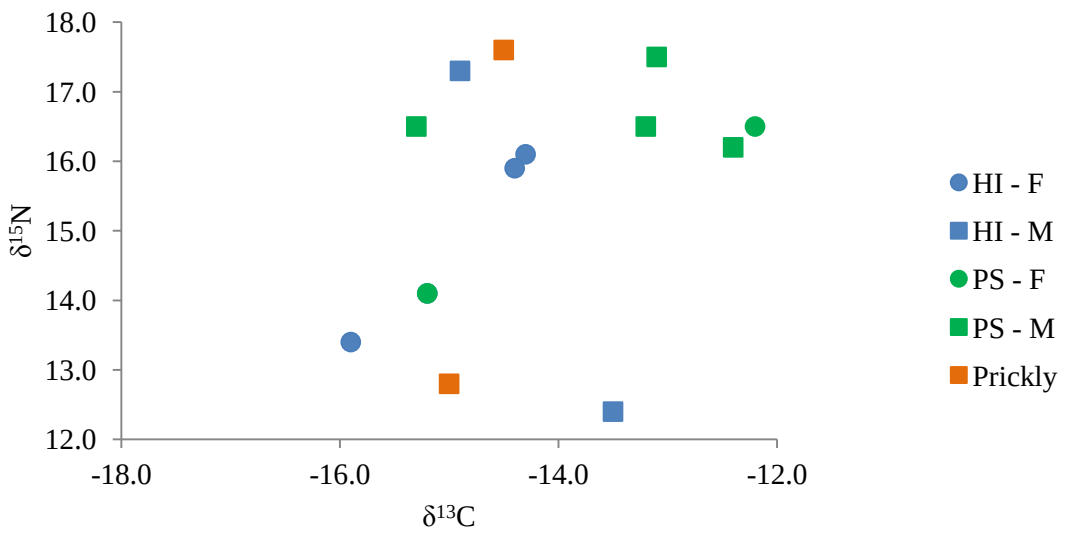



Figure 5. A plot of rinsed $\delta^{13}C$ values versus rinsed $\delta^{15}N$ values reveals no strong clustering or partitioning based on sex or location.

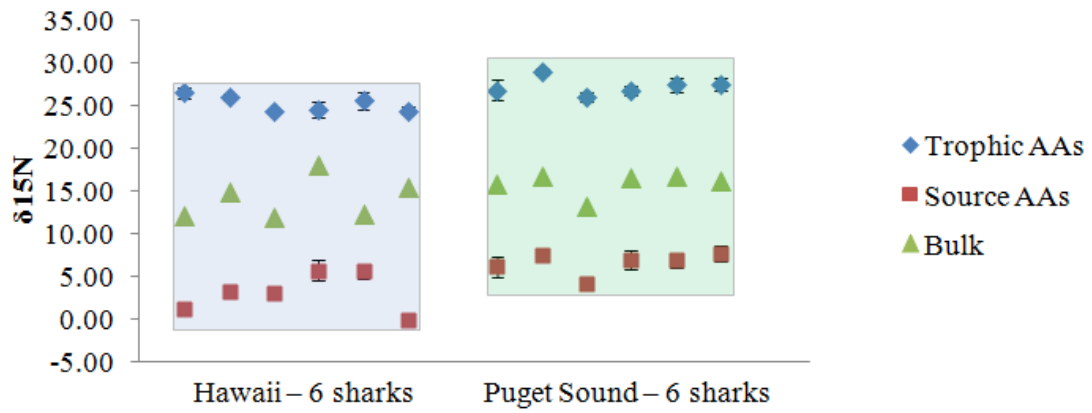



Figure 6. Mean and standard deviations of the trophic and source amino acids plotted for each individual sixgill shark, alongside the bulk tissue $\delta^{15}N$ values. The Hawaii dataset is quite variable, but the third Puget Sound shark (PS-3) was depleted relative to the others. PS-3 was a large, pregnant female that more likely fed on the continental margin, and not inside Puget Sound. Overall, Puget Sound sharks were slightly more enriched based on amino acid data, although the pattern is not visible in bulk data.

| | Trophic/Source | Hawaii | | Puget Sound | |
|---|---|---|---|---|---|
| | | Mean | STD | Mean | STD |
| Alanine | Trophic | 26.5 | 0.94 | 29.2 | 0.76 |
| Glycine | Source | 2.9 | 1.05 | 7.5 | 0.94 |
| Threonine | | -32.1 | 1.8 | -27 | 1.32 |
| Serine | Source | 2 | 1.32 | 6.1 | 1.5 |
| Valine | Trophic | 23.2 | 1.4 | 24.3 | 1.45 |
| Leucine | Trophic | 24.2 | 0.98 | 25.7 | 1.11 |
| Isoleucine | Tophic | 24.9 | 1.21 | 26.1 | 0.79 |
| Proline | Trophic | 24.2 | 1.06 | 26.8 | 1.28 |
| Aspartic acid | Trophic | 22.4 | 0.71 | 24.2 | 1.13 |
| Methionine | | 13.2 | 1.27 | 13.9 | 1.36 |
| Glutamic acid | Trophic | 27.4 | 0.73 | 29.4 | 0.89 |
| Phenylalanine | Source | 4.3 | 1.02 | 6 | 1.3 |
| Tyrosine | Source | 7.8 | 0.82 | 11.8 | 0.03 |
| Lysine | Source | 6.5 | 0.59 | 7.6 | 1.26 |
| Arginine | | 12.5 | 0.36 | 12.4 | 0.76 |

