DIET COMPOSITION AND ONTOGENETIC SHIFTS OF SKIPJACK TUNA (*KATSUWONUS PELAMIS*) IN HAWAIʻI INFERRED FROM STOMACH CONTENTS AND STABLE ISOTOPE ANALYSES

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ABSTRACT

Skipjack tuna (*Katsuwonus pelamis*) contribute high socio-economic value to tuna fisheries in the Pacific Ocean. Understanding their feeding ecology and the food webs that support them is critical to predict how environmental variability will affect their stocks. Skipjack tuna are opportunistic predators and consume a wide range of prey, including fish, squid, and crustaceans. Previous studies have found dietary differences across oceanographic domains, with varying regional importance of fish and crustaceans. Additionally, studies have documented ontogenetic diet shifts, where different sizes of tuna consume different prey. This is the first study describing the diet composition of skipjack tuna, ranging from 40 to 84 cm fork length (FL), in Hawai‘i using stomach-contents and stable isotope analyses. Tuna stomach contents were classified into three broad groups: fish, squid, and crustaceans. DNA barcoding was used to identify prey to the lowest taxonomic level possible (family, genus or species). From the stomach-contents, a species richness of 24 prey taxa (16 fishes, 5 crustaceans, 3 mollusks) was described, with the majority of the species diversity consisting of reef-associated fishes. However, the most important prey group were crustaceans with an index of relative importance (IRI) of 78.4%. Using time-integrated diet estimates from Bayesian isotope mixing models, two crustacean functional groups together contributed the highest proportion (61.1%) to the diet. The complementary approaches of stomach-content and stable isotope analyses demonstrated crustaceans dominate the diet of skipjack tuna around Hawai‘i. This study did not detect an ontogenetic diet shift for skipjack tuna based on their $\delta^{15}N$ values. However, skipjack tuna showed a significant change in their carbon source at a size of 68.8 cm fork length. Based on this $\delta^{13}C$ difference, this result indicates that small (< 68.8 cm FL) and large (> 68.8 cm FL) skipjack are supported by different food webs and likely foraging at different locations.

HAWAI'I PACIFIC

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by

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This thesis is submitted in partial fulfillment of the requirements for the degree of Master of Science in Marine Science at Hawai'i Pacific University. We the undersigned have examined this document and have found that it is complete and satisfactory in all respects, and all revisions required by the final examining committee have been made.

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Skipjack (*Katsuwonus pelamis*) and Yellowfin (*Thunnus albacares*) Tuna Distribution and Foraging Ecology in the Pacific Ocean

Introduction

Tuna species (Family Scombridae*,* tribe Thunnini) have global socio-economic value to fisheries and contribute towards food security and trade income to island nations, and coastal communities (FAO, 2022; Miyake & Nakano, 2004; Miyake et al. 2010). Tuna species are targeted by industrial purse-seine, pole-and-line, and longline fisheries that contribute the majority of global capture fisheries production (FAO, 2020; Miyake & Nakano, 2004). While tunas are widely distributed, occurring in every ocean basin, and ranging from tropical to temperate regions, the Pacific Ocean provides the highest yields of global tuna production (Ely et al., 2005; FAO., 2022; Lehodey et al., 1997). Pacific tuna fisheries target five species in the Thunnini tribe (skipjack, yellowfin, bigeye, albacore, and bluefin), with skipjack tuna being the predominant species caught (FAO., 2022; ISSF., 2021). In fact, skipjack tuna is the top pelagic fish caught worldwide.

In 2020, skipjack and yellowfin tuna were the top third and fifth producing wild fish species, with a catch of 2.8 and 1.5 million tonnes, respectively (FAO, 2022). In the Pacific Ocean, skipjack and yellowfin tuna provide the first and second largest tuna yields (Williams & Terawasi, 2010; ISSF., 2021). Over the years, fisheries have developed increasingly sophisticated methods (deep longlines, purse-seines, fish aggregating devices) to catch tuna, and have expanded their fishing ranges, resulting in higher catches. When the increased fishing effort is considered, stock assessments have determined that skipjack and yellowfin stocks are being fished sustainably because exploitation rates are below maximum sustainable yields and their biomass remains above an exploited limit (Brouwer et al., 2018; Hare et al., 2019). However, yellowfin was previously exploited in the Western and Central Pacific Ocean in 2009, potentially

due to a reduced spawning biomass or high fishing mortality rates (Bailey et al., 2013; Langley et al., 2009). Although skipjack and yellowfin tuna are high-producing species and reach maturity faster than other tuna species, their productivity is influenced by oceanographic conditions (Brock, 1954; Sun et al., 2005; Uchiyama & Struhsaker, 1981).

The year-to-year variability of the climate and the ongoing greenhouse warming raises concerns on the future sustainability of tuna stocks (Bell et al., 2021; Lehodey et al., 2013). Climate models predict that sea surface temperatures will increase in the Pacific Ocean due to the expansion of the Western Pacific warm pool by 250% in areal extent by 2035 (Ganachaud et al., 2011; Lehodey et al., 2013). Furthermore, the biomass of skipjack and yellowfin tuna is expected to decline by 13% around Pacific Small Islands Developing States' EEZ's by 2050, due to tuna redistribution from greenhouse gas emission effects (Bell et al., 2021). Eventually, sea surface temperatures will be too warm for tuna spawning grounds and productivity will suffer (Wexler et al., 2011).

Tuna species are expected to decline due to unsuitable oceanographic conditions and likely overfishing on declining stocks (Wexler et al., 2011). Moreover, ecosystem modelers expect changes to cascade through pelagic marine food webs, and as a result, reduce the abundance of these top pelagic predators (Hunsicker et al., 2012). Due to future potential biotic and abiotic perturbations, it is important to better understand the life history and feeding ecology of the most abundant tuna in the Pacific Ocean, skipjack and yellowfin. These tunas are the top two abundant species in the Pacific and are often found foraging together. To date, more studies have focused on understanding the distributions and diet of yellowfin tuna. Since yellowfin and skipjack share

similar life history traits and inhabit shared spaces, yellowfin tuna can be an indicator to better understand and predict the ecology of skipjack tuna.

The purpose of this literature review is to compare the distributions and foraging ecology of skipjack tuna (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) in the Pacific Ocean. First, I will describe the life history and behavior of these two species, focusing on the horizontal and vertical distributions of different age classes (larvae, juvenile, and adults). Next, I will summarize and compare the published knowledge of the diet and ontogenetic shifts of these two tuna species. Then, I will discuss the advantages / disadvantages of various methods used to describe pelagic fish diets and will address their potential biases. Lastly, I will identify some unresolved issues I plan to address in my thesis project.

Tuna Physiological Traits

Tuna have unique physiological and morphological features that influence the way they interact with the environment. Some key characteristics include a fusiform body shape, posterior finlets, thunniform swimming, high metabolic rates, high aerobic capacity, and endothermic abilities (Graham & Dickson, 2004; Korsmeyer & Dewar, 2001). These features make tuna high-performance swimmers, and are the underlying influence of their movement patterns, habitat utilization, and foraging mechanisms. Tuna species are the only teleost with thunniform swimming, characterized by propulsion focused on the undulation of the caudal fin with minimal body movement (Syme & Shadwick, 2002). Furthermore, the posterior finlets give tuna additional propulsion by reducing drag and improving the flow of water across their caudal peduncle (Wang et al., 2020). As highly mobile fish, their locomotory muscles require high

aerobic metabolism (Bertrand et al., 2002; Estess et al., 2014). In the process, these muscles produce heat and allow tunas to maintain their internal temperature higher than the surrounding water. By freeing tuna from thermal constraints, endothermy increases their vertical and horizontal range (Figures 1; Block et al., 1993). Their distinct physiological and morphological characteristics play a crucial role in shaping their habitat range and distribution (Graham & Dickson, 2004).

Oceanographic Properties Influence on Tuna

Although tuna have traits and adaptations that drive their foraging, migrations, and distributions, their abundance is influenced by oceanographic conditions like ambient oxygen levels, sea surface temperatures, and oceanic fronts (Block et al., 1993; Lehodey et al., 1997). The cost of being highly mobile fishes includes a high aerobic capacity, and therefore tuna distribution is driven by ambient oxygen levels. The maximum oxygen consumption for skipjack and yellowfin is 2500 and 2200 mg O2/kg/h, respectively (Dewar & Graham, 1994; Gooding et al., 1981). Studies have shown a difference in hypoxia tolerance between tuna species, where yellowfin are more tolerant to hypoxia than skipjack tuna (reviewed in Pecoraro et al., 2017). Yellowfin tuna can withstand hypoxic levels for prolonged periods because they can maintain oxygen consumption at a lower partial pressure of oxygen (90-50 mmHg) than skipjack (130-90 mmHg) (Bushnell & Brill, 1992). This tolerance creates a vertical niche separation between species based on their hypoxic tolerance, as yellowfin perform larger vertical migrations into deeper depths with reduced oxygen levels.

In addition, sea temperatures and dissolved oxygen also define tuna species' vertical range. Skipjack and yellowfin tuna are surface-schooling tuna highly abundant in warm waters (>24℃), with minimum water temperature of 18℃ and dissolved oxygen thresholds of 3.5 ml/L (skipjack) and 2.0 ml/L (yellowfin) (Barkley et al., 1978; Sharp, 2012). Tuna aggregation areas are driven by boundaries of water masses. Fronts, convergence zones, and upwelling zones consist of high nutrients that support primary production and indirectly create an aggregation area for tuna (Lehodey et al., 1997). For example, the Western Pacific warm pool is a region characterized as oligotrophic, low primary production, low salinity, and sea surface temperatures above 24℃ (Barkley et al., 1978; Lehodey et al., 1997; Reglero et al., 2014). However, the warm pool sustains the highest biomass of tuna because of the convergent zone located in the eastern edge of the warm pool (Ganachaud et al., 2011; Lehodey et al., 1998). The convergence zone on the eastern edge where the sea surface temperatures are 29℃ and chlorophyll a levels are 0.17 ± 0.03 mg m^{-3}, while sea surface temperatures are above 30℃ and chlorophyll a levels are lower (0.08 ± 0.02 mg m^{-3}) (Radenac et al., 2013).

Another aggregation area for tuna is the tropical Eastern Pacific Ocean (EPO), characterized by nutrient-rich and cold sea surface temperatures due to upwelling, which supports elevated primary productivity (Lezama-Ochoa et al., 2019; Pennington et al., 2006; Schaefer, 2001). While elevated primary production does not necessarily attract upper-level predators, it stimulates secondary production of the nektonic prey that tuna prey upon (Lehodey et al., 1998). For instance, coastal upwelling along Mexico generates plumes of nutrient-rich surface waters. Additionally, the northward deflection of the Equatorial Counter-Current combines with the

Costa Rica Current to create the Costa Rica Dome, a region with a shallow thermocline and sea surface temperatures between 25 and 28°C (De Anda-Montañez et al., 2004; Fiedler, 2002).

Tuna Abundance in the Pacific Ocean

Understanding how tuna distributions across the Pacific Ocean shift temporally is crucial for the operation of fisheries. Most tuna species have similar horizontal distribution patterns relating to their life cycle: seasonal latitudinal shifts and the ontogenetic return to spawning grounds at maturity (Kiyofuji et al., 2019; Schaefer et al., 2007). However, tuna species differ in their vertical space range and their habitat utilization in relation to foraging behaviors.

Skipjack and yellowfin tuna are abundant across the tropical Pacific Ocean and managed by two separate stocks, the Western and Central Pacific Ocean (WCPO) and the Eastern Pacific Ocean (EPO). Many studies identify tuna to be highly abundant in the Western Pacific warm pool because it is a region of favorable oceanographic features for the sustainability of tuna stocks, causing catches to be significantly higher (Ashida, 2020). In the WCPO, over 2 million tonnes of skipjack tuna and 722 thousand tonnes of yellowfin tuna were caught in 2019 and 2020, respectively (IATTC, 2021). While in the EPO, 365 and 254 thousand tonnes of skipjack tuna and yellowfin tuna were caught in 2021 (IATTC, 2021).

Larval Tuna Distribution

Tropical tuna species, like skipjack and yellowfin, spawn in tropical regions year-round because these regions provide restricted environments for the survivability for eggs and larval development (Muhling et al., 2017; Schaefer, 2001). During this early stage for a tuna life cycle,

most of their energy budget is spent towards growth (Aoki et al., 2020). Wexler et al. (2011) experimentally determined the optimal temperature window for yellowfin egg and larval survivability is between 26℃ and 30℃. Temperatures that were below 21°C and above 33°C were lethal to the yolk-sac and larval stage. Like yellowfin, skipjack also spawn in warm water and happen to share spawning grounds (Reglero et al., 2014). The optimal temperature window for skipjack larvae growth and development ranges from 24℃ to 30℃ (Lehodey et al., 1997; Reglero et al., 2014; Strasburg, 1960; Schaefer, 2001). The Western Pacific warm pool is an important spawning ground for tuna because water temperatures in this region are above 24℃. When the warm pool is spatially displaced due to the downstream effects of El Nino Southern Oscillation (ENSO), skipjack tuna schools respond by shifting eastwards with the warm pool during ENSO periods (Lehodey et al.,1997). This association further depicts the strong dependency for larvae and mature adults on the warm pool, a crucial spawning and foraging habitat.

Two studies described the vertical distribution of larval skipjack and yellowfin in the Hawaiian archipelago (Boehlert & Mundy, 1994; Strasburg, 1960). Using plankton tows, larvae (less than 11 mm) were collected during 24-hour periods at various depths. Studies have suggested larval skipjack tuna abundance is higher at night in shallow waters between the surface and 60 meters (Strasburg,1960), 50 meters (Matsumoto, 1958), and 40 meters (Boehlert & Mund, 1994). Yellowfin larvae showed no diel pattern and are most abundant in the upper 20 m (Boehlert & Mund, 1994). Tuna larvae for both species were observed in warm water layers restricted to the upper mixed layer and a 24℃ isotherm, as predicted by Margulies et al. (2007) and Wexler et al. (2011). Interestingly, because both tuna species were less abundant in shallow waters during the

day up to sunset, they were predicted to be following the vertical-migrating deep scattering layer for foraging opportunities. As tuna continue to grow, we start to observe a species-specific separation in their vertical patterns.

Juvenile Tuna Distribution

The duration of the larval stage is approximately 12 to 30 days, and larval individuals are considered juveniles when they reach about 13 mm standard length (Kaji et al., 1999; Murua et al., 2017). Studies of juvenile tuna distributions are limited because individuals > 13 mm SL become more challenging to catch due to their enhanced vision and swimming ability (Tanabe et al., 2001). The vertical distribution of juvenile skipjack tuna (10 to 100+ mm SL) was explored in the Western Equatorial Pacific Ocean using midwater trawls (Tanabe et al., 2017). Compared to the larval optimal temperature of >24℃ (Strasburg, 1960), the temperature window for juveniles expands to 20-30℃. Tanabe et al. (2017) observed a year-to-year variation in skipjack vertical distribution as a response to thermocline shifts. In 1992 and 1993, skipjack juveniles were collected at shallow depths (40-80 m), in relation to a shallow thermocline. During 1995 and 1996, skipjack juveniles were collected deeper in the water column (40-100 m), in response to a deeper thermocline. In the tropical Western Pacific, juvenile skipjack (5.5-171.6 mm SL) and *Thunnus* spp. (8-148.8 mm SL) occur at a high abundance between 40 to 120 m and from the surface to 80 m, respectively (Tanabe et al., 2001). The depths for juvenile skipjack correspond to temperatures of 20-28℃ and the depths of *Thunnus* spp. correspond to the mixed layer (28℃). These results suggest that although the range of temperature in which juvenile skipjack tuna are found is broader than the larval stage, temperature still drives the vertical distribution of juveniles, which is influenced by the depth of the thermocline.

In addition, in the Western Pacific Ocean, near the Philippines, archival tags were used to track the vertical behavior of juvenile yellowfin (Mitsunaga et al., 2013). Juvenile yellowfin (20.5-24 cm FL) exhibited a diurnal vertical pattern, where they were present in shallow areas during the night and deeper during the daytime (maximum depth = 159 m). Another study analyzed the movement of 5 juvenile yellowfin (52.5-92 cm FL) near Japan (Matsumoto et al., 2013). The behavior of each yellowfin varied by day, but overall, they exhibited a diurnal pattern with a vertical range between the surface and 150 meters, corresponding to the depth of the thermocline. This behavior was seen in yellowfin (35 - 81 cm FL) near Taiwan, who were also in deeper water (> 40 m) during the day than at night, reaching a maximum depth of 250 meters (Weng et al., 2013). Juvenile skipjack and yellowfin inhabit a similar vertical range, in relationship to the depth of the thermocline. As tuna develop from larvae to juveniles, their vertical habitat expands because they acquire physiological capabilities and are no longer constrained by the 24°C isotherm (Tanabe et al., 2001; Tanabe et al., 2017; Wexler et al., 2011).

Adult Tuna Distribution

Yellowfin and skipjack tuna reach maturity when they are about 112.5 cm and 40-45 cm fork length (FL), respectively (Itano, 2000). The maximum length of yellowfin ranges from 148 to 200 cm and skipjack ranges from 60 to 141 cm fork length (Murua et al., 2017). By the time tunas reach maturity and their adult size, their endothermic ability allows them to forage in deep cold waters (Neill et al., 1976). In the Eastern Equatorial Pacific Ocean, 20 yellowfin off Baja California remained in temperatures above 18.3°C (Schaefer et al., 2007). Yellowfin in this study (60-135 cm fork length) exhibited four distinct diving behaviors: type I) they remained in between 0-50 meters during the night and 0-100 meters during the day, type II) they remained

between 0-50 meters during the night and 50-300 meters during the day, type III) deep-diving, and type IV) surface-oriented. The mean percent of days that were classified as a type 1 and type 2 for individual fish was 78.1% and 21.2%, respectively. On average, a few yellowfin (> 92 cm FL) exceeded depths greater than 1000 meters (maximum depth = 1160 m). Their diving below the surface suggests that adult yellowfin is foraging at or below the thermocline during the day.

A tag study near the Main Hawaiian Islands (MHI) examined the vertical distribution of 5 adult yellowfin (146-167 cm FL) and found they spent most of their time in shallow depths (< 100 m), with a maximum depth of 270 m (Brill, 1999). In a recent study around the MHI, 19 yellowfin (149-188 cm FL) mainly occupied the surface mixed layer (0-100 m, 27-28°C), during the day they were at depths < 190 m and at night < 88 m, while the maximum depth reached was 1,592 m (Lam et al., 2020). While yellowfin can withstand colder water temperatures and lower oxygen levels, these studies, in aggregate, indicate yellowfin do not regularly take advantage of their endothermic abilities.

The distribution and movement patterns of 5 skipjack (66-69 cm length) and 5 yellowfin (51-60 cm length) tuna were observed in the Eastern Pacific Ocean (Schaefer et al., 2009). An adult skipjack (66 cm size) tuna depicted a bouncing behavior and a diurnal vertical cycle. During the night, it remained in shallow water above the 20°C isotherm. During the day, it moved below that isotherm and consistently “bounced” between 50 to 350 meters depth. The deepest dive by a skipjack tuna was 595 m, with an internal peritoneal temperature lowered to 22°C in an ambient temperature of 10°C. In contrast, an adult yellowfin (52 cm size), remained in shallow waters above the 20°C isotherm at night, and in the daytime exhibited a bounce diving behavior similar

to skipjack tuna, ranging between the surface and 300 m. The deepest dive by yellowfin tuna was 1022 m, with the peritoneal temperature lowered to 14°C in an ambient water temperature of 6°C. This study recorded different vertical depth use by adult skipjack and yellowfin tuna. Overall, yellowfin tuna depicted a greater vertical distribution than skipjack because their endothermic abilities allowed them to tolerate deeper and colder depths (Schaefer et al., 2009).

Through their life, skipjack and yellowfin tunas have similar vertical behavioral patterns as they develop from larvae to juveniles to adults. Larvae of both species are constricted to surface waters of a narrow temperature range (Margulies et al., 2007; Schaefer, 2001; Strasburg, 1960; Wexler et al., 2011). The vertical distribution of juveniles expands from the surface to below the thermocline (0 to 250 meters) (Tanabe et al., 2017; Mitsunaga et al., 2013; Weng et al., 2013). Once they reach maturity, tuna gain endothermic abilities capable of deep diving to cold water temperatures and further expand their vertical habitat (Neill et al., 1976; Schaefer et al., 2009).

Diet Analysis Methodologies

Because species in the Thunnini tribe have high aerobic metabolic rates, they have high energetic requirements and feed on a wide variety of prey (Bertrand et al., 2002; Graham & Dickson, 2004). Therefore, tunas are considered opportunistic predators, whose prey consists of crustaceans, teleost fish, cephalopods, and other pelagic mollusks. The diversity and composition of their prey changes, as tuna expand their vertical distribution.