Table 1. Mean and standard deviation of nitrogen isotopic composition for all amino acids, separated by region. In general, amino acids in Puget Sound samples were more enriched in $^{15}N$ relative to those in samples from Hawaii.

| | Sex | Total length | TP (glu-phe) | St Dev | TP (trophic-source) | St Dev |
|---|---|---|---|---|---|---|
| **HI-1** | F | 282 | 3.01 | 0.23 | 3.86 | 0.51 |
| **HI-2** | F | 299 | 3.71 | 0.14 | 4.11 | 0.45 |
| **HI-3** | M | 305 | 3.57 | 0.18 | 3.48 | 0.41 |
| **HI-4** | M | 313 | 3.65 | 0.26 | 3.66 | 0.37 |
| **HI-5** | F | 333 | 3.73 | 0.22 | 4.37 | 0.4 |
| **HI-6** | F | 457 | 3.91 | 0.28 | 4.5 | 0.49 |
| | | | | | | |
| **PS-1** | M | 208 | 3.75 | 0.29 | 3.77 | 0.42 |
| **PS-2** | M | 225 | 3.4 | 0.12 | 3.9 | 0.43 |
| **PS-3** | M | 247 | 3.66 | 0.27 | 3.63 | 0.43 |
| **PS-4** | F | 261 | 3.67 | 0.37 | 3.75 | 0.38 |
| **PS-5** | M | 278 | 3.9 | 0.2 | 3.62 | 0.41 |
| **PS-6** | F | 450 | 3.4 | 0.31 | 3.96 | 0.39 |
| | | | | | | |
| **EC-1** | M | 193 | 3.67 | 0.19 | 4.63 | 0.52 |
| **EC-2** | M | 234 | 3.69 | 0.15 | 4.53 | 0.52 |

Table 2. Trophic positions calculated by Method 1 (glu-phe) and Method 2 (trophic-source) for each individual shark. HI = Hawaii sixgill; PS = Puget Sound sixgill, and EC = *Echinorhinus cookei*, also captured in Hawaii.

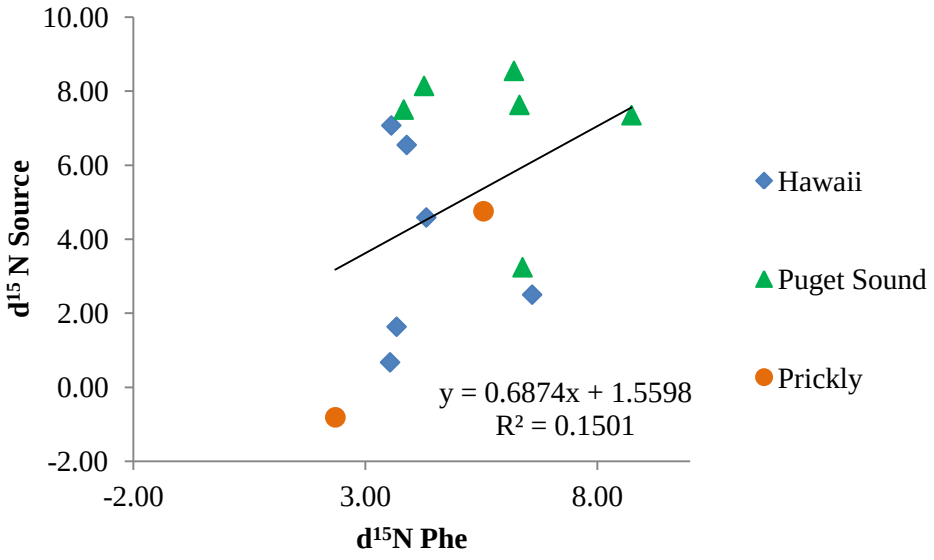



Figure 7. A plot of $\delta^{15}N_{Phe}$ vs. $\delta^{15}N_{Source}$ values reveals poor correlation between phenylalanine and the average of all other source amino acids that were quantified ($r^2$=0.15). The source amino acids in this plot include serine, glycine, and lysine.