Studies have associated the vertical distribution and foraging behavior yellowfin and skipjack, with three consistent patterns: (i) bounce diving (frequent dives from the surface to the thermocline), (ii) crepuscular feeding (deep diving at dusk and dawn), and (iii) diurnal cycles

(deep diving during the day to the thermocline) (Bertrand et al., 2002; Matsumoto et al., 1984; Kitagawa et al., 2007; Kitagawa et al., 2010). These studies underscore that tuna behavioral patterns and diet are inextricably linked. Thus, intraspecific differences across size classes and interspecific differences in tuna diet reflects their habitat-specific prey distributions (Bertrand et al., 2002).

Tuna diet has been analyzed using a variety of methods:

(i) Stomach gut content analysis of dead fish (via necropsy) and live fish (via stomach flushing), followed by stomach fullness quantification, and morphological identification of prey taxa by presence/absence (frequency of occurrence), and relative abundance (number and volume/mass).

(ii) Genetic analysis of gut contents (metabarcoding of whole gut contents - dDNA - and barcoding of individual specimens) to identify new prey items or improve prey taxonomic classification of prey that cannot be identified morphologically.

(iii) Stable isotopes to determine the trophic level of individuals in the food web and estimate the relative importance of prey sources over a longer time scale using isotope mixing models.

Each method used to analyze fish diet has its advantages and disadvantages, therefore, many studies use a combination of methods. Literature reviews have examined and scrutinized gut content methods (Hyslop, 1980; Amundsen & Sánchez-Hernández, 2019). For example, Hyslop (1980) and Amundsen and Sánchez-Hernández (2019) agree that stomach fullness is a subjective method because it is based on a visual interpretation and assigning a relative score of fullness.

Furthermore, stomach fullness does not account for the size or amount of each prey taxa. Prey occurrence (presence/absence) is a fast method, but it fails to give the relative amount of prey taxa. Prey abundance is also a fast method by measuring the relative amount of prey taxa, but it may overemphasize small prey. Prey volume and mass account for the bulk of each prey, but are time consuming and disregard small, digested prey. Therefore, researchers use several indices capable of incorporating frequency of occurrence and relative abundance to combine diet metrics to understand the importance of prey taxa, known as index of relative importance.

Feeding Ecology of Yellowfin Tuna

The diet of yellowfin tuna has been well-studied worldwide since the early 1950s (Reintjes & King, 1953). Near French Polynesia, the prey taxa found in yellowfin stomach contents consisted of fishes (myctophids, reef-fishes, piscivorous fishes), crustaceans, cephalopods, and gelatinous organisms (Bertrand et al., 2002). Bertrand et al. (2002) observed a spatial difference in prey across three sites: 1) the zone closest to the islands had the highest abundance of juvenile reef-fishes, 2) the only zone where myctophids were consumed, and 3) the zone further north of the islands had the highest proportion of piscivorous fishes. In a separate study, the highest proportion of prey was also fish across three locations in the Pacific Ocean - New Caledonia, New Guinea, and French Polynesia (Allain, 2005). The type of fishes consisted of surface fishes (e.g., flying fishes, juvenile skipjack tuna, juvenile reef-fishes) and deep-sea fishes (e.g., lancetfish), indicating that yellowfin foraging grounds consist of surface and deep-water prey. By identifying prey functional groups, such as epipelagic, mesopelagic, bathypelagic, juvenile reef or coastal species, this allows us to gain an understanding of their foraging strategies and how they can change by age and region (Allain, 2004).

As opportunistic foragers, yellowfin utilize regional prey availability, but once they reach a certain age or size, they have shown to shift their prey selectivity, known as an ontogenetic diet shift. The diet of juvenile yellowfin tuna was analyzed around Hawai‘i using stomach contents and stable isotopes (Graham et al., 2007). The diet of smaller fish (20-49.9 cm FL) contained a higher relative abundance and volume of crustaceans: Stomatopoda (larvae) and Decapoda (megalopae). The prey taxa for the larger fish (> 50 cm FL) consisted of epipelagic fishes, reef-associated teleosts, and Oplophorids - midwater shrimp. A shift in diet was evident after the yellowfin reached 45-50 cm FL, supported by a significant increase of nitrogen ($\delta^{15}N$) isotope composition of 5‰ (Graham et al., 2007). Nitrogen 15 is a stable isotope that infers the trophic level of an individual in the food web and traces long-term assimilated diet in a predator. Similarly, stomach contents of yellowfin tuna near Taiwan also portrayed a diet shift at 50 cm FL, where small tuna primarily feed on crustaceans and squid, and larger tuna primarily feed on teleost fish (Weng et al., 2015). Stable isotopes further confirmed $\delta^{15}N$ values of white muscle tissue were significantly lower in tuna < 50 cm FL than tuna > 50 cm FL. Moreover, these studies highlight the value of comparing results from several diet methodologies (stomach contents and stable isotopes) to confirm patterns.

In addition, the stomach contents of 339 juvenile yellowfin (41.1-75.8 cm FL) were analyzed and the predominant prey group was teleost fish, *Auxis* spp. (Varela et al., 2017). A variation of prey composition was found across 3 tuna size classes: class I (≤ 50 cm FL), class II (50-60 cm FL), class III (≥ 60 cm FL), reflecting how their feeding behaviors can relate to their size. Yellowfin in class I mainly consumed deep-water fish (myctophids and jumbo squid), suggesting that they forage in deep waters. Yellowfin in class II and III mainly consumed epipelagic fish (bullet and

frigate tuna), suggesting that they feed in shallow waters. Furthermore, the results in this study support an ontogenetic shift of juvenile yellowfin at 50 cm and 60 cm FL, close to the threshold size of 45 and 50 cm found in Graham et al. (2007) and Weng et al. (2015). These studies support the prediction that endothermy allows larger-sized tunas to expand their foraging niche by diving in deeper water or to feeding at higher trophic levels.

Feeding Ecology of Skipjack Tuna

The diet of skipjack tuna, like yellowfin, occupy similar foraging grounds and feed on crustaceans, cephalopods, and teleost fishes. In the tropical Western Pacific, juvenile skipjack tunas (8.5-66.8 mm SL) diet consisted of fish larvae, Euphausiacea, Copepoda, Amphipoda, and Cephalopoda (Tanabe, 2001). The main prey by occurrence, abundance, and mass was fish larvae. Tanabe (2001) observed a diel change in skipjack feeding behavior. From morning to evening, about 50% of the stomach contents had a stomach fullness score of 4 or 5 (more than half-full or full, respectively). From evening to night, the stomachs were either empty or almost empty. Studies suggest skipjack tuna exhibit a diurnal vertical cycle, where they are shallow at night and bounce-dive throughout the day (Schaefer et al., 2009). This result stresses the link between their vertical behavior and the feeding habits of skipjack tuna, with the fish most likely feeding during the day at or below the thermocline.

In the tropical Eastern Pacific, the diet of skipjack quantified in terms of volume consisted of crustaceans (59%), fish (37%), and cephalopods (3%) (Alverson, 1963). The top three prey items by volume and occurrence were family Euphausiidae, a small bathypelagic fish from the family Gonostomatidae (*Vinciguerria lucetia*), and flying fishes (Exocoetidae). Alverson (1963) also

documented a diet that varied with size (age) in skipjack, where the relative importance by volume of fishes increased and the relative importance of crustaceans decreased with an increase in skipjack size, implying a shift in their diet. In particular, skipjack tuna has shown evidence for an ontogenetic diet shift at 54.7 cm FL, where small tuna primarily feed on anchovies, Humboldt squid, and krill and large tuna primarily eat epipelagic fish and red crabs (Fuller et al., 2021). In food webs, the trophic level of skipjack tuna depends on their age, where the trophic level of small skipjack is 3.87, juvenile skipjack is 4.01, and large skipjack is 4.36 (Griffiths et al., 2019), showing how larger-sized tuna utilize prey at higher trophic levels.

The results of stomach contents and stable isotope analyses support that the feeding ecology of skipjack is region-specific, like yellowfin tuna. The Eastern Pacific Ocean was partitioned into unique biogeographical regions: upwelling and coastal/tropical offshore provinces. The diet of skipjack tuna differed across these two regions, where two species of anchovies dominated skipjack diet in upwelling provinces, and mesopelagic and epipelagic fishes were dominant prey in coastal/offshore provinces (Fuller et al., 2021). Additionally, the prey taxa composition was higher in tropical offshore provinces than upwelling provinces. Stable isotopes also display regional differences between $\delta^{15}N$ values; skipjack in western Taiwan (11.2 +/- 0.7‰) were significantly higher than eastern Taiwan (9.8 +/- 1.2‰) (Chang et al., 2022). Applying Stable Isotope Mixing Models has allowed studies to explore the relative importance of prey taxa relative to their predator. Here cephalopods were the most important prey in western Taiwan, while crustaceans and cephalopods were the most important prey in eastern Taiwan. Bayesian Mixing Models are useful because they reconstruct a time-integrated diet for predators and resolve the limitation of recently ingested prey in stomach contents.

Summary and Unresolved Issues

Although skipjack and yellowfin tuna share foraging grounds, they target different prey species and sizes. Skipjack tuna eat smaller-sized prey, like euphausiids (krill), than yellowfin tuna (fish and squid) (Magnuson & Heitz, 1971; Menard et al., 2006; Alatorre-Ramirez, 2007). Generally, teleost fishes comprise a larger proportion of yellowfin tuna diet than other taxonomic groups for tuna populations in the Pacific Ocean (Allain, 2005; Bertrand et al., 2002; Graham et al., 2007; Weng et al., 2015; Varela et al., 2017). Overall, crustaceans play a more important role in skipjack tuna diet than in yellowfin tuna diet (Olson et al., 2016). Niche partitioning is also observed in the spatial feeding ecologies of skipjack and yellowfin tuna: skipjack tuna forage in surface waters while yellowfin tuna forage between the surface and the thermocline. As top predators, skipjack and yellowfin tuna are strongly impacted by changes in species composition in lower trophic levels.

Researchers need to continue to explore the diet of top marine predators to learn about changes in prey communities caused by seasonal variability and climate perturbations, like El Nino Southern Oscillation (Olson et al., 2014; Trujillo-Gonzalez et al., 2022). The diet of yellowfin and skipjack tuna has been mostly focused in the Eastern and Western Pacific Ocean; with a few studies in the Central Pacific Ocean. Future research needs to expand their sampling range to understand the prey diversity and ecosystem structure of different oceanographic provinces. The diet of yellowfin tuna has been previously explored around Hawai‘i (Brock, 1985; Graham et al., 2007), while skipjack tuna has not been sampled in this region. Studies have shown that the prey diversity is higher for yellowfin, therefore the diet of skipjack tuna should be explored to understand their niche separation in this region. Additionally, to best understand the diet of

predators, studies need to combine methodologies to analyze recent ingested prey and time-integrated prey using stomach contents and stable isotopes, respectively.

A major gap in diet research entails standardized methods to compare results across studies. In particular, stomach content analyses use different metrics to determine the relative importance of prey taxa, reported in volume, number, weight, occurrence, or index of relative importance. Diet studies need to develop a consensus on reporting results so they can be easily compared. An unresolved issue involves unidentifiable prey in stomach contents due to their digestion rate, which can under- or over-estimate other prey taxa if unidentifiable prey is not accounted for in data analyses. Many studies only characterize broad prey classes (crustaceans, cephalopods, fishes), or identify prey to class, order, or family. Increasingly, DNA barcoding of stomach contents is allowing researchers to identify more prey taxa and assign a taxa to digested contents, to obtain a more descriptive list of prey (Trujillo-Gonzalez et al., 2022). Having a more refined diet can be helpful in identifying functional prey groups (e.g., epipelagic, nearshore, oceanic, vertically migrating prey) and understanding the foraging behavior of a predator.

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Chapter 2

Diet Composition and Ontogenetic Shifts of Skipjack tuna (*Katsuwonus pelamis)* in Hawai‘i inferred from Stomach Contents and Stable Isotope Analyses

Introduction

Skipjack tuna, *Katsuwonus pelamis*, is a highly abundant epipelagic predatory fish that inhabits tropical to subtropical regions (McCluney et al., 2019; Mugo et al., 2010). It accounts for 57% of global tuna production and is ranked the third highest catch in terms of weight (2.8 million tonnes per year) (FAO, 2022; ISSF, 2021). The majority of the catch is harvested by commercial purse-seine and pole-and-line fisheries from two Pacific Ocean stocks, the Western and Central Pacific Ocean (WCPO) stock and the Eastern Pacific Ocean (EPO) stock (Fonteneau, 2003; FAO, 2020; ISSF, 2021; Langley et al., 2005). Additionally, small-scale fisheries operating from oceanic islands take the species for commerce, subsistence, and recreation (Teneva et al., 2018). As a major fishery resource, ensuring sustainable stocks is critical for the future of tuna fisheries and for food security (Lehodey et al., 2021).

Many stocks of highly migratory tuna species, like bigeye (*Thunnus obesus*), bluefin (*Thunnus thynnus*), and yellowfin (*Thunnus albacares*), are fully exploited or reaching maximum sustainable yields (ISSF, 2021; Kirby et al., 2014). Since skipjack tuna has a high productivity and reaches maturity within a year, corresponding to a fork length of 40 to 45 cm, this species is more resilient to fishing pressure when other tuna species are over-exploited (Brock, 1954). In the past 20 years, catches of skipjack have increased from 1.0 to 1.9 million tonnes in the WCPO (FAO, 2022). Today, skipjack tuna stocks are fished sustainably because exploitation rates are below the maximum sustainable yield and their biomass exceeds the sustainable exploitation limit (Hare et al., 2019). However, if fishing effort continues to increase, higher catches may threaten the sustainability of these stocks, especially in the face of changing interannual oceanographic variability and global warming in the tropical Pacific Ocean (Kirby et al., 2014).

Developing an understanding of the diet and foraging ecology of skipjack tuna is critical for predicting how stocks will respond to growing fishing pressure and future climate variability.

As surface-schooling fish, skipjack tuna foraging distributions and breeding grounds are driven by oceanographic conditions, including water masses (e.g., sea surface temperature) and their boundaries (e.g., oceanic fronts), making skipjack tuna distributions susceptible to global warming (Block et al., 1993; Lehodey et al., 1997). The Western Pacific warm pool, an area characterized by oligotrophic water and sea surface temperatures above 24°C, supports the highest yields of skipjack tuna by enhancing secondary production and causing an aggregation-associated area with a strong hydrographic front (Fonteneau, 2003; Ganachaud et al., 2011; Lehodey et al., 1998). Due to physical oceanographic processes during El Niño Southern Oscillation (ENSO) events, there is an eastward spatial shift of the warm pool and in response tuna aggregation areas respond to this habitat disturbance by migrating eastward (Lehodey et al., 1997; Lehodey, 2000). These environmental shifts lead to yearly variations in skipjack distributions, whereby the stock shifts longitudinally and becomes more or less accessible to different island fisheries.

Climate change models predict that future El Niño events will increase in frequency and amplitude, causing downstream changes in ocean conditions and unfavorable feeding and spawning grounds for skipjack in the Western Pacific after 2050 (Lehodey et al., 2013). Skipjack tuna populations in Pacific equatorial waters are expected to respond by 2050, with an overall stock decline and an eastward and northward change in distribution (Bell et al., 2021; Dueri et al., 2014). The biomass for skipjack tuna is expected to decrease in the WCPO and increase in

the EPO, due to a cascade of physical oceanographic changes caused by global warming (Lehodey et al., 2011). These predicted changes underscore the need to better understand how future tuna stocks will respond to fishing pressure and climate change (Bell et al., 2021). Thus, it is important to continue to study the feeding ecology and the food webs that support skipjack tuna to be able to make better projections of their biomass and catch in the future.

Skipjack tuna are opportunistic foragers with high energetic requirements (Bertrand et al., 2002; Brill, 1987; Graham & Dickson, 2004), and a wide range of prey. Their diet includes a wide range of nektonic species, such as crustaceans, cephalopods, and teleost fish (Alverson, 1963; Schaefer, 1960; Varela et al., 2019). In the EPO, fishes are the predominant prey to skipjack tuna, with regional differences in prey species (Fuller et al., 2021). Two anchovy species (Family Engraulidae) account for nearly half of the diet (by mass) in upwelling provinces, whereas mesopelagic Panama lightfish (Family Myctophidae) and epipelagic mackerel scad (*Decapterus* spp., Family Carangidae) are important prey in the coastal and offshore provinces. In the Western Pacific Ocean (WPO), there are dietary differences in skipjack tuna caught by the Taiwanese fishery in the eastern (Pacific Ocean) and western (China Sea) fishing grounds. According to the index of relative importance (IRI), ponyfish (*Leiognathus bindus*), round herring (*Spratelloides gracilis*), and lanternfish (*Benthosema pterotum*) dominate the diet in the west; and squid, carangids, and flying fishes dominate in the east (Chang et al., 2022).

Altogether, previous stomach content analyses suggest the diet of skipjack tuna varies regionally. Fuller et al. (2021) hypothesized that epipelagic fish are more important prey in oceanic regions, therefore, we would expect the diet of skipjack tuna in Hawai‘i to be dominated by flying fish

and mackerel species. However, Chang et al. (2022) showed regional differences due to nutrient-driven pelagic food webs and seasonality of oceanographic environments. The skipjack tuna foraging in the more stable environment (western Taiwan) consisted of coastal prey species (16 prey taxa) and in the more variable oceanographic environment (eastern Taiwan) consisted of epipelagic and pelagic-oceanic prey (25 prey taxa). In addition, reef-associated prey contributes a high proportion to the diet of pelagic predators, like yellowfin and skipjack tuna, but are most important to surface feeders, nearshore feeders, and small predators (Allain et al., 2012). Hawai‘i does not have major seasonal changes, so I would expect skipjack tuna foraging nearshore to islands to utilize reef-associated prey and flying fishes.

Previous studies have also documented that skipjack diet varies with size (age), as skipjack increases in size, the predominant prey (by volume) shifts from crustaceans to fishes. For instance, in the EPO, skipjack tuna experience an ontogenetic shift at a fork length of approximately 54.7 cm: small tuna primarily eat anchovies, Humboldt squid, krill; large tuna primarily eat epipelagic fish and red crabs (Fuller et al., 2021). Similarly, stable isotopes revealed a shift in the skipjack tuna trophic level in the WPO: from 3.87 for small skipjack and 4.36 for large skipjack (Griffiths et al., 2019). These studies provide evidence that skipjack undergo region-specific ontogenetic diet shifts, which have been attributed to the increased capacity to thermoregulate and the onset of deeper diving in larger skipjack (Graham & Dickson, 2001; Kiiyofuji et al., 2019; Schaefer et al., 2009).

While the diet of skipjack tuna has been well described in certain regions of the world, their feeding ecology in nearshore waters of Hawai‘i has not been explored. The aims of this research

are to describe the diet and trophic position of skipjack tuna in nearshore waters around O‘ahu, and to explore ontogenetic shifts in their diet composition and trophic position. This study’s reliance on specimens sampled by island-associated fisheries addresses the importance of reef-associated and nearshore prey. Ultimately, this research will create a robust prey list for skipjack tuna and provide information on the prey available to predatory fish around the Main Hawaiian Islands.

Materials And Methods

Sample Collection

Skipjack tuna were captured by a recreational fisherman, using conventional fishing rods with artificial lures. A total of 121 specimens were collected northeast of O‘ahu (Hawai‘i) between May 20 and September 2 of 2020 (Julian days 141 and 246, respectively) (Figure 1). Upon capture, the fork length of each tuna was measured to the nearest 0.5 cm, ranging from 40.0 to 84.0 cm (Table 1). The gastro-intestinal tract (stomach and intestine) and the tail were removed immediately after length measurements were recorded, the samples were kept on ice during the transportation to the laboratory, and then stored at -80°C.

Stomach Contents Analysis (SCA)

Stomach dissection and prey sorting

The stomach contents of skipjack tuna were thawed at room temperature and dissected in the laboratory following a standardized protocol (Figure 2). In the laboratory, eight steps were taken: (i) stomach length (cm), and (ii) weight (g) were recorded, (iii) stomach fullness was determined using standardized categories (Table 2, Table 3, modified from Tanabe, 2001), (iv) stomach

contents were sorted into four broad categories (identifiable prey items, identifiable prey remains, unidentifiable prey remains, and plastic), (v) prey items were identified to the lowest possible taxonomic level, (vi) the digestion state for each prey item ranging from 1 for intact condition to 3 for poor condition (Table 4, Table 5) (modified from Magnuson, 1969; Bertrand et al., 2002), (vii) for complete prey items the wet weight (nearest 0.1 g) and standard lengths (nearest mm) were recorded, and (viii) contents and prey remains were sieved through a 500 micron mesh and inspected under a microscope with 10X - 40X magnification to identify hard parts (otoliths, squid beaks, and microplastic) (Hyslop, 1980).

The digestion state of prey can impact the diet quantification, by underestimating the size/mass of small/soft prey items and by interfering with the genetic identification of highly digested prey (Amundsen & Sánchez-Hernández, 2019; Donahue et al., 2021; Hyslop, 1980). To account for these biases, prey items were ranked using a three-level digestion state (Table 4). Fresh items (digestion state 1 or 2) were measured and stored in at -80°C freezer for stable isotope analysis and prey identification via DNA barcoding (Table 7).

Metrics for Quantifying Prey

To characterize the relative importance of the ingested prey to skipjack tuna diet, the percent abundance (%N), percent weight (%W), frequency of occurrence (%FO), and index of relative importance (IRI) for each prey species and functional group was calculated, using the equations below. The IRI combines three dietary metrics (abundance, weight, and frequency of occurrence) into a single metric that evaluates the importance of each prey taxon *i* (Hart et al., 2002).

$$\%N_i = (\text{Total number of prey taxon } i \times 100) / (\text{Total number of prey taxon } i \text{ in all stomachs pooled})$$

$$\%W_i = (\text{Total weight of prey taxon } i \times 100) / (\text{Total weight of prey taxon } i \text{ in all stomachs pooled})$$

$$\%FO_i = (\text{Number of stomachs containing prey item } i \times 100) / (\text{Total number of non-empty stomachs})$$

$$IRI_i = (\%N_i + \%W_i) \times \%FO_i$$

$$\%IRI_i = (100 \times IRI_i) / (\sum n_i{=}_1 IRI)$$

Prey Barcoding Analysis

DNA barcoding was used to further characterize prey items to a lower taxonomic level. Between dissections, the lab bench and tools were cleaned between samples with 10% bleach to prevent cross-contamination, and the samples were stored at -18℃.

DNA Extractions

The HotSHOT method was used to extract DNA from prey items (Truett et al., 2000). Briefly, a small piece (~10 mg) of tissue from each prey was extracted and added to a strip tube containing 100 µl of 50 mM NaOH. The extracted samples were incubated in a thermocycler at 95℃ for 25 minutes and held at 15℃ indefinitely. Once the incubation step was completed, tubes were centrifuged briefly and 10 ul of 1M Trish-HCl (pH=8) was added to each strip tube. For squid samples, the extracted DNA was not diluted and used at the original concentration. For fish samples, the extracted DNA was diluted to a 1:10 solution. For crustacean samples, the extracted DNA was diluted to a 1:10 and 1:100 solution. A 1:10 solution was made by adding 27 µl of

MilliQ water and 3 µl of the extracted DNA into a new strip tube. A 1:100 solution was made by adding 27 µl of MilliQ water and 3 µl of the 1:10 DNA solution into a new strip tube.

PCR Amplification

A master mix was prepared with 10 µM of the forward primer (1 uL/sample), 10 µM of the reverse primer (1 uL/sample), 100x BSA (0.5 µl/sample), 2x Biomix (Mango Mix) (10 µl/sample), and water (5.5 µl/sample). The primer pairs used for prey items: fish, squid, and crustaceans were Mi-Fish U-F and U-R 10 µM (Miya et al., 2015), Ceph 16S F and R 10 µM (Peters et al., 2015), and LCO 1498 and HCO 2198 µM (Folmer et al., 1994), respectively. In a new strip tube, 18 µl of master mix was added with 2 µl of extracted DNA. Tubes were placed in the thermocycler with a) an initial temperature of 95℃ for 5 minutes, b) denatured at 94℃ for 30 seconds, c) annealed at 50℃ (for fish and squid samples) or 45℃ (for crustacean samples) for 45 seconds, d) an extension step at 72℃ for 1 minutes, e) steps b-d were repeated for 34 more cycles, and f) a final extension at 72℃ for 10 minute and held at 15℃ indefinitely. PCR products were stored at -20°C until required.

Agarose Gel Run

PCR products were visualized on a 2% agarose gel with smart glow and a 50 base-pair ladder for a DNA fragment size reference. The gel was run in an electrophoresis setup at 100 volts for 30 minutes (amps: 3.0 A, wat: 220 W). The gel was then analyzed using ChemDoc XRS+ with Image Lab Software. A control well with no DNA extraction was added to the analysis to confirm that the products were not contaminated. Wells showing bright bands confirmed successful PCR products and were processed for a PCR cleanup. Wells with no bands were

reanalyzed with a different extraction dilution, re-amplified using a different primer pair, set at a different annealing temperature, or a combination of all of these approaches.

PCR Cleanup Protocol

PCR products were cleaned by mixing ExoSAP-IT$_{TM}$ reagent with post-PCR product in a 2:5 ratio (Werle et al., 1994). The mixture was placed in a thermocycler to incubate at 37°C for 30 minutes to degrade remaining primers and nucleotides, then at 85°C for 15 minutes to inactivate ExoSAP-IT$_{TM}$ reagent. Once complete, the PCR product was sent for Sanger sequencing at the University of Hawai‘i genomics core sequencing facility (ASGPB).

Taxon Assignment to DNA Sequences

DNA sequences were manually annotated using Geneious Prime 2022.1.1. Sequences were trimmed where base peaks were low and did not improve the percent high quality. “N” bases were replaced with A, T, G, or C based on the peak height. When peak height was not strong or had several peak overlaps, a base pair accounting for 2-4 bases was replaced using the ambiguity guidelines. Base pairs that were misassigned were also corrected. Once sequences were edited, sequences were blasted to the NCBI (National Center for Biotechnology Information) nt’s database with BLAST (Basic Local Alignment Search Tool) to assign taxa to sequences. Sequence hits were filtered based on query cover (> 95%), E-value (< 0), and percent identified (> 95%). Top hits following the criteria were checked if they could be a reasonable possibility by checking their habitat and distribution on FishBase (www.fishbase.org). Whenever multiple species of the same genus were matched with high alignment scores, the hit was assigned to the genus level, without specifying a species. Similarly, whenever multiple genera of the same

family were matched with high alignment scores, the hit was assigned to the family level, without specifying a species or a genus.

Species Prey Diversity

Species accumulation curves were used to determine if the number of stomachs sampled (n = 118) was sufficient to describe the diet of skipjack tuna using three diversity indices (Hill numbers): species richness (q=0), Shannon diversity (q=1), and Simpson's diversity (q=2) (Chao et al., 2014). These sampling curves of Hill numbers were made in R using the iNext package (Hsieh et al., 2016).

Stable Isotope Analysis (SIA)

Tissue Collection

A small (2 mL) sample of white muscle tissue was dissected from the caudal peduncle of each skipjack tuna (n=121). Prey items (n = 159) from the stomach and intestine contents were collected for isotope analysis (Table 7). All the fish and squid samples came from skipjack stomach contents. Tissue was taken from the caudal peduncle of 53 fishes and the mantle of 14 squids. A total of 93 whole crustaceans were collected from skipjack stomach and intestine contents. Because crustacean samples were very small and provided a low sample weight (averaging 0.14 - 0.23 g before drying), we combined 2-5 crustaceans that were morphologically identical and came from the same skipjack specimen to increase our sample weight.

Tissue Preparation

Tissue samples were initially stored frozen (-18°C) and then thawed, grounded with a mortar and pestle, and dried in a drying oven at 60°F for 24 to 48 hours following Sulzman (2007). Once dried, the samples (0.4 - 0.6 mg) were homogenized into a fine powder with a mortar and pestle and packaged in a 2 mL vial.

The samples were analyzed for carbon and nitrogen bulk stable isotopic composition at the University of Hawai‘i at Manoa Biogeochemical Stable Isotope facility using a Costech ECS 4010 Elemental Combustion System, with a Zero Blank Autosampler, a ThermoFinnigan MAT Conflo IV, and a ThermoFinnigan DeltaXP. Each sample yielded the ratio of carbon to nitrogen (C:N), derived from the mass of carbon (mg) and nitrogen (mg), and the isotopic values for $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰). The δ values were calculated using the following equation: $\delta X = [(R_{sample}/R_{standard})-1]*1000$, where X is ^{13}C and ^{15}N and R is the ratio of heavy isotope to light isotope, $^{13}C/^{12}C$ and $^{15}N/^{14}N$ (Peterson and Fry, 1987). Lipid contents in samples can influence the isotopic value for ^{13}C, and C:N ratio values indicate if a sample is biased by excess lipids if values are above the threshold of 3.5. Skipjack C:N ratios were below 3.5 (min = 2.97, max = 3.28), implying that the effect of lipid contents in the samples were normal and therefore lipid-extractions were not necessary (Post et al., 2007; Skinner et al., 2016).

The crustacean prey samples were analyzed twice, before and after an acid-wash treatment to remove the inorganic carbon compounds in their exoskeleton that do not get assimilated by their predators (Bunn et al., 1995; Carabel et al., 2006). This method corrects the carbon isotopic ratios but can also increase or decrease the nitrogen isotopic ratio by approximately 3‰,

resulting in food web errors. Therefore, the crustacean samples were analyzed for nitrogen ($\delta^{15}N$) first, then acid-washed, and analyzed for carbon ($\delta^{13}C$).

To check that the carbon isotope for prey samples was not biased with an excess of lipids or carbonates, their molar C:N ratio was compared to their $\delta^{13}C$. This relationship was assessed separately for fish, squid, and crustacean prey samples (See Appendix). After acidification, there was no relationship between crustacean molar C:N ratio and $\delta^{13}C$ values (Linear regression, $r^2 = 0.002$, $F_{1,45} = 0.09$, $p = 0.768$). There was a significant negative relationship between fish molar C:N ratio and $\delta^{13}C$ values (Linear regression, $r^2 = 0.711$, $F_{1,51} = 125.30$, $p < 0.001$). There was a significant negative relationship between squid molar C:N ratio and $\delta^{13}C$ values (Linear regression, $r^2 = 0.736$, $F_{1,12} = 33.38$, $p < 0.001$). While negative relationships between molar C:N ratio and $\delta^{13}C$ values can indicate that some samples contained excess lipids, we concluded our results were not from a lipid bias, due to the small observed ranges of $\delta^{13}C$ (fish: -20.9 to -18.3‰, squid: -20.0 to -18.2‰) and C:N ratios (fish: 3.64 to 5.76, squid: 3.75 to 4.31). Rather, we attributed these $\delta^{13}C$ differences to biologically meaningful species differences. Thus, we did not lipid extract these samples.

Stable Isotope Data Distributions

A sample set of 121 skipjack was analyzed for stable isotopes and the resulting data were assessed for normality and outliers. Shapiro-Wilk tests revealed that neither the $\delta^{13}C$ ($W = 0.896$, $p < 0.001$) nor the $\delta^{15}N$ ($W = 0.969$, $p = 0.008$) were normally distributed, due to one and two outliers, respectively. Skipjack #113 had a very low $\delta^{13}C$ value and #58 and #64 had high $\delta^{15}N$

values. Outliers were identified using Tukey's method, and values beyond 1.5 times the IQR (interquartile range) were considered outliers, significantly different from the rest of the data.

Reproducibility of Stable Isotope Values

To explore the potential reason for three outliers, we re-analyzed these samples, alongside a randomly selected subset of 20% of the other samples (24 of 121). All 27 samples for the second isotope analysis were re-ground with a mortar and pestle. If the samples were not properly ground to a homogenized powder, a result of this could lead to a misreading if the sample contained excess lipids and ultimately cause a change in the C:N ratio and carbon isotopic value. The reproducibility of the values of these 27 samples was assessed by comparing their C:N ratio from the two analyses. A linear regression was used to predict the C:N ratio from the second analysis using the C:N ratio from the first analysis. Assuming these analyses had no error, the intercept would be 0 and the slope would be 1. This regression yielded a non-significant intercept ($t = 1.111$, $p = 0.277$), indistinguishable from 0 ($b_o = 0.629$ +/- 0.567 S.E.), and a significant slope ($t = 4.400$, $p < 0.001$) which was not significantly different from 1 ($b1 = 0.798$ +/- 0.181 S.E.). While the regression residuals were normally distributed (Shapiro-Wilk, $W = 0.968$, $p = 0.558$), there was a large outlier (skipjack #113).

Skipjack #113 was highly significant, with a Z score of -2.75 and an associated value of $p = 0.003$, we replaced the isotope values ($\delta^{13}C$ and $\delta^{15}N$) for #113 from the first analysis with those from the second analysis. This sample had a C:N ratio of 2.97 and 3.08 in the first and second analysis, respectively. While skipjack #113 was characterized as an outlier by the lowest $\delta^{13}C$ value in the dataset (-20.7‰), the reanalysis yielded a substantially higher $\delta^{13}C$ value (-17.4‰).

The other two specimens (skipjack #58 and #64) with the anomalous $\delta^{15}N$ values did not stand out as significant outliers when we compared the values from the first and second analyses, so we kept their original first values.

The results from the reanalysis of the 24 randomly selected samples yielded highly reproducible values, based on Pearson correlations. The $\delta^{15}N$ values had the highest correlation coefficient (Pearson's correlation, $r = 0.965$, $N = 24$, $p < 0.001$), followed by the $\delta^{13}C$ values (Pearson's correlation, $r = 0.953$, $N = 24$, $p < 0.001$). The C:N ratio was more variable, as evidenced by the lower correlation coefficient (Pearson's correlation, $r = 0.690$, $N = 24$, $p < 0.001$). Overall, the r-squared values of the $\delta^{15}N$ (93.1%) and the δ13C (90.9%) data indicate replicate measurements share over 90% of their variability.

After correcting the anomalous $\delta^{13}C$ value of specimen 113, we reanalyzed the data for stable isotope normality. While the $\delta^{13}C$ data (Shapiro-Wilk, $W = 0.967$, $p = 0.005$) and the $\delta^{15}N$ data (Shapiro-Wilk, $W = 0.969$, $p = 0.008$) were not normally distributed, the C:N ratio data were normal (Shapiro-Wilk test, $W = 0.980$, $p = 0.082$).

Exploring Drivers of Stable Isotope Ratios

I related the stable isotope ratios ($\delta^{15}N$ and $\delta^{13}C$) of skipjack white muscle to three drivers: (i) the size of the fish, expressed as the fork length (cm); (ii) seasonal variability during the sampling period, expressed as the Julian Day of capture; and (iii) a potential bias due to changes in the C:N ratio of the samples. Two of these predictors were significantly cross correlated (Pearson correlations with Holm correction with df = 119): the C:N ratio and the Julian date were

negatively correlated (r = -0.213, p = 0.037), and the fork length and the Julian date were positively correlated r = +0.289, p = 0.004). Yet, the C:N ratio and the fork length were not correlated (r = -0.006, p = 0.948). While these cross-correlations were relatively weak ($r^2 <$ 0.083), we used the variance inflation factor (VIF) to assess the collinearity of the predictors, after we performed the multiple linear regressions (Belsley et al., 2005). All VIF values were less than 4, the threshold value commonly used to identify collinearity (fork length = 1.095, Julian date = 1.147, C:N ratio = 1.052), indicating that the three predictor variables were independent (O'Brien, 2007).

First, we related $\delta^{15}N$ and $\delta^{13}C$ to each variable using simple linear regression with a single predictor. Then, we developed more complex models with two and three predictors using multiple linear regression. Overall, we built eight models for $\delta^{13}C$ and $\delta^{15}N$: three with a single predictor, three with two predictors (FL and Julian Day, FL and C:N ratio, Julian Day and C:N ratio), one full model with all three predictors (FL, Julian Day, and C:N ratio), and a three-predictor model including the interaction between tuna size and date of capture (Julian day, fork length, and Julian date * fork length).

To meet the assumptions for linear regression for each model, we analyzed the residuals for normality and quantified the leverage using the Cook's distance, to ensure all observations had similar influence on the best-fit slope (Montgomery et al., 2021; Schmidt & Finan, 2018). Lastly, we used Akaike information criterion (AIC) to compare models with the same outcome variable by estimating each model's fit given the number of parameters, with the following equation:

AIC = -2log(maximum likelihood) +2k,

where the log of maximum likelihood is the conformity of the fitted model to the real data and k is the number of independently adjusted parameters in the model (Akaike, 1974; Cavanaugh & Neath, 2019). The regression model with the lowest AIC represents the best fit candidate model at predicting the $\delta^{13}C$ and $\delta^{15}N$ of skipjack tuna (Akaike, 1987).

Describing Ontogenetic Changes in Stable Isotopic Ratios

To investigate potential ontogenetic shifts in the diet composition of skipjack, we related the isotopic signatures of $\delta^{13}C$ and $\delta^{15}N$ to their fork length using nonlinear regression models with package drc (Analysis of Dose-Response Curves) in R. Following Graham et al. (2007), I fitted the same 4-parameter sigmoid model used previously for yellowfin tuna (*Thunnus albacares*) in Hawai‘i, using the equation:

$$y = y_o + (a-y_o) / (1 + \exp(-(x-x_o)/b),$$

to relate the response variable y ($\delta^{13}C$ or $\delta^{15}N$) to x (fork length), given y_o (the lower horizontal asymptote value), a (the upper horizontal asymptotes of y), x_o (the fork length that falls mid-way between y_o and a), and b (the slope between the lower and the upper horizontal asymptotes).

Prey Functional Groups

A total of 114 prey items (53 fishes, 47 crustaceans, and 14 squids) from stomachs were analyzed for $\delta^{13}C$ and $\delta^{15}N$. Prey sources were grouped into taxonomically and ecologically similar groups. Fish families were organized into functional groups using vertical and horizontal distributions from Boehlert et al. (1992) and Leis and Miller (1976). Chaetodontidae and Monacanthidae were categorized as Inshore Reef with a depth range from 0 to 50 meters. Their mean $\delta^{13}C$ and $\delta^{15}N$ were not significantly different so these families were combined to: Inshore

Reef (0-50 m). Mullidae, Acanthuridae, and Balistidae were categorized as Inshore Reef with a depth range from 0 to 100 meters, their mean $\delta^{13}C$ and $\delta^{15}N$ were not significantly different so these families were combined. Blenniidae was classified as Inshore Reef with a depth range from 0 to 200 meters, and they were not significantly different from the mean $\delta^{13}C$ of Inshore Reef (0-100 m), so Blenniidae was combined with Inshore Reef (0-100 m) to form the functional group: Inshore Reef (0-200 m). Carangidae were classified as Pelagic-Neritic and Scombridae were classified as Pelagic-Oceanic: their mean $\delta^{15}N$ were significantly different from each other, so both functional groups were kept separate.

A one-way ANOVA was conducted to test if there was a significant difference in the mean $\delta^{13}C$ and $\delta^{15}N$ for four crustacean taxa (Welch's t-test, $F(3.000, 19.264) = 10.993$, $p < 0.001$). A Games Howell post-hoc test showed which groups of crustacean taxa were significantly different from each other. Family Odontodactylidae, Order Decapoda[1] (crab megalops), and Order Decapoda[2] (larval shrimp) were not significantly different from each other in both isotopes, so they were grouped as Crustacean 1. The mean $\delta^{13}C$ and $\delta^{15}N$ of Family Carpiliidae were significantly different from the other crustacean taxa ($p < 0.001$), so they were classified as Crustacean 2. Family Ommastrephidae were kept as their own functional group: Squids. Overall, 7 functional groups were used as sources in the mixing model (Figure 7).

Bayesian Isotope Mixing Model

To relate the trophic links and dietary composition of skipjack tuna, I integrated the stable isotope analysis (SIA) and stomach contents analysis (SCA) perspectives using Bayesian dietary source mixing models. SIA quantifies the assimilation of prey into a consumer's tissue and SCA

identifies taxon-specific groups for selecting prey sources. Mixing models relate the different prey sources ($\delta^{13}C$ and $\delta^{15}N$ of prey) to a consumer's tissue ($\delta^{13}C$ and $\delta^{15}N$ of skipjack tuna muscle), to estimate the relative contribution of these prey groups to a predator's diet (Phillips et al., 2014).

I used the Bayesian dietary source mixing model, implemented with R package simmr, Stable Isotopes Mixing Models in R (Parnell & Ingler, 2019; Halpin et al., 2021). The trophic enrichment factors (TEFs) were calculated using the following equation:

$$\Delta X_{\text{tissue-prey i}} = \delta X_{SJ} - \delta X_{\text{Prey i}},$$

where δX_{SJ} represents the stable isotopic value ($\delta^{13}C$ or $\delta^{15}N$) for skipjack tuna white muscle and $\delta X_{\text{Prey i}}$ represents the stable isotopic value ($\delta^{13}C$ or $\delta^{15}N$) for prey source i. The mean and standard deviation TEF's were calculated for each stable isotope. The concentration dependency was derived for each prey source using the percent weights of carbon and nitrogen in prey samples using the following equation:

$$\text{Concentration dependency} = \text{X weight } (\mu g) / \text{Sample weight } (g),$$

where X is the weight of carbon or nitrogen in the sample (Halpin et al., 2021). A mixing polygon simulation was used to evaluate any consumers that were not explained in the model using packages 'sp' and 'splancs' in R (Smith et al., 2013). The Deviation Information Criterion (DIC) is a measure of fit for Bayesian models, and the model having the lowest DIC provides the best fit to the data (Spiegelhalter et al., 2002).