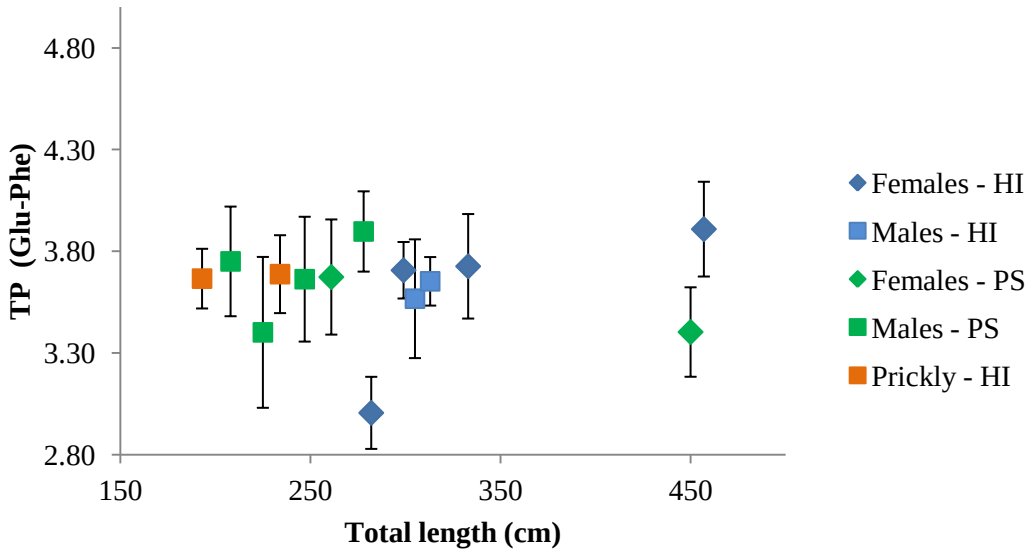



Figure 8. Calculation of trophic position based on glutamic acid and phenylalanine. Using this method, few clear trends in the data were observed except for a trend of higher trophic position with larger size for the Hawaii sixgill sharks only.

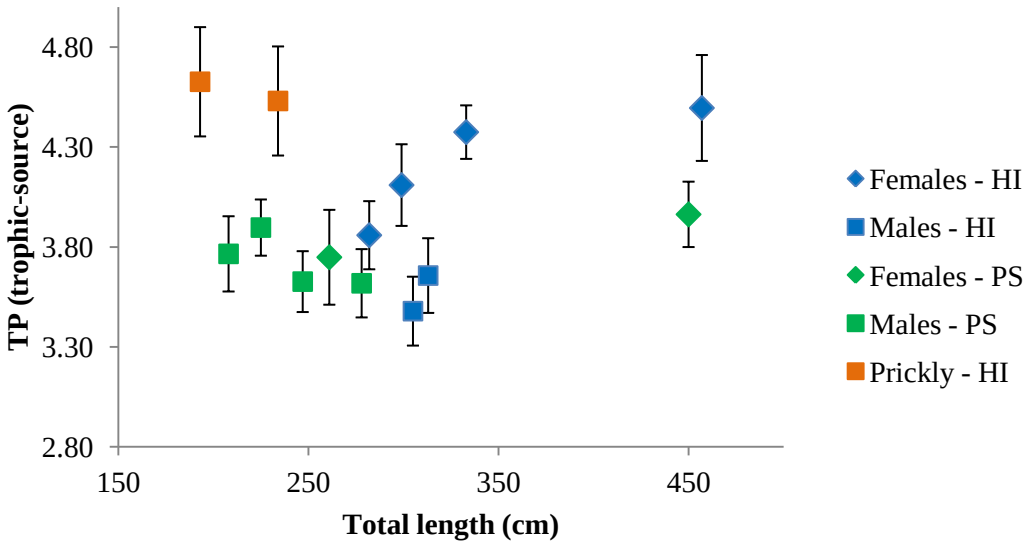



Figure 9. Calculation of trophic position based on three source amino acids (gly, phe, ser) and three trophic amino acids (glu, pro, leu) better elucidates ontogenetic shifts and species differences. Using this method, there is evidence for a trophic shift with increasing size in the Hawaii sixgills, and a smaller shift in the Puget Sound sixgills. Prickly sharks were found to have a high trophic position, in agreement with limited stomach content analysis.

| | Estimate | Standard Error | t value | p value |
|---|---|---|---|---|
| **TL** | -0.005369 | 1.561074 | -0.971 | 0.3863 |
| **Sex*TL** | 0.008685 | 0.003399 | 2.555 | 0.0630* |
| **Location*TL** | -0.003009 | 0.001368 | -2.201 | 0.0926* |
| **Maturity*TL** | -0.005945 | 0.004274 | -1.391 | 0.2366 |

R-sq (adjusted)=0.854
Deviance explained = 94.7%

Table 3. GAM results from a model explaining 94.7% of the variability observed. No factors were significant at α=0.05, but sex and location (including their relationship to total length) appeared to be relatively important.

## 4. DISCUSSION

This study is the first to analyze the muscle isotopic compositions of the deepwater elasmobranchs *Hexanchus griseus* and *Echinorhinus cookei* using AA-CSIA techniques, adding to the knowledge base of deepwater shark isotope ecology (Condon, 2011; Pethybridge, 2010), and it is among the first studies to use AA-CSIA on elasmobranchs. To our knowledge, previous trophic ecology studies on the two species addressed here have not separated specimens based on habitat, though the food web structure may be very different in the wide range of habitats these species inhabit, including temperate, shallow estuaries, along an upwelling zone of the continental shelf, or in a mesophotic oligotrophic system. This study suggests that ontogenetic diet shifts between two habitat types may differ, reveals a higher variability of trophic position in Hawaii versus Puget Sound sixgill sharks, and supports the high trophic position of prickly sharks suggested by limited stomach content analysis.