Results

Tuna Size

Skipjack tuna were caught between May 20, 2020 (Julian day = 141) and September 2, 2020 (Julian day = 246). Their fork length size ranged from 40 to 84 cm, with the majority (94.2%) of the samples ranging between 50 to 80 cm fork length (Table 1). Neither Julian day ($W = 0.876$, $p < 0.001$) and skipjack size ($W = 0.969$, $p = 0.007$) were normally distributed. There was a positive correlation (Pearson's correlation, $r = 0.29$, $N = 121$, $p = 0.001$) between the size of tuna (fork length) and the date they were caught (Julian Day). From the growth curve of skipjack tuna in Hawai'i, they reach 40 cm fork length at age 1 and approximately 70 cm at age 2 (Brock, 1954). We cannot conclude that skipjack grew substantially between the 4 months of the sampling period because it takes them approximately 30 months (~2.5 years) to go from 40 to 80 cm fork length (Brock, 1954).

Stomach Samples

Only 2.5% (3 of 121) of the samples had empty stomachs, with a stomach fullness ordinal score of 0. Overall, 61.1% of the tuna had mostly full stomachs, with fullness scores ≥ 3 (Table 2, Table 3). There was no correlation between the size (fork length) of the tuna and their stomach fullness score (Spearman rank correlation, $rs = 0.03$, $N = 121$, $p = 0.73$).

There was a significant, albeit weak ($R^2 = 4.5\%$) relationship between their size (fork length) and the mass of their stomach contents (digested remains and fresh items) (Linear regression, $r^2 = 0.045$, $F_{1,116} = 5.4$, $p = 0.02$). Yet, there was no relationship between tuna size (fork length) and the mass of identifiable prey they contained (Linear regression, $r^2 = 0.012$, $F_{1,116} = 1.4$, $p =$

0.24). Thus, the overall diet characterization was not biased towards the stomach contents of the larger sampled individuals.

Of the total contents mass from all stomachs (2,420 g), 41.5% was identified to a prey taxon level (species, genus, or family) and 58.5% of the content mass was highly digested and unidentifiable. On average 34.2% (SD = 33.3%) of the mass for each stomach was classified to the lowest taxonomic level, this proportion varied by individuals from 0% to 100%. A total of 3450 prey items, weighing 1,006 g, were sorted and quantified: 4.4% were assigned a digestion score of 1 ("intact condition"), 15.7% were a digestion score of 2 ("fair condition"), and 79.9% were a digestion score of 3 ("poor condition").

Stomach Contents Analysis

Stomach contents were classified and quantified into three broad taxonomic groups: crustaceans, fishes, or mollusks (cephalopods and pteropods). Overall, crustaceans contributed the highest proportion to the diet, as evidenced by the four dietary metrics (FO = 71.2%, N = 89.6%, W = 40.3%, IRI = 78.4%) (Table 6). While fishes contributed a similar weight proportion of the diet (W = 40.0%), their frequency of occurrence (FO = 43.2%) and numerical abundance (N = 9.5%) were substantially lower, leading to a lower index of relative importance (IRI = 18.2%). Cephalopods contributed the least proportion to the diet, across all four dietary metrics (FO = 19.7%, N = 0.9%, W = 19.5%, IRI = 3.4%) (Table 6).

Prey Identification

From a total of 3450 prey items, 93 (2.7%) were successfully barcoded and identified (mean percent identity = 95.4%, SD = 6.0%, range = 78.9 - 100%). A sample size of 133 fishes, 36 squids, and 206 crustaceans were selected for barcoding, but only 71, 12, and 10 were successfully barcoded, respectively (Table 8). The number of prey items identified by digestion scores 1, 2, and 3 were 37, 30, and 26, respectively. Once a prey item was identified to species or family level, morphologically similar prey were assigned the same taxonomic identification.

The stomach contents contained undigested hard remains from prey. The most frequent hard remains were squid beaks (FO = 22%), followed by squid pens, and fish otoliths (Table 9). The presence of squid prey and indigestible remains (beaks and pens) in the stomach samples were independent (Fisher's Exact test, $p = 0.053$). Yet, there was a significant correlation between the number of squid prey and squid remains in a given stomach (Spearman rank correlation, $rs = 0.20$, $N = 121$, $p = 0.03$).

In total, 24 unique taxa (16 fishes, 5 crustaceans, and 3 mollusks) were identified in skipjack tuna stomach contents listed in Table 10. At the species level, the three most common prey by occurrence were from the Order Decapoda; an unidentified crab megalopae (50.0%), *Odontodactylus* spp. (28.0%), and an unidentified shrimp (21.2%) (Figure 4). The unidentified crab megalopae contributed the highest quantity to the diet by numerical abundance (N = 82.7%) and weight (W = 35.2%), making it the most important prey (IRI = 85.6%). In fact, the largest number of crustaceans in a stomach was 511 crab megalopae consumed by a 76-cm FL specimen. However, *Odontodactylus* spp. and the unidentified shrimp contributed a low

abundance (N = 1.9% and 1.4%, respectively) and weight (W = 2.0% and 1.1%, respectively) to the diet of skipjack.

Two cephalopod families (Ommastrephidae and Ocythoidae) and a pteropod (Family Cavolinidae) were identified. The most frequently observed cephalopod species was the purpleback flying squid, *Sthenoteuthis oualaniensis* (FO = 18.6%), despite having a low abundance (N = 0.9%). Yet, its high contribution to the diet, by weight (W = 19.6%), made it the second most important prey (IRI = 5.5%). The largest squid had a mantle length of 14.3 cm, consumed by a 62-cm FL tuna.

From the 16 fish taxa identified, 13 were larval to pre-juvenile stages of reef-associated species. Three fish taxa were pelagic-oceanic juvenile fish (*Katsuwonus pelamis*, *Euthynnus affinis*, and *Decapterus* spp.). The most encountered fish was the *Parupeneus* spp. goatfish (FO = 13.6%), contributing 15% of the diet by weight and 2.4% by number, and being the third most important prey (IRI = 3.4%). The next two most frequent fish were the butterflyfish, *Chaetodon kleinii* (FO = 11.9%), and the blenny, *Cirripectes* spp. (8.5%). The other 13 fish taxa had low dietary importance of IRI $\leq$ 0.5%. The largest prey was a 14.5 cm juvenile skipjack tuna, consumed by a 58-cm FL tuna.

Diet Changes in Stomach Contents

Using the $\delta^{13}C$ threshold found in the section "Describing Ontogenetic Changes in Stable Isotopes", I used the midpoint 68.8 cm FL to differentiate two size classes: small and large tuna. The most important prey for small (< 68.8 cm FL) and large (> 68.8 cm FL) tuna was

crustaceans (IRI = 64.6% and 85.9%, respectively). Small tuna consumed a high relative number of crustaceans (N = 71.6%) with a disproportionately low relative weight (W = 14.9%), while large tuna primarily consumed crustaceans (N = 92.4%), which accounted for most of their prey, by mass (W = 61.3%). Accordingly, there was a significant difference between the number (Welch's t-test, F (1.0, 54.64) = 5.874, p = 0.019) and weight (Welch's t-test, F (1.0, 53.25) = 6.818, p = 0.012) of crustaceans consumed by small tuna and large tuna, where large tuna consumed a higher proportion of crustaceans by number and weight (Table 11).

Small tuna consumed a low number of fish (N = 18.9%), however they contributed the highest weight to their diet (W = 58.4%) and had an IRI of 30.9%. The proportion of fish nearly halves for the diet of large tuna (N = 7.1%, W = 24.8%, IRI = 11.4%). Cephalopoda contributed the smallest proportion of the diet of small (N = 2%, IRI = 4.5%) and large (N = 0.6%, IRI = 2.7%) tuna. However, the occurrence of cephalopods increased from small tuna (FO = 16.4%) to large tuna (23.5%). Cephalopoda contributed the second highest weight (W = 26.7%) to the diet of small tuna and about half as much in large tuna (W = 13.9%) (Table 11).

Species Prey Diversity

Species accumulation curves calculated three diversity indices: species richness (24), Shannon diversity (13.4), and Simpson's diversity (9.4). All three curves reached an asymptote (Figure 3), indicating that the number of stomachs sampled in this study was adequate to describe the diet of skipjack tuna in this region.

Because there was no significant correlation between the size of tuna (fork length) and the species richness of their stomach contents (Pearson's correlation, $r(116) = 0.023$, $p = 0.803$), separate species accumulation curves were not developed for the small and the large tuna size classes.

Skipjack Tuna Bulk Stable Isotopes

Skipjack white muscle tissue $\delta^{15}N$ values ranged from 7.8 to 12.4‰ (*Mean* = 9.4 ± 0.9‰ SD) and $\delta^{13}C$ values ranged from -19.0 to -16.8‰ (*Mean* = -17.8 ± 0.5‰ SD) (Table 12). The distributions for $\delta^{15}N$ and $\delta^{13}C$ were not normally distributed ($W = 0.969$, $p = 0.008$; $W = 0.967$, $p = 0.005$, respectively).

Predictors of $\delta^{15}N$

I considered the fork length of skipjack, their Julian day of collection, and their C:N ratio as individual predictors for skipjack isotopic values. I used these three predictors to test their linear relationship with the $\delta^{15}N$ of skipjack tuna (Figure 5). The fork length was marginally not significant (Linear regression, $r^2 = 0.028$, $F1,119 = 3.49$, $p = 0.064$), the Julian day of collection had a significant negative relationship (Linear regression , $r^2 = 0.186$, $F1,119 = 27.27$, $p < 0.001$), and the C:N ratio was not significant (Linear regression , $r^2 = 0.050$, $F1,119 = 0.56$, $p = 0.567$).

However, the observed variability in $\delta^{15}N$ was best explained by a two-parameter linear model, including fork length and Julian day (Table 13). $\delta^{15}N$ increased significantly for larger sized

specimens but decreased significantly over the sampling period. Overall, this model had the lowest AIC value with an explanatory power of $R^2 = 28.05\%$.

Predictors of $\delta^{13}C$

All three predictors I used to model $\delta^{13}C$ of skipjack tuna using simple linear regression were statistically significant (Figure 5): the fork length had a significant positive relationship (Linear regression , $r^2 = 0.189$, $F_{1,119} = 27.81$, $p < 0.001$), the Julian day of collection had a significant positive relationship (Linear regression, $r^2 = 0.138$, $F_{1,119} = 18.98$, $p < 0.001$), and the C:N ratio had a significant negative relationship (Linear regression , $r^2 = 0.103$, $F_{1,119} = 13.71$, $p < 0.001$).

The observed variability in $\delta^{13}C$ was best explained by a three-parameter linear model, including fork length, Julian day, and the C:N ratio (Table 14). $\delta^{13}C$ increased significantly for larger specimens and over the sampling period but was negatively related to the C:N ratio. Overall, this model had the lowest AIC value with an explanatory power of $R^2 = 32.73\%$.

Describing Ontogenetic Changes in Stable Isotopes

The nonlinear regression identified a step-like increase in the $\delta^{13}C$ results (Figure 6), at a fork length mid-point (threshold) of 68.8 cm ± 2.0 S.E., which defined two tuna size classes: smaller specimens had an asymptotic value of -17.98‰ ± 0.07 S.E. and larger specimens had an asymptotic value of -17.48‰ +/- 0.09 S.E. (Table 12). The shift between the lower and the upper asymptote spans the fork length range from 66.8 and 70.8 cm. The mean $\delta^{13}C$ of small tuna (-

18.0‰ ± 0.5 SD) was significantly lower than the mean of the large tuna (-17.5‰ ± 0.3 S.D.) (Welch's test not assuming equal variances, F,1.00, 109.09 = 37.17, $p < 0.001$).

The nonlinear regression did not find a step-like increase in the $\delta^{15}N$ results (Figure 6), as evidenced by the model's inability to estimate the threshold and slope of the 4-parameter sigmoidal model (Table 15). The mean $\delta^{15}N$ of the small (FL < 68.8 cm) tuna (9.3‰ ± 0.8 S.D.) was significantly lower than for the large (FL > 68.8 cm) tuna (9.6 ‰ ± 1.1 S.D.) (Welch's test not assuming equal variances, F,1.00, 92.33 = 4.09, $p = 0.05$).

Prey Functional Groups Stable Isotopes

There was a significant difference among the prey functional groups $\delta^{13}C$ means (ANOVA, F6, 23.05 = 51.84, $p < 0.001$). The Games Howell post-hoc test found the mean $\delta^{13}C$ value of Crustacean 1 was significantly lower ($p < 0.001$) from all functional groups, except Inshore Reef (0-50 m). The mean $\delta^{13}C$ of Crustacean 2 was significantly higher than Crustacean 1 and significantly lower than Pelagic-Oceanic ($p < 0.001$). The mean $\delta^{13}C$ of Inshore Reef (0-50 m) was significantly lower than Inshore Reef (0-200 m), Pelagic-Neritic, Pelagic-Oceanic, and Squids ($p < 0.001$). The mean $\delta^{13}C$ of Inshore Reef (0-200 m) was also significantly lower than Pelagic-Oceanic ($p < 0.001$).

There was a significant difference between the prey functional groups $\delta^{15}N$ means determined by (ANOVA, F,6, 24.63 = 56.93, $p < 0.001$). The Games Howell post-hoc test revealed that the mean $\delta^{15}N$ value of Crustacean 1 and Crustacean 2 (lowest mean $\delta^{15}N$) were significantly different from each other and from all 6 other functional groups ($p < 0.001$). The mean $\delta^{15}N$ of

Pelagic-Neritic was significantly lower than Inshore Reef (0-200 m), Pelagic-Oceanic, and Squids ($p < 0.001$). See Table 16 for the mean and standard deviation $\delta^{15}N$ and $\delta^{13}C$ of each functional group. A total of 7 functional prey groups were classified for the isotope mixing model (Figure 7).

Stable Isotope Mixing Model

The mixing polygon simulation confirmed that the calculated trophic enrichment factors (TEFs) could explain the skipjack isotopic values in this model because all consumers were above the 5% contour threshold, so the proposed model was suitable (Smith et al., 2013). The TEF used in this Bayesian Model was $\Delta^{13}C = 2 \pm 0.9‰$ and $\Delta^{15}N = 4.6 \pm 2‰$ based on the average difference between skipjack and prey isotopes. All the points were within the 95% mixing region and in particular, all consumers are within 75% of the mixing region (Figure 8).

The Bayesian dietary source mixing model estimated the relative contribution of seven prey sources to the diet of skipjack tuna (Figure 9). Crustaceans accounted for over half (61.1%) of the skipjack tuna diet: Crustacean 2 contributed an average of 37.3% +/- 7.6% (SD) and Crustacean 1 contributed an average of 23.8% +/- 4.8% (SD). Fish, comprising four prey sources, contributed approximately one third (32.4%) of the skipjack diet. Inshore Reef (0-50 m) fish contributed 7.6% +/- 3.7% (SD), and Inshore Reef (0-200 m) fish contributed 8.4% +/- 4.3% (SD). Pelagic-Neritic fish contributed 11.2% +/- 5.1% (SD) and Pelagic-Oceanic fish made up the smallest proportion (5.2% +/- 2.7% SD) of the diet. Squids were the least important prey group, only accounting for an average of 6.5% +/- 3.4% (SD) of the diet. The DIC for this model

with 7 sources was the lowest (486.2) compared to Bayesian mixing models using 6 (493) or 5 (526.9) sources.

Discussion

Marine top predators provide a unique perspective of an environment's composition by monitoring bottom to top effects in the marine food web (Hazen et al., 2019). Tuna species are biological indicators of oceanic ecosystems due to their broad distribution, generalist diet, and response to climate variability (Hyrenbach et al., 2021; Ueno et al., 2003; Ueno et al., 2004). As a highly mobile fish from nearshore waters to the open sea, tunas provide an efficient way to observe a vast environment through the lens of a top predator's diet. Stomach-content analysis of skipjack tuna allows us to indirectly observe the composition and diversity of regional and local ecosystems, trophic dynamics, and provides an indication of the environment's health.

Importance of Crustaceans in the Diet

The two complementary approaches used to characterize the diet of skipjack tuna around O'ahu, stomach-content analysis (SCA) and stable isotope analysis (SIA), revealed that crustaceans were the primary prey taxa. SCA provides a short-term picture of recently ingested prey, which can misrepresent diet composition due to differential digestion rates (Hyslop, 1980; Amundsen and Sánchez-Hernández, 2019). Conversely, SIA integrates the assimilated diet over time using biochemical tracers ($\delta^{13}C$ and $\delta^{15}N$) of consumers and their prey (Phillips et al., 2014; Zanden and Rasmussen, 2001). The dietary metrics from the SCA showed that crustaceans were the most important prey taxa (IRI = 78.4%), with the highest frequency of occurrence (71.2%), relative abundance (N = 89.6%) and relative weight (W = 40.3%). The isotope mixing model showed

two distinct crustacean sources contributed the highest proportion to skipjack tuna diet, accounting for an estimated contribution of 61.1%. As skipjack tuna size increased, the relative importance (IRI) of crustaceans increased from 64.6% to 85.9% from small tuna (< 68.8 cm FL) to large tuna (> 68.8 cm FL).

These results agree with other studies that conclude crustaceans are the predominant prey in skipjack tuna diet, due to their epipelagic feeding habits. For instance, crustaceans made up the highest proportion of the diet by volume (59%) and occurrence (76%) (Alverson, 1963) and number (86.3%) in the tropical Eastern Pacific Ocean (Bernard, 1985). Off the coast of California, crustaceans (krill and pelagic red crabs) were the most important prey by IRI (Alatorre Ramírez, 2007). In Fuller et al. (2021), two arthropod species (pelagic red crab and krill) comprised the second most important prey in two regions in the Eastern Pacific Ocean by weight (W=21% and 35%, respectively). Compared to other tuna species in the Pacific Ocean, crustaceans dominate skipjack tuna diets (Olson et al., 2016; Young et al., 2010).

The results in this study suggest that skipjack tuna benefit from consuming crustaceans because they may be easier to catch, allowing tuna to spend less energy foraging (Menard, 2006). Additionally, skipjack tuna have small gill raker gaps which gives them the advantage of retaining smaller prey, like crustaceans (Magnuson & Heitz, 1971). The size of a predator has a relationship with their gill raker gap. Skipjack tuna are the smallest tuna, and they have the smallest mean gill raker gap (0.51), compared to other larger scombrid species (Magnuson & Heitz, 1971). This may explain why crustaceans contribute a low portion to the diet of larger

tuna species (yellowfin, bluefin, bigeye) because they cannot retain small crustaceans as well as skipjack tuna (Olson et al., 2016).

These results contrast with the general pattern documented in the Eastern Pacific Ocean. Alverson (1963) used conventional stomach-content analyses for skipjack in offshore waters along California to Chile, and overall, the top three prey by volume were crustaceans (49%, Family Euphausiidae), bathypelagic fish (10%, Family Gonostomatidae), and epipelagic flying fish (9%, Family Exocoetidae). In particular, skipjack in offshore waters of the EPO consumed a high volume of deep-water fish like *Vinciguerria lucetia* (Gonostomatidae, 10%) and lantern fishes (Myctophidae, 6%), which revealed skipjack foraging grounds expand below the surface. In a different study in the EPO, fishes were the predominant prey; two species of anchovies (*Cetengraulis mysticetus* and *Engraulis mordax*) dominated in upwelling provinces, while mesopelagic (*Vinciguerria lucetia)* and epipelagic (*Decapterus* spp.) fish dominated in coastal/offshore provinces (Fuller et al., 2021). In the Western Pacific Ocean, the diet of skipjack tuna is dominated by fish and squid, ranging from fish who are epipelagic, demersal, pelagic-neritic, and benthopelagic, indicating skipjack in this region are foraging below the surface and away from shore (Chang et al., 2022). Here in Hawai‘i, skipjack are foraging at the surface for epipelagic prey because there was no evidence for deep-water species or vertically migrating mesopelagic prey from their stomach contents or indications from their $\delta^{15}N$ values.

Importance of Reef Prey

Tuna are opportunistic predators who consume a variety of prey species and adapt to utilize regionally available prey in habitats that maximize their food intake (Bertrand et al., 2002;

Graham & Dickson, 2004). Near oceanic islands, this opportunistic diet involves nearshore crustaceans and larval and juvenile fishes from reef-associated families, such as Acanthuridae, Balistidae, Carangidae, Chaetodontidae, Mullidae, and Priacanthidae (McCoy et al., 2018; Randall, 2010). Due to the proximity of our samples to nearshore waters around O‘ahu, it is not surprising that nearshore and reef-associated prey dominated the skipjack diet.