### 4.1 Bulk Tissue

Removing lipids and urea from bulk tissue samples substantially changed the observed molar C:N ratio, similar to results observed for other large sharks (Hussey et al., 2010). Removal of lipids increased the $\delta^{13}C$ values, and removal of urea increased the $\delta^{15}N$ values. Deepwater sharks tend to be rich in lipids compared to shallower sharks (Pethybridge 2010). In this study, larger sharks tended to have higher C:N ratios indicating higher lipid concentrations. The presence of lipid in the tissue was also visible in artificially low $\delta^{13}C$ values of muscle tissue (Hussey et al. 2010). Removal of lipids shifted the molar C:N ratio to about 3.8, which is consistent with literature values for teleost and elasmobranch protein (e.g. Pethybridge 2010; Wallsgrove 2011). Similarly, urea is depleted in $^{15}N$ compared to protein (e.g. Hussey et al. 2010), and a DI rinse to remove urea increased the $\delta^{15}N$ values, indicating that the urea in the tissue altered bulk tissue $\delta^{15}N$ values.

Rinsed carbon isotopic ratios for bulk tissue of Hawaii deepwater sharks (-15.9‰ to -13.5‰) were enriched in $^{13}C$ compared to particulate organic matter (POM) collected from the depth range of sixgill sharks at Station ALOHA, a site 100km north of Oahu, Hawaii (-26.5‰ to -24.5‰) (Benner et al., 1997; Sannigrahi et al., 2005). If deepwater sharks were feeding mostly on the *in situ* food web based on particulate input from the

upper ocean, the carbon isotopic values should be more similar to that of the POM unless there was an effect of proximity to Oahu. Sixgill sharks were caught on the south and west slope of Oahu, which is in the lee of the island. Isotopic data from POM collected on the leeward island slope would help resolve this question more accurately. Increased importance of scavenging may explain the enrichment in $\delta^{13}C$ values in deepwater sharks, since epipelagic carrion is typically enriched in $\delta^{13}C$ values versus *in situ* benthic prey in deep benthic ecosystems (Benner et al., 1997; Drazen et al., 2008).

Small sixgill sharks feeding in the Puget Sound estuary are likely to have littoral carbon inputs to their food web. In a study of $\delta^{13}C$ values of Puget Sound benthic algae, most measurements ranged between -22‰ to -14‰ (Hellquist and Black, 2004), and these species probably influence $\delta^{13}C$ values in the shark tissue in the typical pattern of higher $\delta^{13}C$ values in littoral versus pelagic food webs (France, 1995). For comparison, resident killer whale pods in Puget Sound had average epidermal $\delta^{13}C$ values of about -16.1 to -15‰ (lipid-rinsed) (Krahn et al., 2007), which is similar to the $\delta^{13}C$ values observed in resident sixgill sharks. Similar isotopic compositions of carbon and nitrogen in killer whales and sixgill sharks resident to Puget Sound suggest that they may share food sources in that ecosystem.

**4.2 Trophic position**

Bulk isotope analysis revealed no correlation between $\delta^{15}N$ values and size, which is inconsistent with stomach content analyses of *H. griseus* off South Africa, which showed ontogenetic shifts at multiple size classes (Ebert 1994). This suggests that the base of the food web in which sixgill sharks feed might change as they grow and move into different habitats, which has been observed for the brown stingray *Dasyatis lata* in Hawaii (Dale et al., 2011). The measurements for source amino acids were variable for Hawaii, but in Puget Sound, the five immature sharks had consistent $\delta^{15}N$ values of source amino acids (6.1-7.7‰) but the only mature shark was apparently feeding in a food web that is depleted in $^{13}C$ relative to that in Puget Sound (4.1‰). This female shark was found carrying pups in Puget Sound, and this supports the suggestion that sixgill sharks may utilize shallow estuaries like Puget Sound as a pupping ground, but mostly spend their mature life in deeper water out on the continental shelf (Andrews et al., 2010; Dunbrack and Zielinski, 2003). Andrews (2010) found a significant positive

relationship between the size of sixgill sharks and their probability of leaving Puget Sound. Many elasmobranchs have different preferred habitats as juveniles than as adults, and may inhabit special "nursery" habitats when young (Cartamil et al., 2010a; Cartamil et al., 2010b; Heupel et al., 2007; Weng et al., 2007), so this result should further caution the use of $\delta^{15}N$ values of bulk tissue to quantify ontogenetic changes in trophic position for highly mobile animals, especially since the source $\delta^{15}N$ values can be very difficult to quantify for wide-ranging species without AA-CSIA.

The isotopic compositions of individual amino acids revealed a significant trend of higher trophic position with increasing size, which was more pronounced for sharks captured in Hawaii than sharks captured in Puget Sound. This ontogenetic shift was not evident in the bulk tissue isotopic data, and underscores the usefulness of the AA-CSIA technique for elucidating trophic shifts. There are several possibilities that could lead to differences in the amount of ontogenetic shift.