Reef-associated prey play an important role in tuna foraging near islands (Bertrand et al., 2002; Fernandez and Allain, 2011). A study examining the diet of yellowfin in the Central and Western Pacific highlights the importance of reef crustaceans (stomatopods and megalop crab stages) and juvenile reef-fish, specifically around Papua New Guinea and French Polynesia (Allain, 2004). In addition, yellowfin tuna caught near O‘ahu identified similar prey found in skipjack like Stomatopoda, Decapod Megalopae, Ommastrephidae, and reef fish (Mullidae) (Graham et al. 2007). This is the first study to describe the diet composition of skipjack tuna around Hawai‘i, which highlights the importance of inshore reef fish and reef crustaceans (stomatopods, megalop stages, shrimp) to tuna around islands. Surface slicks are areas where the ocean surface converges and diverges, creating a highly productive region and providing a nursery habitat for early stages of reef prey. Alternatively, this creates a suitable foraging ground for tuna (Whitney et al., 2021).

In this study, 16 fish taxa were identified and 75% (n=12) were reef-associated fishes and 25% (n=4) were pelagic fishes. Although reef-associated species dominated the fish prey taxa, their index of relative importance was relatively low (4.88%) due to their low abundance and weight as small larval and juvenile fish. Skipjack tuna are epipelagic predators, foraging on nearshore

prey, evident by the diversity of juvenile reef fish and the contribution of reef crustaceans to their diet. Studies predict the size of prey gradually increases as predators get larger due to an increase in predator gape size, enhanced swimming speed, or endothermic abilities (Block et al., 1993; Magnuson & Heitz, 1971; Menard et al., 2006). In this study, skipjack tuna were primarily consuming nearshore prey.

Changes in Skipjack Tuna Carbon Sources

Nitrogen and carbon isotopes are used to understand food webs and the trophic levels of prey and predators. Each trophic level is enriched by 3-4‰ $\delta^{15}N$ and 0.5-1.0‰ $\delta^{13}C$ between prey and predator (Michener & Kaufman, 2007). Previous studies have found evidence for ontogenetic diet shifts in skipjack tuna. In southwestern Atlantic Ocean, isotope mixing models portray a decrease of lanternfish and krill and an increase of cephalopods and pelagic fish with skipjack size (Coletto et al., 2021). Additionally, skipjack in the southern area had a greater mean $\delta^{15}N$ relative to those in the northern area, suggesting different prey sources. Reglero et al. (2014) found that skipjack and yellowfin tuna (*Thunnus albacares*) have similar life histories because they are often found together. In nearshore waters of Hawai‘i, the diet of yellowfin showed a $\delta^{15}N$ shift at a fork length of 45 cm, with the smaller juveniles mainly consuming crustaceans in shallow water and the larger juveniles consuming *Oplophorus gracilirostris* (shrimp) and fish in deeper water (Graham et al., 2007). These studies underscore that the size (fork length) where an ontogenetic diet shift is apparent is region-specific and species-specific.

Although there was no increase in $\delta^{15}N$ for larger-size skipjack, there was a shift in the linear increasing trend of $\delta^{13}C$ with skipjack size. In fact, there was a significant step-like $\delta^{13}C$

increase, indicative of a shift in foraging grounds at a fork length size of 68.8 cm. While the $\delta^{13}C$ difference between the mean of the small (< 68.8 cm FL) and the large (> 68.8 cm FL) tuna was 0.5‰ there was no concurrent $\delta^{15}N$ step-like increase. Thus, rather than a trophic level increase, the observed difference in $\delta^{13}C$ suggests there was a change in carbon source for a food web, driven by dissolved inorganic carbon (DIC) variations with location (Peterson & Fry, 1987). In other words, while the larger and smaller tunas consume similar trophic level prey, they are supported by different food webs. A difference in $\delta^{13}C$ values can indicate nearshore versus offshore feeding grounds because consumers in nearshore ecosystems are enriched in $\delta^{13}C$ values while offshore and open-ocean ecosystems are depleted in $\delta^{13}C$ values (Hobson, 1994; Johnson & Schindler, 2009). Another possibility for their shift in $\delta^{13}C$ may represent skipjack tuna that recently migrated from productive regions (enriched $\delta^{13}C$) to the Hawaiian Islands. If prey sources vary by regions, this hypothesis is not strongly supported because their $\delta^{15}N$ does not change. I hypothesize that as skipjack tuna get larger, they forage in nearshore waters around islands due to their enrichment in $\delta^{13}C$ values with fork length size.

Future Considerations

The results of this study show that skipjack tuna consume primarily small nearshore prey. As they forage around the Hawaiian Islands, like O‘ahu, their diet consists of reef-associated larval, pre-settlement fish and primarily reef crustaceans. Within the skipjack size range of this sample (40-84 cm FL), a shift in carbon source was detected at a fork length of 68.8 cm, with a significant increase in $\delta^{13}C$, implying that smaller and larger tuna are utilizing different food webs with a change in carbon source baselines. Ongoing diet studies may capture an earlier ontogenetic diet shift for skipjack tuna by expanding the size range of sampled skipjack to

include juvenile tuna. Additional prey species could be identified by DNA metabarcoding highly digested contents (sensu Himmelsbach 2021; Nimz et al., 2021), using other extraction techniques to obtain higher quality DNA, and trying different PCR primer sets to acquire better species matches in GenBank. Future studies around Hawai‘i should consider sampling tuna offshore to test if their diet changes.

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Tables

Table 1. Skipjack tuna sample sizes for 10-cm size classes. Total sample size is 121 fish, spanning 40 cm to 84 cm FL.

Fork length interval (cm)	Sample size	Proportion
40-49.9	2	1.7
50-59.9	28	23.1
60-69.9	42	34.7
70-79.9	44	36.4
80-89.9	5	4.1

Table 2. Stomach fullness criteria used to determine tuna stomach content, with examples (modified from Tanabe (2001)).

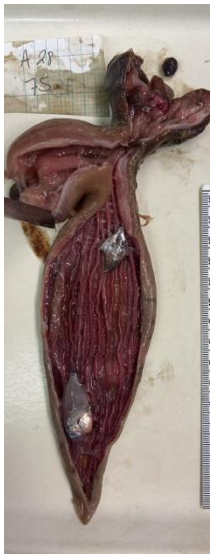
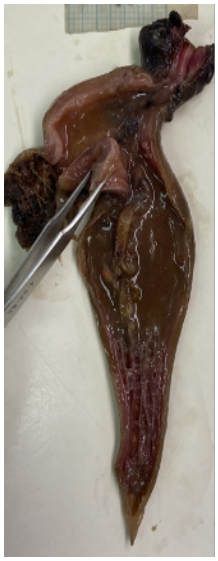
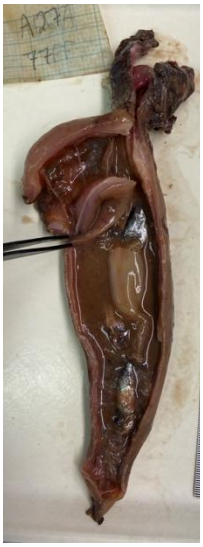
Stomach Fullness Scores					
0	**1**	**2**	**3**	**4**	**5**
Stomach is completely empty	Stomach is empty with the exception of a few remains/hard parts that are unidentifiable or at most 2 small prey items	Stomach is less than half-full with remains/hard parts and/or more than 2 prey items	Stomach is half-full with a mixture of prey items and parts that are identifiable	Stomach is more than half-full with prey items and parts that are identifiable, stomach is not completely full	Stomach is full, with abundant or identifiable prey
					

Table 3. Proportion of stomachs in this study with designated stomach fullness scores described in Table 2.

Stomach Fullness Score	Number of Stomachs (Proportion %)
0	3 (2.5)
1	24 (19.8)
2	22 (18.2)
3	29 (23.9)
4	25 (20.7)
5	18 (14.9)

Table 4. Description of digestion state scores (modified from Donahue et al., 2021).

Digestion state scores			
Prey	1 (intact condition)	2 (fair condition)	3 (poor condition)
Description of prey item	'Perfectly intact': identifiable prey item with colorful skin/scales, all body parts are present, able to take standard measurements of prey item	'Somewhat intact': identifiable prey type with signs of discoloration and some intact skin/scales, most body parts are present, some tissue missing, standard measurements are not always guaranteed if head or tail are missing, or squid pen is sticking outside the mantle	'Incomplete': might or might not be identifiable, poor condition, no coloration of skin/scales, missing body parts, crustaceans are soft-bodied, squid mantle is not firm, fish are showing vertebrae and digested tissue, standard measurements cannot be taken

Table 5. Examples for digestion state scores for three broad prey categories (fish, crustaceans, and squid).

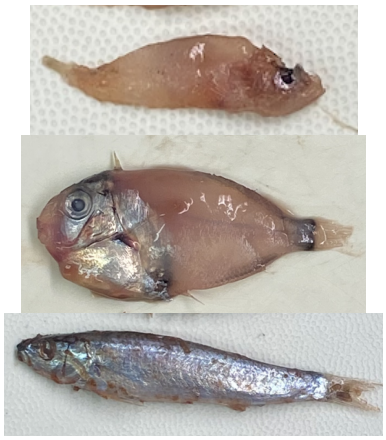
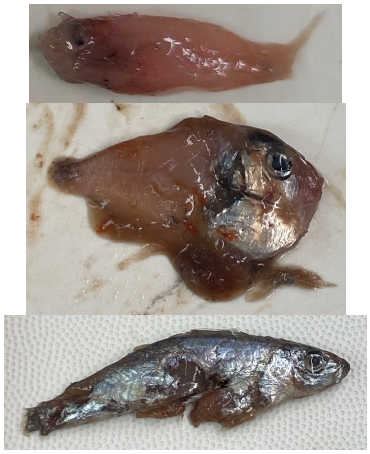
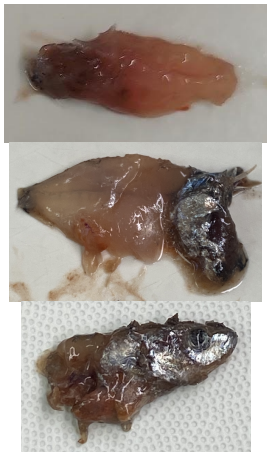
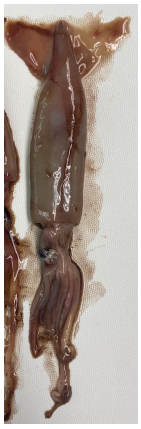
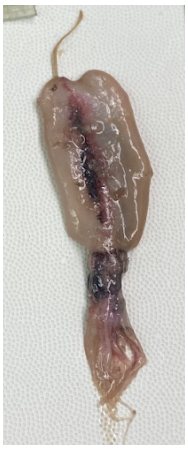
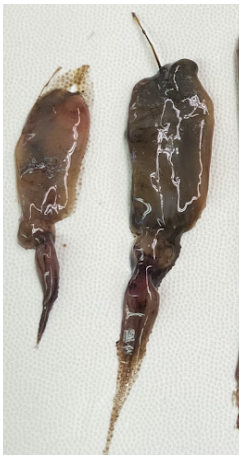
Digestion state scores			
Prey	**1 (intact condition)**	**2 (fair condition)**	**3 (poor condition)**
Fish			
Crustacean			
Cephalopoda			

Table 6. Dietary composition of skipjack tuna identified as three broad taxonomic groups: crustacean, fish, cephalopod by percent by weight (proportion of the total mass of a prey item to the total mass of all prey items, W%), percent by number (proportion of the total abundance of a prey item to the total abundance of all prey items, N%), frequency of occurrence (proportion of each prey item present relative to the total number of stomachs containing prey, FO%), and index of relative importance (proportion of composite measure of each prey item to the total index of relative importance of all prey items, IRI%).

Prey Taxa	W %	N %	FO %	IRI %
Crustacean	40.3	89.6	71.2	78.4
Fish	40.0	9.5	43.2	18.2
Cephalopod	19.7	0.9	19.5	3.4

Table 7. Prey taxa and sample size for stable isotopes analysis (S.I.A).

Prey Taxa	**Total Abundance in Stomachs**	**Sample size for S.I.A**
Fish	383	53
Crustaceans	2972	47
Cephalopods	36	14
Total	**3391**	**114**

Table 8. Prey taxa and the number of prey selected for barcoding from the total abundance found in skipjack stomachs. From the number of prey selected, only a certain number were successfully barcoded, shown by the barcoding success rate in percent.

Prey Taxa	**Total Abundance in Stomachs**	**Prey selected for barcoding**	**Successfully barcoded prey**	**Barcoding success rate (%)**
Fish	383	133	71	4.4
Crustaceans	2972	206	10	0.34
Cephalopods	36	36	12	33.3
Total	**3391**	**375**	**93**	**2.7**

Table 9. Hard part prey remains of found in skipjack stomachs by total number in stomachs (N) and frequency of occurrence (proportion of each hard part item present relative to the total number of stomachs with contents, FO%).

Hard Part Remains	**N**	**FO %**
Squid beaks	56	22.0
Squid pens	7	3.4
Otoliths	18	5.9
Hard Part TOTAL	81	28.8

Table 10. Dietary composition of skipjack tuna identified in this study by: percent by weight (proportion of the total mass of a prey item to the total mass of all prey items, W%), percent by number (proportion of the total abundance of a prey item to the total abundance of all prey items, N%), frequency of occurrence (proportion of each prey item present relative to the total number of stomachs containing prey, FO%), and index of relative importance (proportion of composite measure of each prey item to the total index of relative importance of all prey items, IRI%). Empty cells are prey items that could only be identified to family level. Unidentified prey are marked with an asterisk*.

Phylum	**Class**	**Order**	**Family**	**Species**	**FO%**	**N%**	**W%**	**IRI%**
Arthropoda	Malacostraca	Decapoda		Order Decapoda* (crab megalopae)	50.0	82.7	35.2	85.6
				Order Decapoda* (larval shrimp)	21.2	1.4	1.1	0.8
			Carpiliidae	*Carpilius convexus*	13.6	2.3	1.8	0.8
		Stomatopoda	Odontodactylidae	*Odontodactylus* spp.	28.0	1.9	2.0	1.6
		Amphipoda	Hyperiidae*		3.4	0.2	0.05	0.01
Mollusca	Cephalopoda	Oegopsida	Ommastrephidae	*Sthenoteuthis oualaniensis*	18.6	0.8	19.6	5.5
		Octopoda	Ocythoidae	*Ocythoe tuberculata*	0.8	0.03	0.1	0.002
	Gastropoda	Pteropoda		Family Cavolinidae	2.5	0.1	0.05	0.01
Chordata	Actinopterygii	Mulliformes	Mullidae	*Parupeneus* spp.	13.6	2.4	15.0	3.4
				Mulloidichthys flavolineatus	0.8	0.03	0.3	0.004
		Tetraodontiformes	Balistidae	*Sufflamen* spp.	5.1	0.3	0.4	0.05
			Monacanthidae	*Cantherhines* spp.	7.6	0.4	0.7	0.1
			Blenniidae	*Cirripectes* spp.	8.5	0.5	0.8	0.2
			Ostraciidae	*Lactoria fornasini*	2.5	0.1	0.4	0.02
		Acanthuriformes	Acanthuridae	*Acanthurus nigrofuscus*	5.9	0.3	1.5	0.2

			Chaetodontidae	*Forcipiger flavissimus*	0.8	0.03	0.03	0.001
				Chaetodon kleinii	11.9	0.8	1.5	0.4
		Perciformes	Priacanthidae	*Heteropriacanthus* spp.	1.7	0.1	0.3	0.01
			Lutjanidae	*Lutjanus kasmira*	0.8	0.03	0.1	0.001
		Scombriformes	Scombridae	*Katsuwonus pelamis*	1.7	0.1	6.6	0.2
				Euthynnus affinis	2.5	0.3	4.9	0.4
		Carangiformes	Carangidae	*Selar crumenophthalmus*	5.1	0.8	4.9	0.4
				Decapterus spp.	0.8	0.03	0.1	0.002
		Aulopiformes	Synodontidae	*Synodus variegatus*	7.6	3.1	1.5	0.5

Table 11. Dietary composition of small tuna (less than 68.8 cm fork length, N=68) and large tuna (greater than 68.8 cm fork length, N=53) by three broad prey taxonomic groups (crustacean, fish, cephalopod) measured in percent by weight (proportion of the total mass of a prey item to the total mass of all prey items, W%), percent by number (proportion of the total abundance of a prey item to the total abundance of all prey items, N%), frequency of occurrence (proportion of each prey item present relative to the total number of stomachs containing prey, FO%), and index of relative importance (proportion of composite measure of each prey item to the total index of relative importance of all prey items, IRI%).

Prey Taxa	Dietary Measure	Small Tuna (< 68.8 cm FL)	Large Tuna (> 68.8 cm FL)
Crustacean	N%	71.6	92.4
	W%	14.9	61.3
	FO%	71.6	70.6
	IRI%	64.6	85.9
Fish	N%	18.9	7.1
	W%	58.4	24.8
	FO%	41.8	45.1
	IRI%	30.9	11.4
Cephalopod	N%	2.0	0.6
	W%	26.7	13.9
	FO%	16.4	23.5
	IRI%	4.5	2.7

Table 12. Mean and standard deviation $\delta^{15}N$, $\delta^{13}C$, and C:N Ratio of all skipjack tuna, small skipjack (< 68.8 FL cm), and large skipjack (> 68.8 FL cm). The sample size (*n*) for each group.

	n	***$\delta^{15}N$*** **(Mean+/- S.D)**	***$\delta^{13}C$*** **(Mean+/- S.D)**	**C:N Ratio (Mean+/- S.D)**
Small Skipjack	68	9.3 +/- 0.8	-18.0 +/- 0.5	3.1 +/- 0.1
Large Skipjack	53	9.6 +/- 1.1	-17.5 +/- 0.3	3.1 +/- 0.04
Skipjack Tuna	121	9.4 +/- 0.9	-17.8 +/- 0.5	3.1 +/- 0.1

Table 13. Summary of $\delta^{15}N$ model results, including simple and multiple linear regressions and Akaike Information Criteria (AIC) criteria for selecting the model with the best fit. The p-value for significant predictor intercept(s) are highlighted in bold font. The best-fit model, with the smallest AIC is highlighted in bold font.

Predictor(s)	Regression Model	P-value for Predictor(s)	Model F (DF)	Model p (Result)	Adjusted R^2	AIC
Fork length	y = 8.16 + 0.018 * (FL)	0.064	(1,119) 3.49	0.064	28.5	326.7
Julian day	y = 11.69 - 0.011 * (JD)	**<0.0001**	(1,119) 27.27	**<0.0001**	18.6	305.3
C:N ratio	y = 6.012 + 1.088 * (C:N)	0.453	(1,119) 0.56	0.453	0.47	329.7
Fork length Julian Day	y = 9.83 + 0.035 * (FL) - 0.014 * (JD)	**FL: 0.000145 JD: <0.0001**	(1,119) 23.0	**<0.0001**	28.0	**292.4**
Fork length C:N Ratio	y = 4.71 + 0.018 * (FL) + 1.10 * (C:N)	FL: 0.063 C:N: 0.441	(2,118) 2.03	0.134	3.33	328.1
Julian day C:N Ratio	y = 12.93 - 0.011 * (JD) - 0.38 * (C:N)	**JD: <0.0001** C:N: 0.773	(2,118) 13.57	**<0.0001**	18.7	307.2
Fork length Julian day C:N Ratio	y = 12.00 + 0.035 (FL) - 0.014 * (JD) - 0.68 (C:N)	**FL: 0.000139 JD: <0.0001**	(3,117) 15.34	**<0.0001**	28.2	294.1
Fork length Julian day Interaction: FL x Julian Day	y = 9.45 + 0.04 * (FL) - 0.012 * (JD) - 0.000029 * (INT.)	FL: 0.372 JD: 0.446 Int.: 0.899	(3,117) 15.21	**<0.0001**	28.0	294.4

Table 14. Summary of $\delta^{13}C$ model results, including simple and multiple linear regressions and Akaike Information Criteria (AIC) criteria for selecting the model with the best fit. The p-value for significant predictor intercept(s) are highlighted in bold font. The best-fit model, with the smallest AIC is highlighted in bold font.