The simplest situation is that sharks in Hawaii prey on higher trophic position animals as they grow, while sixgill sharks in Puget Sound maintain a more consistent diet throughout their life. It is possible that live prey is more abundant in the more productive, temperate habitat, and that sixgill sharks can prey on certain preferred prey items consistently throughout their life to maintain most of their energy needs (hake has been noted as a common prey item in sixgill shark stomachs of multiple sizes (Ebert 1994)). The sixgill sharks in the oligotrophic habitat of Hawaii, conversely, may become more reliant on scavenging as they grow. Scavenging on carcasses allows the sharks to consume higher trophic level prey, such as large pelagic fishes and other sharks, and additionally, the community of scavengers associated with a carrion fall (including shrimps, crabs, etc) are likely to have enhanced $^{15}N$ enrichment due to the trophic transfer from high trophic position carrion → small-bodied scavenger, and it is likely that the sharks would ingest some of those organisms as well. Finally, an important sixgill shark prey species, hake, has undergone a 90% population decrease in Puget Sound since 1980, mostly due to fishing and other anthropogenic impacts (Gustafson, 2000). If sixgill shark preferred prey resources are diminished, they may be forced to feed at a lower trophic level despite increasing body size. Alteration of food web resources due to fishery

pressure is a common concern for sixgill sharks throughout their distribution, especially as fisheries exploit deeper and deeper slope habitats.

Despite a much smaller body size, the prickly sharks analyzed in this study had a high relative trophic position based on Method 2 (3 source and 3 trophic amino acids), comparable to the largest sixgill sharks in Hawaii. Stomach content analysis of prickly sharks has been limited, but observations of food items include teleosts, cephalopods, and other elasmobranchs, including young sixgill sharks (Compagno, 1984b). Most of the same prey items have also been found in the more robust sixgill shark stomach content studies, and additionally, sixgill sharks have been observed to prey/scavenge on large pelagic fishes such as swordfish and dolphinfish, seals, whale carrion, and smaller shrimps and crabs (potentially enriched in $^{15}N$ due to scavenging on large organisms) (Cortes, 1999; Ebert, 1986, 1994). Since their diets contain many of the same animals but the trophic position of the prickly sharks was slightly higher, it is possible that prickly sharks feed more heavily in the scavenging food web than the young sixgill sharks, or that high trophic position prey simply makes up a greater proportion of their diet compared to a more generalist strategy in the sixgill shark. Prickly sharks also may feed in shallower water than the sixgill sharks (Chapter 1). Sixgill sharks are likely to forage primarily between 275-325m at night based on vertical activity level data, and may also forage between 550-700m at night. Preliminary movement data for prickly sharks in the same ecosystem suggests that they feed exclusively at night between about 200-300m. There is also evidence that both sixgill sharks and prickly sharks may leave the benthos (Chapter 1; Dawson and Starr, 2009), so they may both have access to food resources in the water column. Sixgill and prickly sharks in Hawaii might have different available prey resources mainly because of the potential deep foraging habitat for sixgill sharks.

The $\delta^{15}N$ values in the source amino acids varied substantially among sixgill sharks and prickly sharks. This observation was particularly surprising in the two prickly shark samples, since movement and behavior data suggests that sixgill sharks are generally more mobile than prickly sharks (Dawson et al., 2009; Andrews et al., 2010; Chapter 1). Based on this movement data, prickly sharks captured in the same location would likely be feeding in the same habitat, but the large difference in the composition of the source amino acids suggests differing food sources. Further study of movement and

food web interactions is needed to clarify the differences between sixgill and prickly shark foraging strategies and locations.

### 4.3 Calculation of trophic positions versus expected values

Compared to estimates of trophic position based on stomach content analysis and EcoPath modeling, the trophic positions calculated for sixgill sharks are slightly lower than expected. For both species, method 2 produced estimates of trophic position that were closer to values based on stomach content analysis (Cortes 1999; Fishbase.org). However, relative trophic positions between sixgill sharks and prickly sharks were in agreement with Fishbase, with the sixgill sharks having a slightly lower trophic position. Calculation of accurate trophic position based on stable isotope analysis has often been problematic in sharks (Hussey et al. 2010, Dale et al. 2011). As discussed in section 1.2, urea retention in sharks can cause bulk tissue to be more depleted in $^{15}N$, but even when urea is removed by rinsing the tissue or extracting only amino acids, the trophic position estimations are still lower than expected (Hussey et al., 2010; Dale et al., 2011). Instead, the importance of urea production may reduce glutamate catabolism, leading to a lower than expected $\delta^{15}N$ value in glutamic acid (Dale et al. 2011). Controlled feeding studies are required to establish accurate TEFs for the final trophic transfer to elasmobranchs, given their unique metabolic pathways.