Predictor(s)	Regression Model	P-value for Predictor(s)	Model F (DF)	Model p (Result)	Adj R^2	AIC
Fork length	y = -19.340989 + 0.023501(FL)	**<0.0001**	(1,119) 3.49	**<0.0001**	18.9	133.4
Julian Day	y = -18.733032 + 0.004894(JD)	**<0.0001**	(1,119) 27.27	**<0.0001**	13.7	140.9
C:N Ratio	y = -9.9620 - 2.5015(C:N)	**0.00032**	(1,119) 0.56	**0.00032**	10.3	145.6
Fork length Julian Day	y = -19.76 + 0.019(FL) - 0.003(JD)	**FL: <0.0001 JD: 0.00164**	(1,119) 23	**<0.0001**	25.5	125.2
Fork length C:N Ratio	y = -11.59 + 0.023(FL) - 2.48(C:N)	**FL: <0.0001 C:N: <0.0001**	**(2,118) 2.03**	**<0.0001**	29.1	119.2
Julian day C:N Ratio	y = -12.43 + 0.0041(JD) - 1.97(C:N)	**JD: 0.00027 C:N: 0.0032**	(2,118) 13.57	**<0.0001**	19.9	134.0
Fork length Julian day C:N Ratio	y = -12.96+ 0.02 (FL) + 0.002(JD) - 2.14(C:N)	**FL: <0.0001 JD: 0.013 C:N: 0.00056**	(3,117) 15.34	**<0.0001**	31.0	**114.9**

Table 15. A 4-parameter model (f = yo+a/(1+exp(-(x-xo)/b))) was fitted to $\delta^{13}C$ and $\delta^{15}N$ using fork length as the predictor (x).

$\delta^{13}C$ Parameters	Estimate	S.E.	2.50%	97.50%	t value	p value
b (slope)	-0.525	0.568	-1.65	0.599	-0.925	0.357
a (upper asymptote)	-17.983	0.074	-18.13	-17.836	-242.382	**<2e-16**
yo (lower asymptote)	-17.483	0.096	-17.673	-17.292	-181.898	**<2e-16**
xo (mid-point)	68.782	2.026	64.770	72.794	33.955	**<2e-16**

$\delta^{15}N$ Parameters	Estimate	S.E.	2.50%	97.50%	t value	p value
b (slope)	-0.052	0.035	-0.121	0.016	-1.508	0.134
a (upper asymptote)	-	-	-	-	-	-
yo (lower asymptote)	9.763	0.266	9.236	10.290	36.697	**<2e-16**
xo (mid-point)	-	-	-	-	-	-

Table 16. Classification of identified prey families used in stable isotopes analysis into seven functional groups based on FishBase (www.fishbase.org) and previous studies examining the depth range (Boehlert et al., 1992; Choy et al., 2009) and horizontal distribution of larval reef fish (Leis & Miller, 1976). Note: Order Decapoda[1] was an unidentified crab megalopae and Order Decapoda[2] was an unidentified larval shrimp. The sample size (n) is depicted in parentheses next to each family.

Functional Groups	**Depth Range (m)**	**Family (n)**	**Mean $\delta^{15}N$ +/- SD (‰)**	**Mean $\delta^{13}C$ +/- SD (‰)**
Crustacean 1	0-150 0-200 0-200	Odontodactylidae (11) Order Decapoda[1] (9) Order Decapoda[2] (11)	3.8 +/- 1.3	-20.8 +/- 0.5
Crustacean 2		Carpiliidae (16)	2.4 +/- 0.8	-19.6 +/- 0.8
Inshore Reef Fish (0-50 m)	0-50 0-50	Chaetodontidae (6) Monacanthidae (4)	6.2 +/- 1.2	-20.1 +/- 0.8
Inshore Reef Fish (0-200 m)	0-100 0-100 0-100	Mullidae (17) Acanthuridae (5) Balistidae (4)	5.8 +/- 0.8	-19.3 +/- 0.4
	0-200	Blenniidae (9)		
Pelagic-Neritic	0-100	Carangidae (4)	5.0 +/- 0.1	-18.9 +/- 0.1
Pelagic-Oceanic	0-260	Scombridae (4)	6.8 +/- 0.6	-18.6 +/- 0.2
Squids	0-1000	Ommastrephidae (14)	6.2 +/- 0.4	-19.0 +/- 0.6

Figures

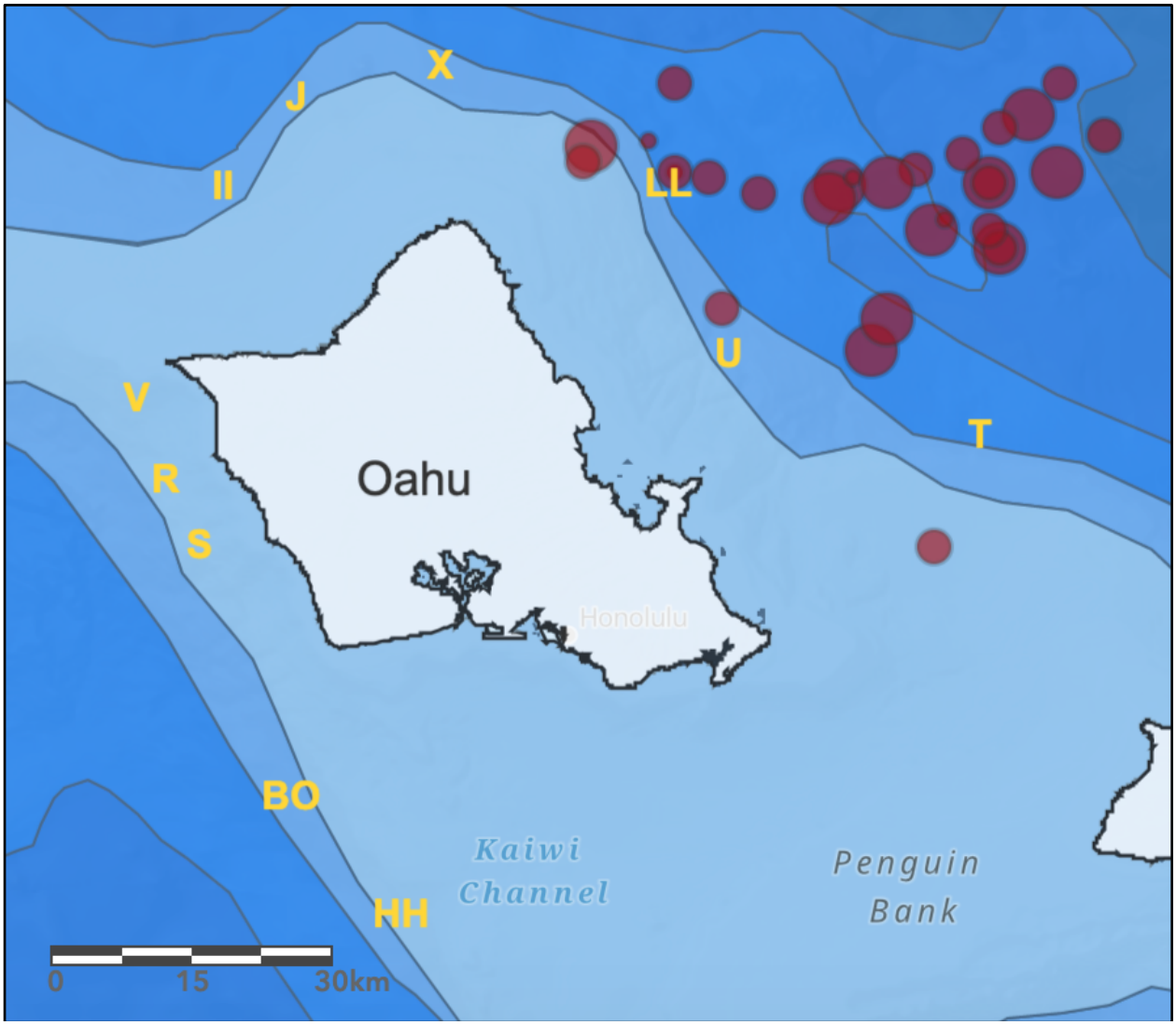


Figure 1. Red circles represent sampling sites where skipjack tuna (*Katsuwonus pelamis,* n=116) were caught between May and September 2020. Three size classes are represented by the number of tuna caught in each site, the small red circles represent 1 tuna caught, the median red circles represent 2 to 3 tuna caught, and the large red circle represent 4 to 10 tuna caught. An additional 5 fish were collected from Penguin Bank during this time. Yellow letters represent fish aggregating devices (FADs) around O‘ahu. The bathymetry is shown by the contour lines in increments of 1000 meters, the innermost contour line represents 1000 meters.

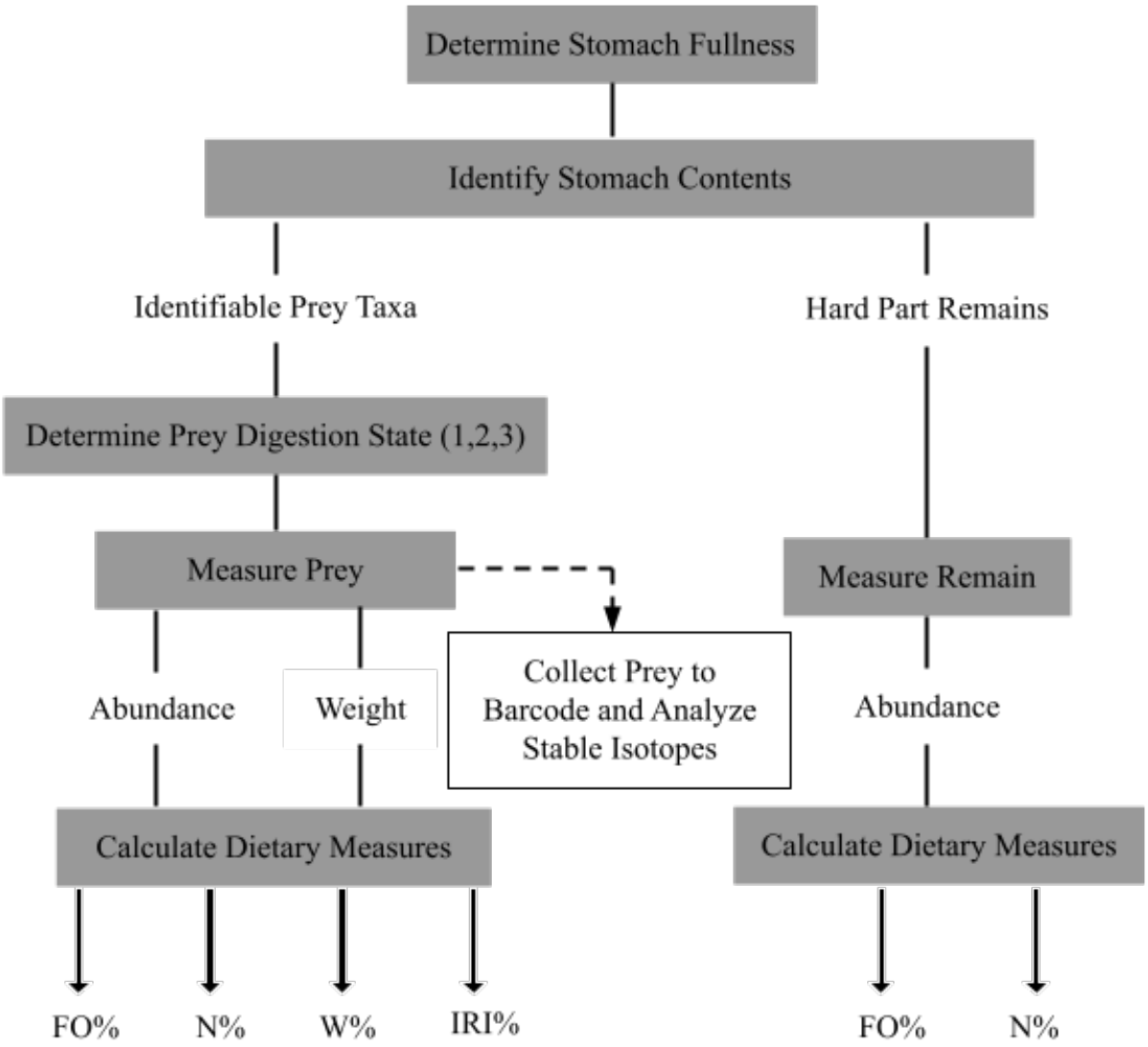


Figure 2. Flow chart listing the sequential steps of diet analysis. Identifiable prey taxa are identified as crustaceans, cephalopods, or fish. Hard part remains were identified as squid beaks, squid pens, otoliths. Digestion states of 3 are not recorded for measurements (weight) because they were incomplete items. Prey from stomach contents were collected to genetically identify with DNA barcoding and analyze for stable isotopes ($\delta^{15}N$ and $\delta^{13}C$).

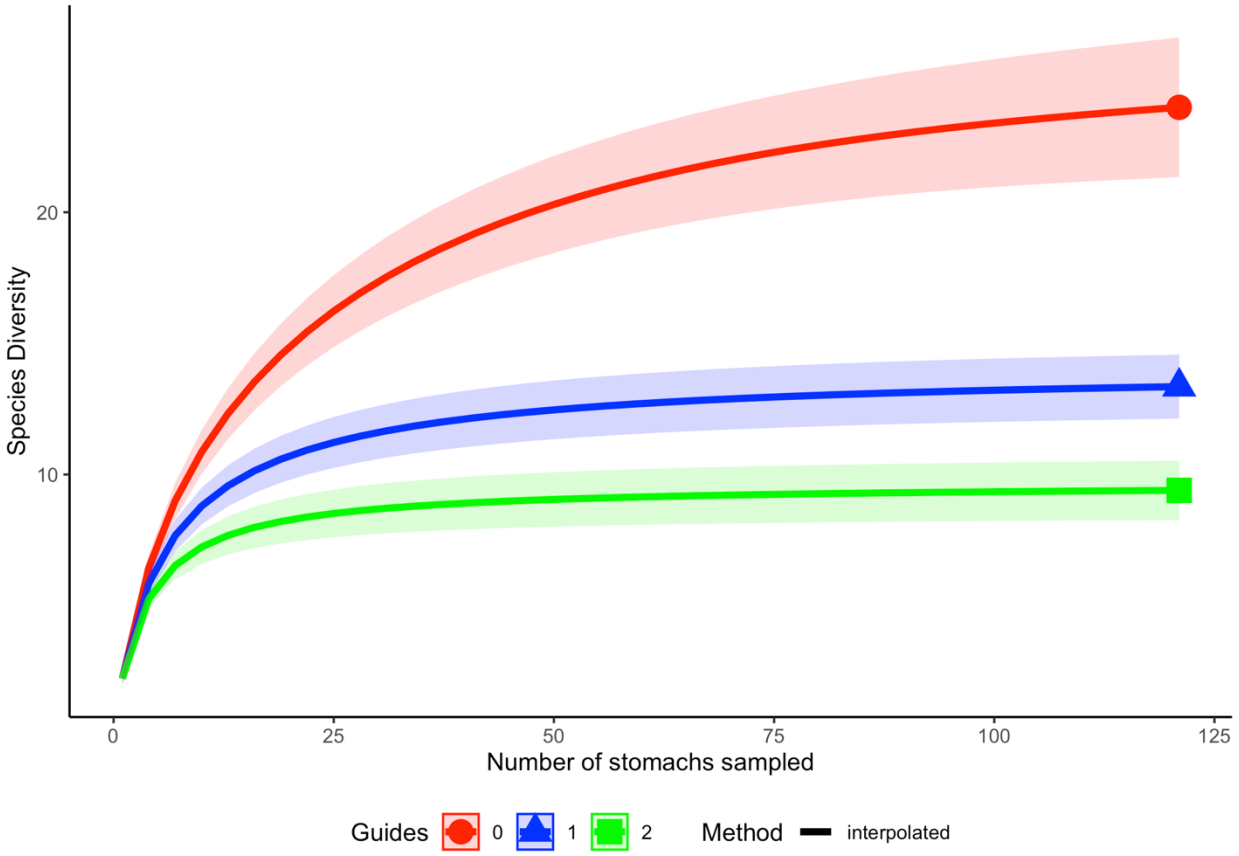


Figure 3. Species accumulation curve for 24 individual taxa. Each line represents a measure of Hill numbers in terms of q: species richness (q=0), Shannon diversity (q=1, the exponential of Shannon entropy), and Simpson diversity (q=2, the inverse of Simpson concentration). For each diversity measure, the observed sample of incidence data (presence/absence) computes the diversity estimates, the associated 95% confidence intervals, and plots the interpolated curves.

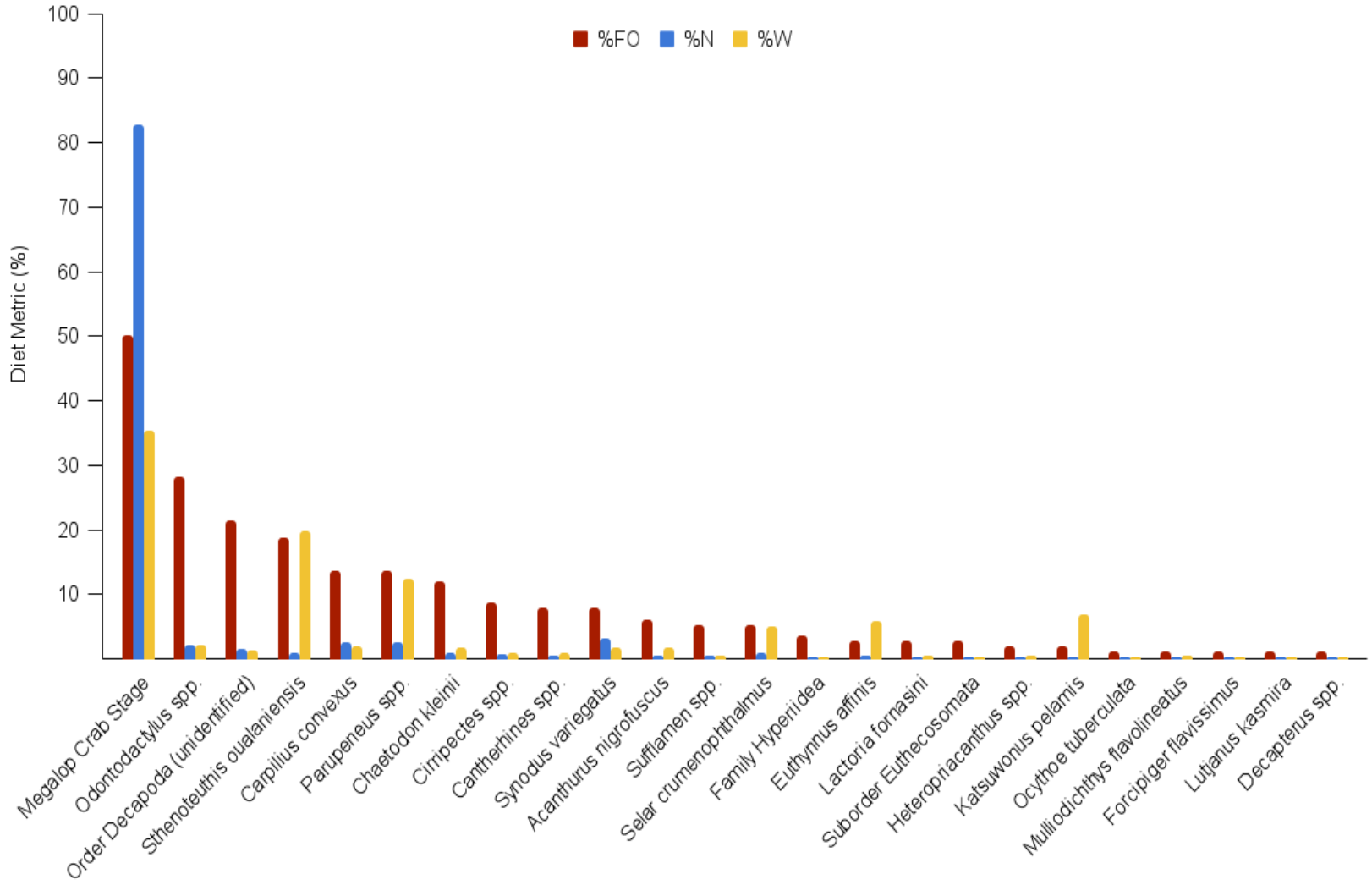


Figure 4. Proportion of individual prey taxa in skipjack tuna stomachs measured in three diet metrics: frequency of occurrence (% FO, red bars), abundance (%N, blue bars), and weight (% W, yellow bars).

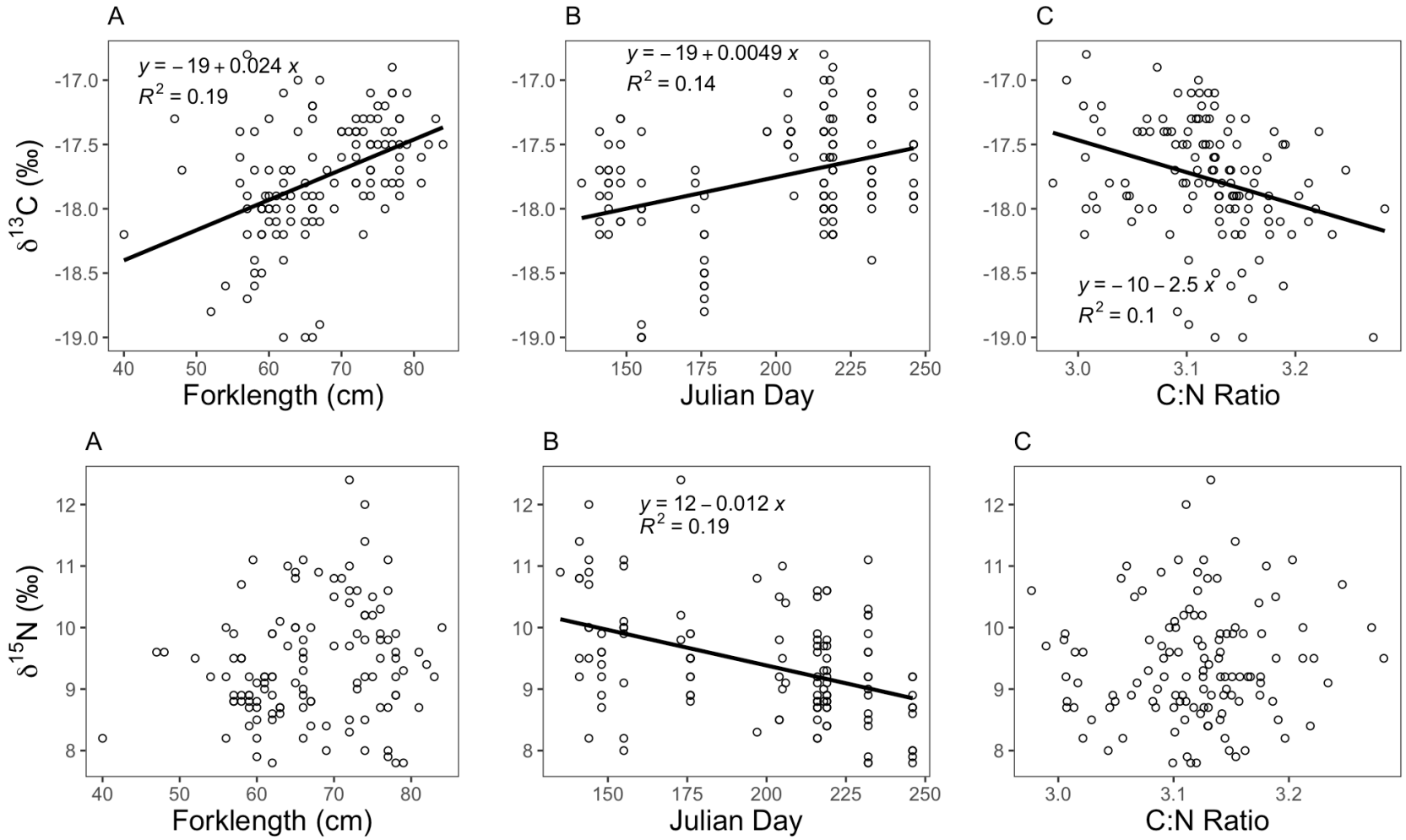


Figure 5. A $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values of skipjack tuna white muscle tissue over their fork length. **B** $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values of skipjack tuna white muscle tissue over the date they were caught (Julian Day). **C** $\delta^{13}C$ (top) and $\delta^{15}N$ (bottom) values of skipjack tuna white muscle tissue over their C:N Ratio. A linear regression was fitted to each model (solid line) and their linear regression equation and R^2 are shown in each graph. Graphs with no solid line means that the linear regression model was not significant.

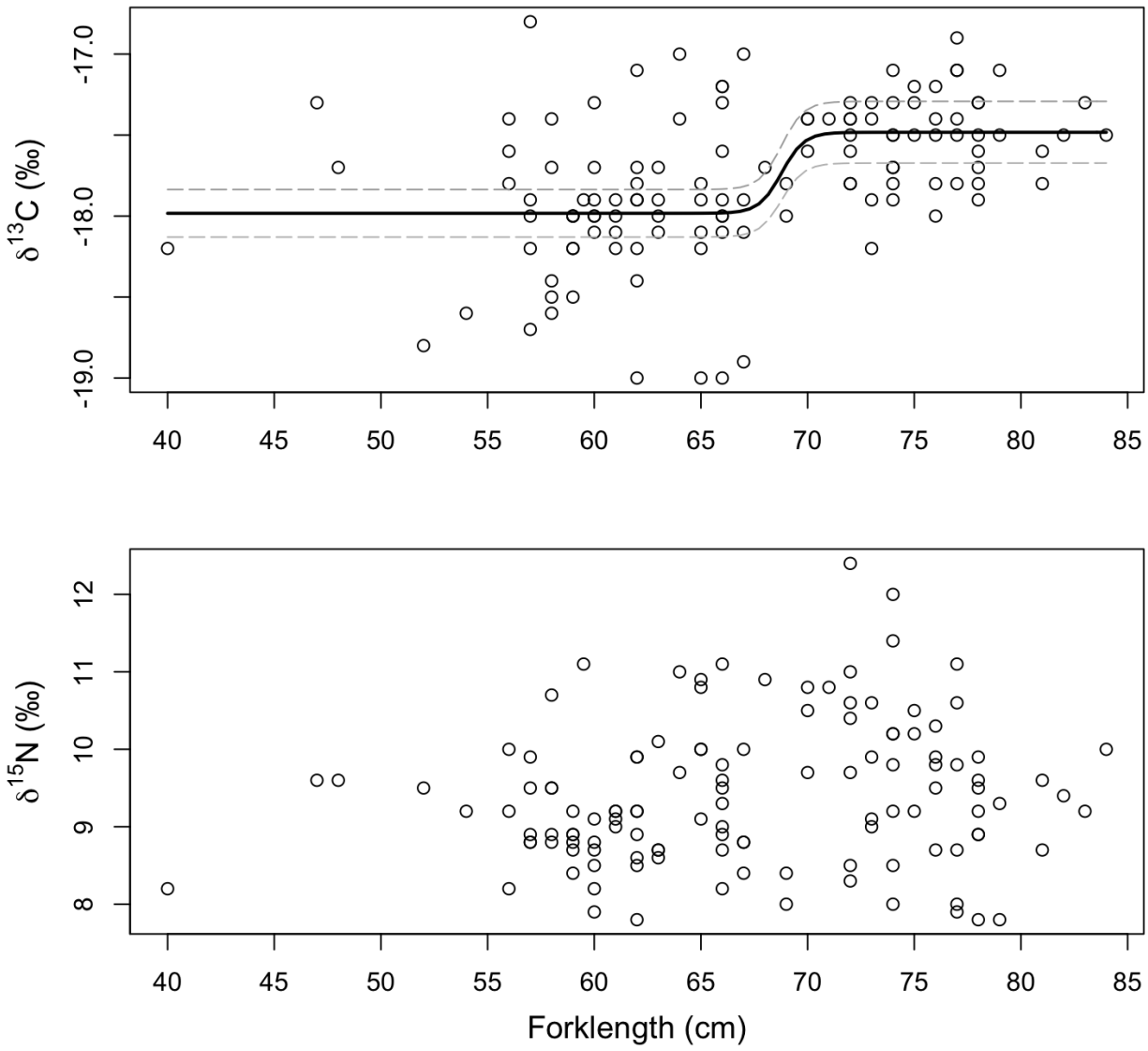


Figure 6. $\delta^{13}C$ and $\delta^{15}N$ values of skipjack tuna white muscle tissue over a range of fork lengths. A 4-parameter sigmoid model (solid line, f = -17.483 + (-0.5)/(1+exp(-(x-68.782)/-0.525), $R^2 = 0.23$) was fitted to $\delta^{13}C$ and there was no 4-parameter model detected to fit $\delta^{15}N$. The gray dashed lines represent the 95% confidence interval of the model.

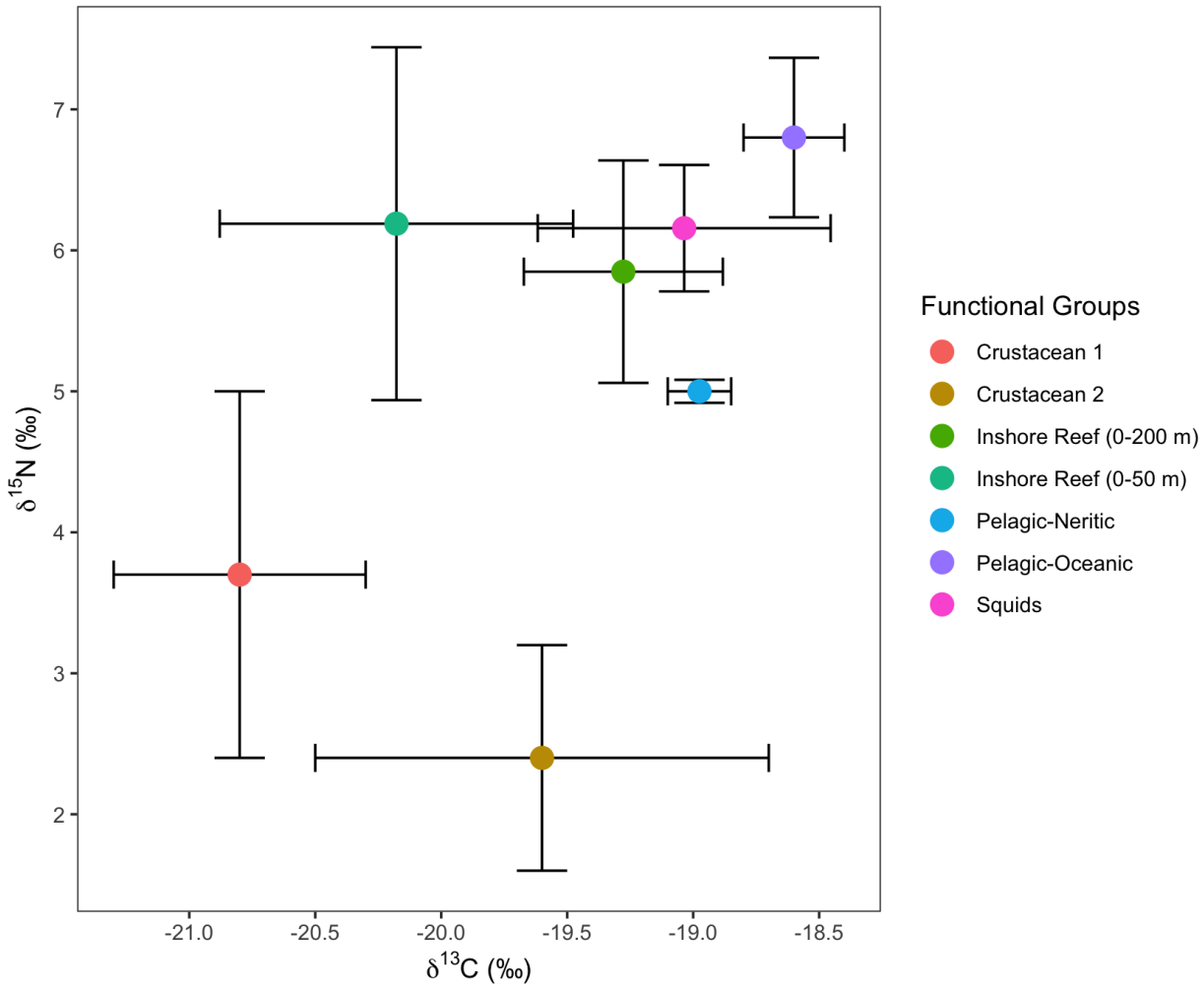


Figure 7. Mean $\delta^{13}C$ and $\delta^{15}N$ values for functional groups (Inshore Reef (0-50 m), Inshore Reef (0-100 m), Inshore Reef (0-200 m), Pelagic Neritic, Pelagic Oceanic, and Squids) of prey muscle. Error bars represent the standard deviation.

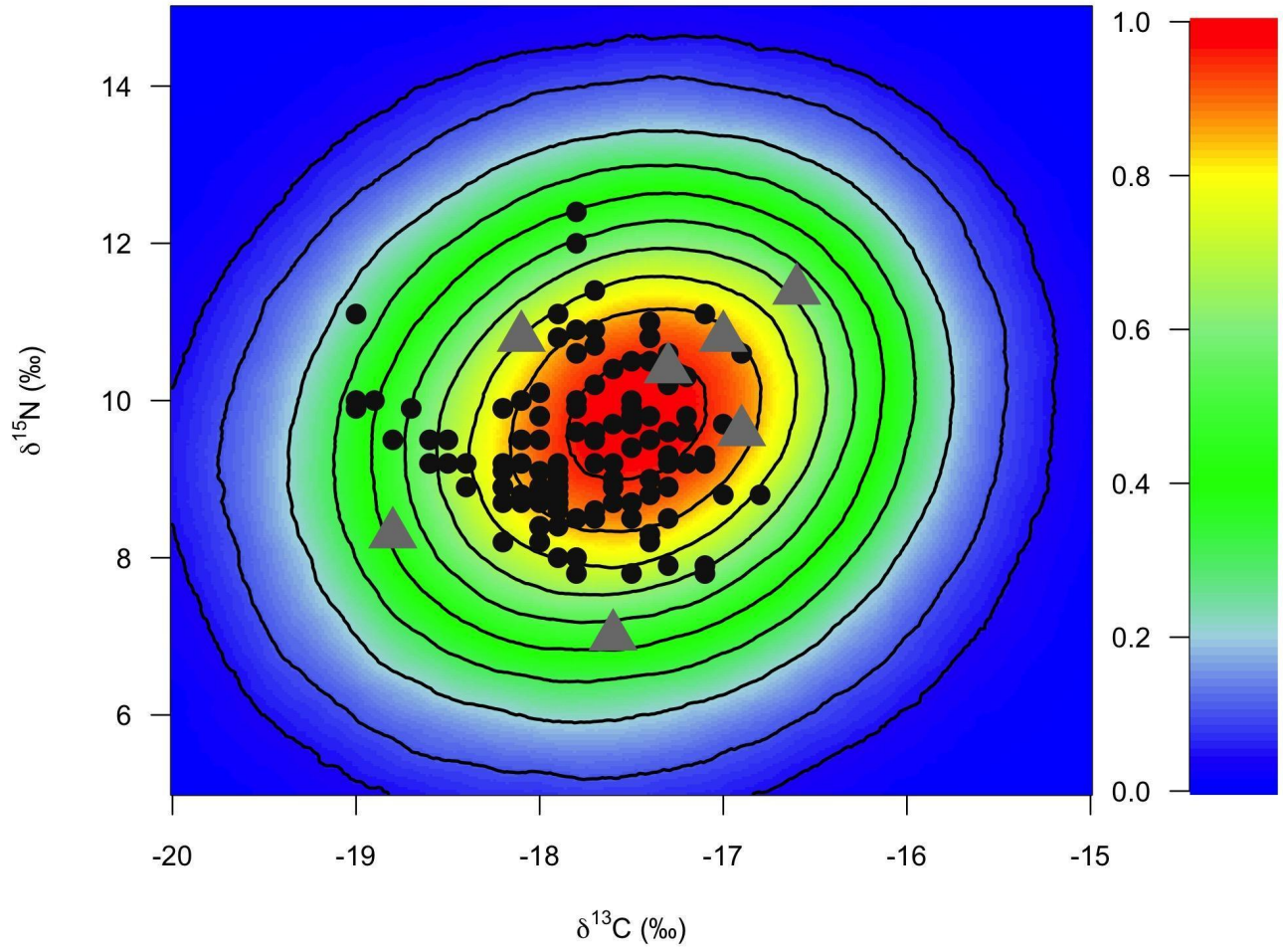


Figure 8. The mixing polygon simulation, following Smith et al. (2013) for the bivariate isospace plot of skipjack $\delta^{13}C$ and $\delta^{15}N$ values (black circles) and the average isotope values for 7 sources (gray triangles). Probability contours are shown in increments of 10%, starting at 5% (outermost contour). The simulation was run with 5,000 iterations.