### 4.4 Implications and future directions

Based on the isotopic composition of amino acids in sixgill sharks, this study has provided support for the ontogenetic shift in diet that was suggested by Ebert (1994) based on stomach content analysis, and revealed trends that were not visible using traditional bulk tissue isotopic analysis. Similarities in trophic position between mature male prickly sharks (~300cm) and mature female sixgill sharks (~450cm) indicate that they could be niche competitors on the Hawaii slope, especially in light of the evidence for overlapping depth ranges for these two species in Hawaii (Chapter 1). A combination of the bulk $\delta^{13}C$ data with the trends observed in AA-CSIA analysis suggest that scavenging could be a more important feeding strategy for sharks inhabiting the deep oligotrophic slope compared to a shallow estuary, and potentially could become more important with larger size.

In future work, a larger number of samples and wider size range of both sixgill and prickly sharks would enable a more robust comparison of the two species and geographic locations. Future studies can also aim to collect samples from more locations within the wide range of sixgill and prickly sharks, including other ocean basins. The trophic ecology results presented here can be used in interpretation of behavioral observations, and contribute to the essential management question of where and how deepwater sharks feed throughout their lifespan and geographic range.

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# SUMMARY AND CONCLUSIONS

Isothermal submergence in low latitudes allows some species to have exceptionally wide geographic ranges while maintaining a suitable thermal habitat (Hubbs, 1952). Following an isotherm north to south across an ocean basin reveals that the depth (pressure), salinity, primary productivity, light regime, prey availability, and many other factors change dramatically. A species that's distribution follows a particular temperature window across latitudes must have adaptations and resilience to wildly different conditions, but may still display behavior patterns driven by similar environmental factors throughout their range. Understanding the biological adaptations to varied conditions and important environmental factors influencing behavior helps to understand the ecology of wide-ranging species on a global scale.

This investigation of the spatial and trophic ecology of sixgill sharks in two distinct habitats contributes to a growing understanding of sixgill shark habitat use across its extensive latitudinal range. By understanding environmental drivers of movement and depth selection, the habitat envelope of sixgill sharks may be estimated throughout its range and be used when considering ecosystem-based management practices or species-specific regulations. Behavior patterns, activity levels, and depth ranges also help define what food resources the sharks may come in contact with, and further investigation of the sharks' trophic ecology will continue to elucidate trends and shifts in foraging throughout the geographic range.

Across the range of potential sixgill shark habitat, there are great variations in temperature stratification, light penetration, oxygen concentration, and productivity. Sixgill sharks can move deeper or shallower to find their preferred temperature regime, but must then adapt to changing light levels, to which they may have an ontogenetic shift in sensitivity, and oxygen concentrations, which are much more limiting in deep water. In the California Current upwelling system, primary productivity is high (Huyer, 1983) and sixgill sharks traverse the euphotic zone (Andrews et al., 2009). In deep oligotrophic water, the same species never enters the euphotic zone and must adapt to a completely different food web based on scavenging and in situ slope productivity from marine snow. These are a sample of the many variables that sixgill sharks must adapt to across their

geographic range, and their plasticity in ecological strategy and tolerance is now better understood.

The environmental variables that appeared to be most important in sixgill shark depth selection and movements are light level and/or a thermal ceiling. Changing light levels drive a very consistent diel vertical migration which coincides quite closely with the Hawaiian mesopelagic boundary community. Sixgill sharks were slightly more active at night than during the day, but potentially forage in both depth regimes. These same patterns were also observed in Puget Sound, but the depth range and activity levels were shallower and slower, with less difference between shallow and deeper rates of movement.

This study successfully utilized amino acid compound-specific isotopic analysis of nitrogen (AA-CSIA) to compare elasmobranch ecology between habitat types and species. The use of this method allowed assessment of the isotopic composition of the integrated base of the food web and a determination of relative trophic position from predator tissue only, which bypasses uncertainty in the contributions of various primary producers to the final isotopic composition of a top predator. This method allows direct comparisons across geographic distance and depth habitats, was useful in identifying trends across size and location, and was consistent with trends identified in stomach content analyses. The use of AA-CSIA elucidated trends in trophic position not visible in bulk tissue isotopic data. This portion of the study provided some support that sixgill sharks in a less productive habitat may rely more on scavenging as a foraging strategy, while they may be selective predators where prey is abundant. Alternatively, high fishing pressure in Puget Sound may lower trophic position in even very large sixgill sharks. Further investigations of trophic positions in other habitats and size classes can increase the understanding of variations in sixgill trophic ecology. To eventually arrive at absolute trophic positions, future research should address the lack of defined trophic enrichment factors specific for elasmobranch metabolic processes.

One of the most surprising and important findings in this study was evidence that sixgill sharks left the slope of Oahu and swam in the pelagic. These are the first reported records of long-distance pelagic movement for sixgill sharks, and may indicate higher degrees of population connectivity than previously realized. Interestingly, the two sharks

which left the Oahu slope were the only two mature sixgills tagged, and were both male, so it is possible that long-distance movements may be related to breeding events. The potential for population connectivity on large spatial scales is critical to conservation efforts, and needs to be evaluated using genetic techniques to determine gene flow between geographically distant populations of sixgill sharks.

In conclusion, this work has investigated some of the adaptive strategies that organisms adopt when changing their depth of occurrence, and added valuable knowledge of sixgill shark ecology and habitat use by assessing environmental drivers of movement, comparative trophic ecology, and revealing the potential for gene flow between distant habitats. There remains much to learn about sixgill shark population connectivity and trophic ecology throughout their range, and future work should also include genetic analysis. The results of this project have increased the scientific understanding of *Hexanchus griseus* and can inform conservation efforts including ecosystem-based management plans and fishery bycatch mitigation.

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**Appendix A. Isotopic measurements of bulk tissue, rinsed bulk tissue, and amino acids for each shark specimen**
*Hexanchus griseus* specimens captured in Hawaii

| | HI-1 | | HI-2 | | HI-3 | | HI-4 | | HI-5 | | HI-6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bulk $\delta^{13}C$** | -19.6 | | -17 | | -16.8 | | -17.3 | | -17.6 | | -17.7 | |
| **Bulk $\delta^{13}C$ (rinsed)** | -15.9 | | -14.3 | | -15.2 | | -14.5 | | -13.5 | | -14.9 | |
| **Bulk $\delta^{15}N$** | 12.1 | | 14.8 | | 12.0 | | 15.4 | | 16.2 | | 12.6 | |
| **Bulk $\delta^{15}N$ (rinsed)** | 13.4 | | 16.1 | | 14.1 | | 17.6 | | 12.4 | | 17.3 | |
| | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** |
| **Alanine** | 27.10 | 0.37 | 26.78 | 0.40 | 25.49 | 0.63 | 25.54 | 0.14 | 27.41 | 0.36 | 26.51 | 0.19 |
| **Glycine** | 1.15 | 0.24 | 3.15 | 0.29 | 0.19 | 0.49 | 7.67 | 0.81 | 5.73 | 0.17 | -0.25 | 0.17 |
| **Threonine** | -38.37 | 0.65 | -28.48 | 0.24 | -36.48 | 0.02 | -22.65 | 1.57 | -28.00 | 0.45 | -38.83 | 0.32 |
| **Serine** | -1.28 | 0.33 | 2.08 | 0.63 | 2.27 | 0.10 | 5.41 | 0.72 | 7.38 | 0.79 | -3.63 | 0.30 |
| **Valine** | | | 24.74 | 0.45 | 20.73 | 0.91 | 22.97 | 0.85 | 24.44 | 0.44 | | |
| **Leucine** | 25.67 | 0.23 | 24.25 | 0.18 | 23.81 | 0.13 | 23.49 | 0.71 | 24.79 | 0.58 | 23.07 | 0.17 |
| **Isoleucine** | | | 25.22 | 0.59 | 24.59 | 0.46 | 24.57 | 0.42 | 25.14 | 0.85 | | |
| **Proline** | 24.65 | 0.44 | 25.67 | 0.36 | 23.93 | 0.05 | 23.42 | 0.34 | 24.87 | 0.76 | 22.61 | 0.33 |
| **Aspartic acid** | 23.80 | 0.23 | 22.97 | 0.03 | 21.90 | 0.22 | 22.27 | 0.45 | 20.41 | 0.35 | 22.78 | 0.28 |
| **Methionine** | 16.03 | 0.21 | 13.31 | 0.51 | 13.73 | 0.75 | 14.69 | 0.73 | 11.34 | 0.27 | 10.34 | 0.35 |
| **Glutamic acid** | 29.18 | 0.34 | 28.29 | 0.09 | 25.24 | 0.16 | 26.80 | 0.46 | 27.12 | 0.41 | 27.65 | 0.08 |
| **Phenylalanine** | 3.67 | 0.36 | 4.31 | 0.17 | 6.59 | 0.42 | 3.89 | 0.43 | 3.56 | 0.28 | 3.53 | 0.67 |
| **Tyrosine** | | | 12.37 | 0.59 | 10.89 | 0.46 | 10.76 | 0.33 | -2.99 | 0.06 | | |
| **Lysine** | 5.04 | 0.14 | 8.54 | 0.32 | 5.05 | 0.03 | 6.57 | 0.29 | 8.11 | 0.24 | 5.91 | 0.29 |
| **Arginine** | | | | | | | 11.78 | 0.02 | | | 13.23 | 0.36 |
| **Sex** | F | | F | | F | | M | | M | | F | |
| **Total Length (cm)** | 457 | | 299 | | 282 | | 305 | | 313 | | 333 | |

*Hexanchus griseus* specimens captured in Puget Sound

| | PS-1 | | PS-2 | | PS-3 | | PS-4 | | PS-5 | | PS-6 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Bulk $\delta^{13}C$** | -19.8 | | -12.5 | | -18.7 | | -15.7 | | -12.7 | | -11.9 | |
| **Bulk $\delta^{13}C$ (rinsed)** | -15.3 | | -13.1 | | -15.2 | | -13.2 | | -12.2 | | -12.4 | |
| **Bulk $\delta^{15}N$** | 13.3 | | 16.7 | | 15.9 | | 16.7 | | 16.5 | | 16.2 | |
| **Bulk $\delta^{15}N$ (rinsed)** | 16.5 | | 17.5 | | 14.1 | | 16.5 | | 16.5 | | 16.2 | |
| | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** |
| **Alanine** | 27.71 | 0.15 | 30.34 | 0.13 | | | 29.05 | 0.18 | 29.06 | 0.46 | 29.59 | 0.54 |
| **Glycine** | 8.26 | 0.72 | 7.10 | 0.04 | 3.49 | 0.10 | 9.38 | 0.31 | 7.66 | 0.24 | 8.93 | 0.46 |
| **Threonine** | -25.68 | 0.88 | -23.14 | 0.28 | -39.61 | 0.29 | -24.18 | 0.36 | -24.80 | 0.27 | -24.78 | 0.77 |
| **Serine** | 6.31 | 0.90 | 6.55 | 0.32 | 2.46 | 0.46 | 7.08 | 0.67 | 6.45 | 0.72 | 7.91 | 0.39 |
| **Valine** | 22.53 | 0.36 | 22.02 | 0.67 | | | 25.69 | 0.47 | 25.40 | 0.99 | 25.98 | 0.56 |
| **Leucine** | 24.34 | 0.15 | 27.91 | 0.30 | 24.87 | 0.29 | 24.92 | 0.43 | 25.82 | 0.59 | 26.22 | 0.70 |
| **Isoleucine** | 25.00 | 0.04 | | | | | 25.48 | 0.41 | 27.15 | 0.63 | 26.86 | 0.25 |
| **Proline** | 28.09 | 1.10 | 28.74 | 0.19 | 25.32 | 0.28 | 25.51 | 0.21 | 26.41 | 0.51 | 26.52 | 0.13 |
| **Aspartic acid** | 22.74 | 0.54 | 26.58 | 0.12 | 22.46 | 0.08 | 23.93 | 0.27 | 24.63 | 0.83 | 24.99 | 0.45 |
| **Methionine** | 13.38 | 0.72 | 14.80 | 0.21 | 14.02 | 0.05 | 12.16 | 0.63 | 14.48 | 0.46 | 14.62 | 0.82 |
| **Glutamic acid** | 28.13 | 0.60 | 30.39 | 0.04 | 28.06 | 0.36 | 29.69 | 0.26 | 30.04 | 0.37 | 29.84 | 0.31 |
| **Phenylalanine** | 3.83 | 0.35 | 8.74 | 0.00 | 6.39 | 0.61 | 4.26 | 0.83 | 6.32 | 0.20 | 6.20 | 0.69 |
| **Tyrosine** | 11.83 | 0.03 | | | | | | | | | | |
| **Lysine** | 7.94 | 1.03 | 8.41 | 0.09 | 3.78 | 0.33 | 7.96 | 0.36 | 8.78 | 0.16 | 8.81 | 0.52 |
| **Arginine** | | | | | 10.41 | 0.45 | | | 14.63 | 0.12 | 12.22 | 0.60 |
| **Sex** | M | | M | | F | | M | | F | | M | |
| **Total Length (cm)** | 208 | | 225 | | 450 | | 278 | | 261 | | 247 | |

*Echinorhinus cookei* specimens captured in Hawaii

| | EC-1 | | EC-2 | |
|---|---|---|---|---|
| **Bulk $\delta^{13}C$** | -15.5 | | -15.6 | |
| **Bulk $\delta^{13}C$ (rinsed)** | -15.0 | | -14.4 | |
| **Bulk $\delta^{15}N$** | 18 | | 12.3 | |
| **Bulk $\delta^{15}N$ (rinsed)** | 12.8 | | 15.9 | |
| | **$\delta^{15}N$** | **St. Dev** | **$\delta^{15}N$** | **St. Dev** |
| **Alanine** | 27.30 | 0.73 | 24.22 | 0.19 |
| **Glycine** | 3.64 | 0.14 | -2.89 | 0.23 |
| **Threonine** | -29.97 | 0.55 | -38.96 | 0.42 |
| **Serine** | 0.83 | 0.84 | -4.19 | 0.61 |
| **Valine** | 26.48 | 0.62 | | |
| **Leucine** | 25.62 | 0.80 | 21.74 | 0.23 |
| **Isoleucine** | 19.12 | 0.42 | 19.06 | 0.00 |
| **Proline** | 31.65 | 0.59 | 25.96 | 0.22 |
| **Aspartic acid** | 22.35 | 0.25 | 19.70 | 0.05 |
| **Methionine** | 12.01 | 0.63 | 7.63 | 0.89 |
| **Glutamic acid** | 29.38 | 0.35 | 26.01 | 0.09 |
| **Phenylalanine** | 5.55 | 0.18 | 2.35 | 0.21 |
| **Tyrosine** | -5.85 | 0.23 | -5.80 | 0.00 |
| **Lysine** | 9.80 | 0.33 | 4.65 | 0.50 |
| **Arginine** | 14.25 | 1.42 | | |
| **Sex** | M | | M | |
| **Total Length (cm)** | 234 | | 193 | |