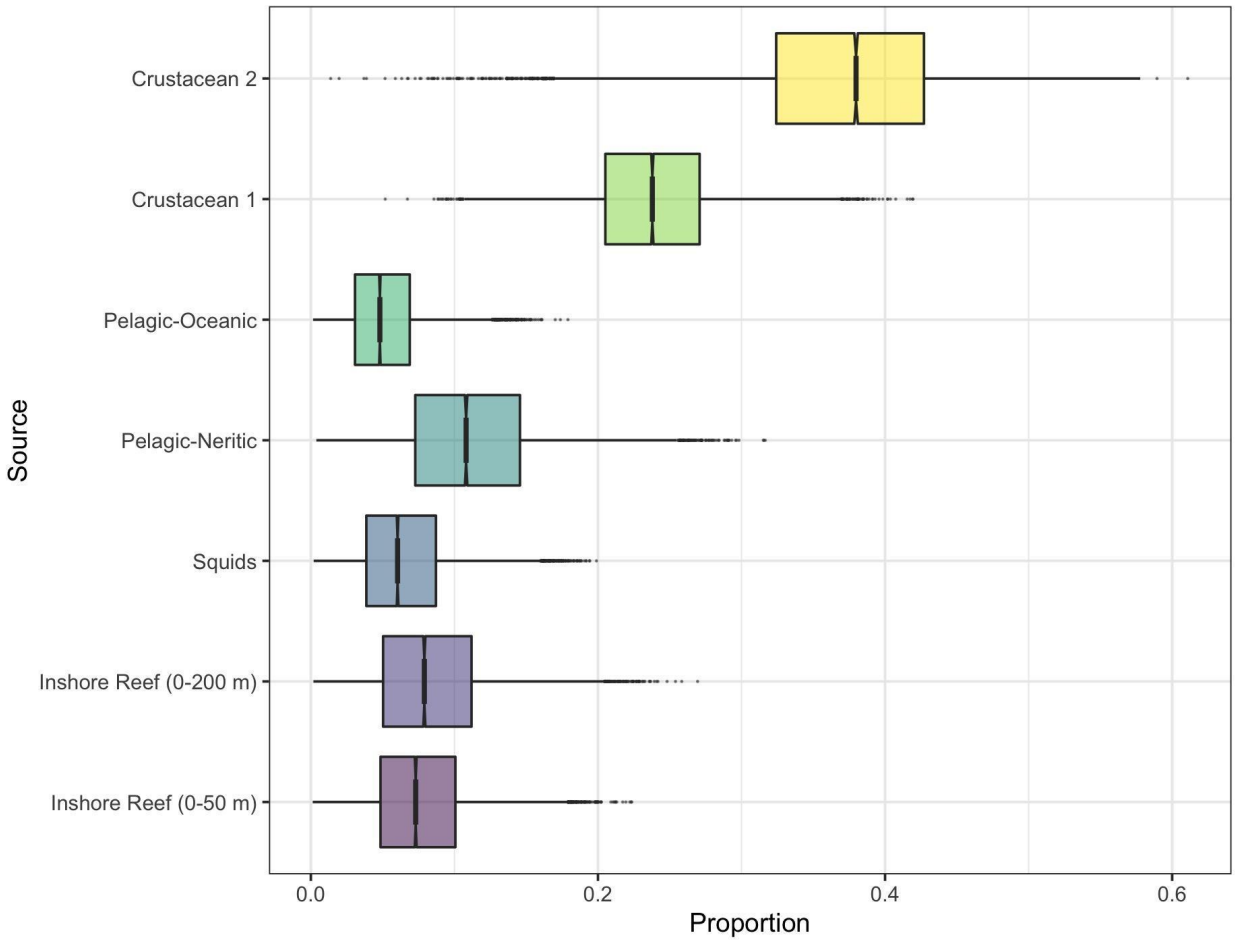
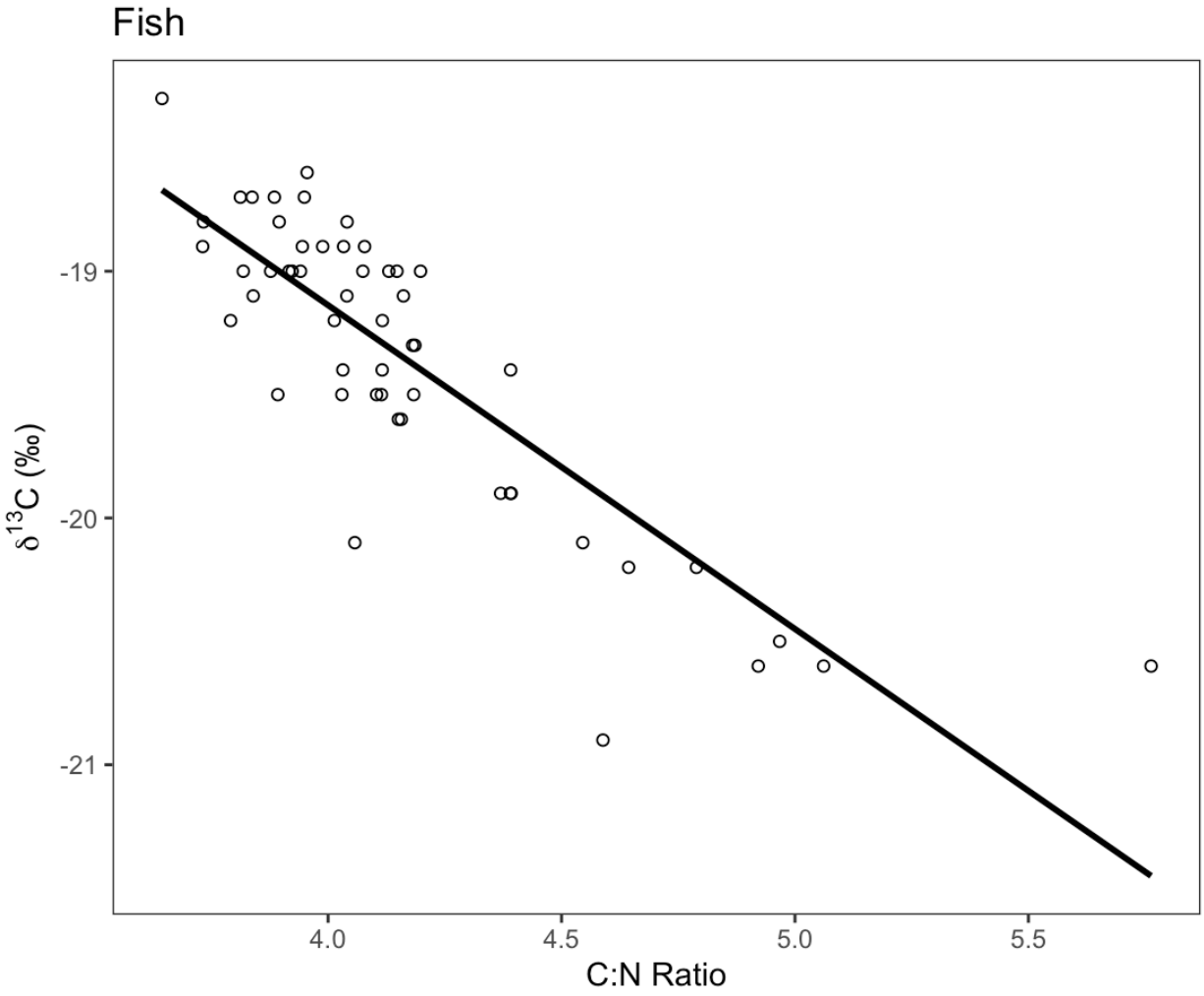
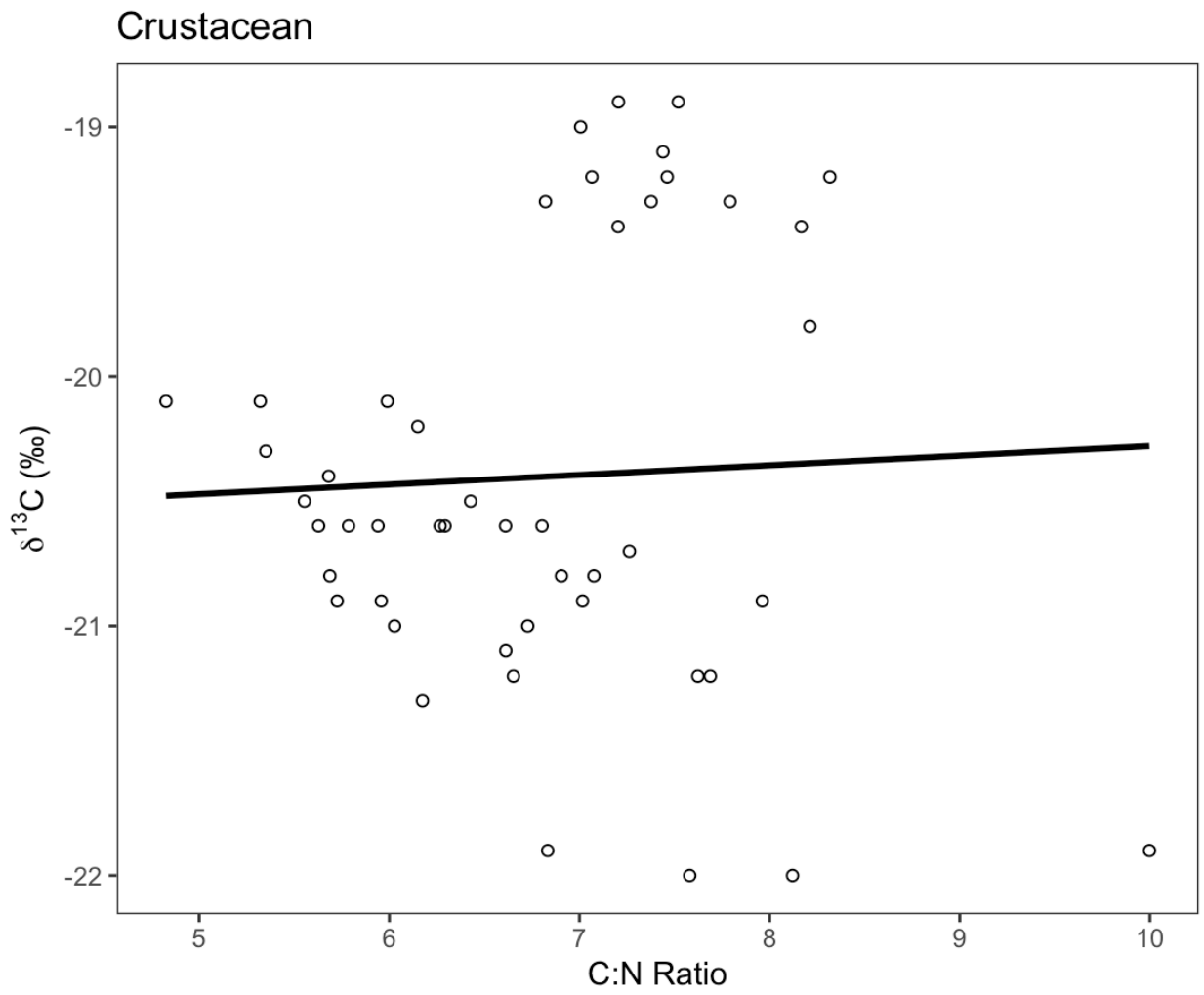


Figure 9. Estimated proportion of prey sources from the diet of skipjack tuna. The Bayesian dietary mixing model shows the minimum, interquartile range, median, and maximum from the posterior probability distribution for each dietary source.

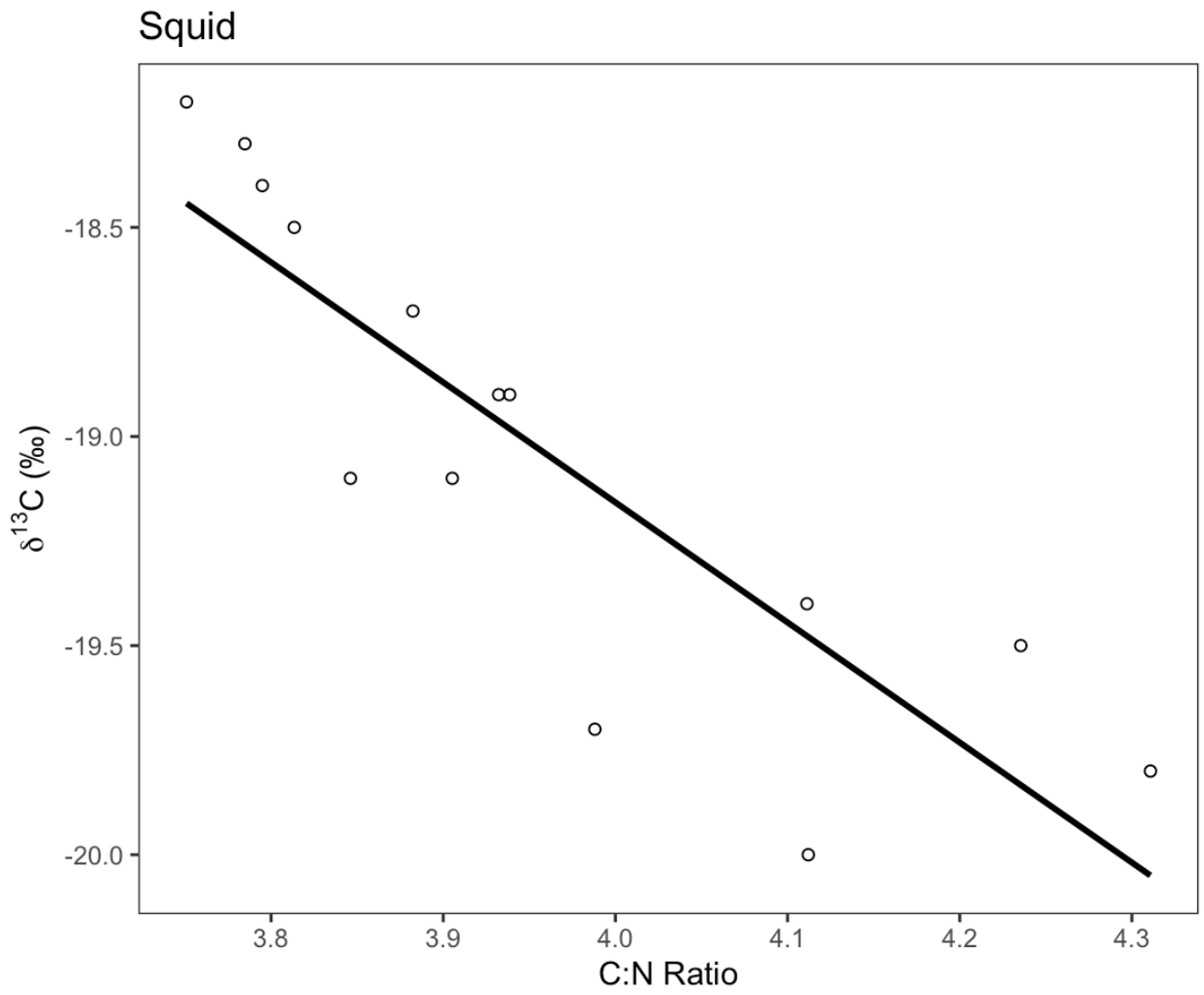
Appendix 1: Prey Stable Isotopes



Appendix Figure 1. $\delta^{13}C$ and molecular C:N ratio of fish prey from skipjack stomach contents. A linear regression was fitted by the solid line and significant ($F(1,51) = 125.3$, $R^2 = 70.51$, $p < 0.001$). The linear equation was: $f = -1.31x - 13.89$.



Appendix Figure 2. $\delta^{13}C$ and molecular C:N ratio of crustacean prey from skipjack stomach contents. A linear regression was fitted by the solid line and not significant ($F(1,45) = 0.09$, $R^2 = -2.02\%$, $p = 0.77$). The linear equation was: $f = 0.04x - 20.66$.



Appendix Figure 3. $\delta^{13}C$ and molecular C:N ratio of squid prey from skipjack stomach contents. A linear regression was fitted by the solid line and significant ($F(1,12) = 33.38$, $R^2 = 71.35\%$, $p < 0.001$). The linear equation was: $f = -2.87x - 7.67$.

Chapter 3

Unresolved Issues and Implications for Management

Unresolved Issues

The objective of this study was to describe the diet of skipjack tuna foraging near Hawai‘i and determine whether they conduct ontogenetic diet shifts with larger and smaller fish consuming different prey and foraging at different depths or distances from shore. Using DNA barcoding methods to identify stomach contents, this study compiled a list of 24 skipjack tuna prey taxa (belonging to 13 orders and 17 families). While the sample size used in this study (118 skipjack with non-empty stomachs) was sufficient to capture the species richness of prey, as evidenced by the rarefaction curves, this study likely underestimated the breadth of the diet. The total prey list consisted of 24 prey taxa, but I identified 6 additional prey species from a single opportunistic skipjack tuna regurgitation sample collected by Chris Cargo: *Coryphaena equiselis, Acanthurus olivaceus, Centropyge flavicauda, Sufflamen fraenatum, Labrus bergylta,* and *Hippocampus* spp. (unidentified seahorse). However, these species were not added to our robust prey list because they came from a tuna that was not included in this study because the date / time of collection and the size of the source skipjack could not be ascertained. Due to the high freshness of regurgitation samples, ideal for barcoding, future studies could involve this type of opportunistic prey sampling.

In addition, two other observations suggest that the importance of other prey may have been underestimated in this analysis. The presence of octopus prey (*Ocythoe tuberculata*) might be underrepresented in the stomachs due to their high digestion rate and the inability to differentiate their hard part remains from those of the much more abundant flying squids. Finally, the presence of hyperiid amphipods is an indicator of likely skipjack ingestion of gelatinous zooplankton. Yet, salps and jellies were likely underestimated, because they are digested rapidly

and do not leave any hard remains. Rapidly digesting prey, like octopus and gelatinous zooplankton species may have a higher chance of detection using dDNA metabarcoding methods (e.g., Himmelsbach et al., 2021; Nimz et al., 2021).

Another limitation to this study was that only 0.3% of the total crustacean contents were identified using barcoding due to challenges with extracting DNA caused by little to no tissue inside the exoskeletons. Thus, most of the crustacean contents were morphologically identified at the Order level, but there may be more than five crustacean prey taxa at the species level in skipjack tuna diet. This foreshadows the need to incorporate metabarcoding practices in future diet studies to add more prey species to this current list and identify crustacean prey to a lower taxonomic level. By expanding our genomic diet information, we can better understand skipjack tuna foraging and additional prey functional groups (nearshore, offshore, deep-water, vertically migrating) and further predict the feeding ecology of predators.

Finally, in this study I used distribution patterns for larval and juvenile fish from studies in 1976 and 1992 to predict the distribution range of the prey in skipjack tuna. The majority of the fish prey of skipjack tuna were larval and pre-juvenile stages. Understanding the life history of their prey (e.g., breeding seasons, age at maturity, migration patterns) allows managers to make predictions on their relative importance supporting skipjack tuna. However, because sampling larvae and pre-juvenile fish can be challenging, there is little information about their vertical and horizontal distributions. Thus, we need to further investigate the seasonality, spatial distribution, and abundance of these prey. This information will allow managers to make more accurate predictions of skipjack foraging grounds, by pinpointing where their prey can be horizontally

(relative to distance from land and water depth) and vertically in the water column. Understanding these predator - prey links will also provide insights into potential skipjack responses to changing prey distributions in the future.

Three factors contributed to the likely underestimation of the overall skipjack tuna diet: (i) the inability to sample the smaller (< 50 cm FL, 1.7%) and the largest (> 80 cm FL, 4.1%) size classes; (ii) low genetic identification rates for crustaceans, and (iii) potential seasonal or interannual variability in their prey. Future studies should address these data gaps to develop a comprehensive perspective of skipjack diet and trophic links in nearshore waters around the Main Hawaiian Islands.

Implications for management

Skipjack tuna are one of the fastest growing tuna because they reach maturity within 1 year, corresponding to an approximate size of 40 cm, and contribute high yields to their stocks. Currently, skipjack stocks in the Pacific Ocean are being fished sustainably (Hare et al., 2019). Skipjack spawning biomass relative to the spawning biomass in absence of fishing ($SB_{recent}/SB_{F=0}$) is 0.44, which is above the limit reference point of 0.2, and suggests that the stock is not overfished. Moreover, the current fishing mortality relative to the fishing mortality at maximum sustainable yield ($F_{current}/F_{MSY}$) is 0.45. Because this ratio is smaller than 1, the stock is not being overfished. Although overfishing is not a current problem for skipjack tuna stocks in the Pacific Ocean, environmental factors might become a threat to their biomass in the future (Lehodey et al., 2011).

In Hawai‘i, skipjack was the largest commercial fishery during the mid-1900s. Detrimental environmental factors and a decline in fishing pressure caused by an issue with baitfish, resulted in a decline of the fishery by the 1970s. Today skipjack are caught by the Hawai‘i deep-set longline fishery, but only account for 4% of the total catch, approximately 0.5 CPUE (fish caught per 1000 hooks) (Polovina et al., 2009). This fishery mainly targets bigeye tuna (*Thunnus obesus*) but captures 13 other species including skipjack tuna. Skipjack tuna are not heavily targeted by this fishery because they are surface-schooling tuna and do not forage at the thermocline. Skipjack are also caught by the pole-and-line fishery (aku boats), main Hawaiian troll, and other small-boat fishers to support local seafood production (Iwane et al., 2020; Sweeney, 2021). Even though they are not heavily fished around Hawai‘i, skipjack tuna contribute an important economic and cultural value as a primary food source around many island nations in the Pacific.

By understanding their foraging ecology around Hawai‘i, we can confirm that skipjack forage on epipelagic prey in the mixed layer and near shore. There is no evidence of mesopelagic or vertically migrating prey; instead, reef-associated prey contributed almost half of their diet based on the IRI of stomach contents and the isotope mixing model results. In fact, reef-associated prey played a key role in the diet of skipjack tuna, by encompassing the majority of their prey species diversity.

Their foraging in the mixed layer makes skipjack tuna available to a variety of commercial fisheries, including shallow-set longlines, trolling, and purse-seines, as well as recreational pole-and-line fishers. The skipjack fishery was once the largest commercial fishery in Hawai‘i, if it

were to re-open, longline, purse seine, and troll fisheries should consider their fishing practices to capture skipjack sustainably. The outcomes of my study provide evidence that small and large tuna are foraging at different locations based on a significant difference between their $\delta^{13}C$ values. A depletion in $\delta^{13}C$ indicates offshore waters and an enrichment in $\delta^{13}C$ indicates nearshore waters. It is important for fisheries to be aware of these habitat differences between different-sized skipjack tuna. Fisheries should refrain from fishing in offshore waters around Hawai‘i because they will target locations where small tuna forage. Removing small and immature tuna can lead to growth and recruitment overfishing and a decline in the spawning biomass to maintain a sustainable stock. Skipjack tuna fisheries in Hawai‘i should consider fishing after skipjack spawning season (between October and April) and closer to land to target larger and mature tuna.

From this study and other studies around islands in the Western and Central Pacific Ocean, my study indicates that reef-associated prey contribute an important role to pelagic predators who forage close in nearshore waters like skipjack tuna (Allain et al., 2012; Oyafuso et al., 2016), and this is likely the case around other islands. With the effects of climate change on coral reefs, the density of reef-associated prey in these areas is expected to decline with a loss of habitat structure and resources (Hempson et al., 2017). I predict that in the future pelagic predators that rely on reef-associated prey, like skipjack tuna, will have to rely more heavily on pelagic species in offshore waters to sustain their energetic requirements. If skipjack tuna rely more heavily on pelagic prey, this may create increased competition with other pelagic predators that also utilize pelagic prey. In the future, these spatial shifts in tuna stocks around islands will impact fishing operations by becoming less accessible to small-scale fisheries if they forage further away from

land. At a basin-wide scale, future spatial shifts in skipjack tuna distributions may further affect their availability to fishers. This spatial shift occurs during El Niño events in the Western Pacific Ocean, when skipjack tuna are displaced eastwards, in response to the longitudinal shift in the warm pool and the secondary production that sustains their stocks (Lehodey et al., 1998). Yet, it is unknown whether island-associated ecosystems will become relatively more productive and important to pelagic predators in a global warming ocean, given the overall decline in productivity anticipated for tropical and subtropical waters (e.g., Polovina et al., 2008; Woodworth-Jefcoats et al., 2019).

Skipjack tuna have a vast distribution in the Pacific Ocean between tropical and subtropical regions (Figure 1). Using climate change models to make projections of their suitable habitat range in 2050 provides insights into likely future changes in their distribution (Figure 2). We can expect tropical regions and the Western Pacific Ocean to become less favorable foraging areas for skipjack, causing them to move eastwards and northwards, into subtropical regions. These changes may also cause skipjack tuna to be less associated with the surface and forage in deeper water, with a deepening of the thermocline. Current research should focus on tagging studies to explore skipjack foraging behavior and migration patterns (vertically and horizontally) to learn about their response to annual variability and their ranging patterns. Gaining an understanding of the feeding ecology of pelagic predators, like skipjack tuna, has important implications for fisheries management, by allowing managers to develop predictions about shifting tuna distributions, the fisheries that target them, and the status of their stocks (Bell et al., 2018; Lehodey et al., 2011; Senina et al., 2016).

Figures

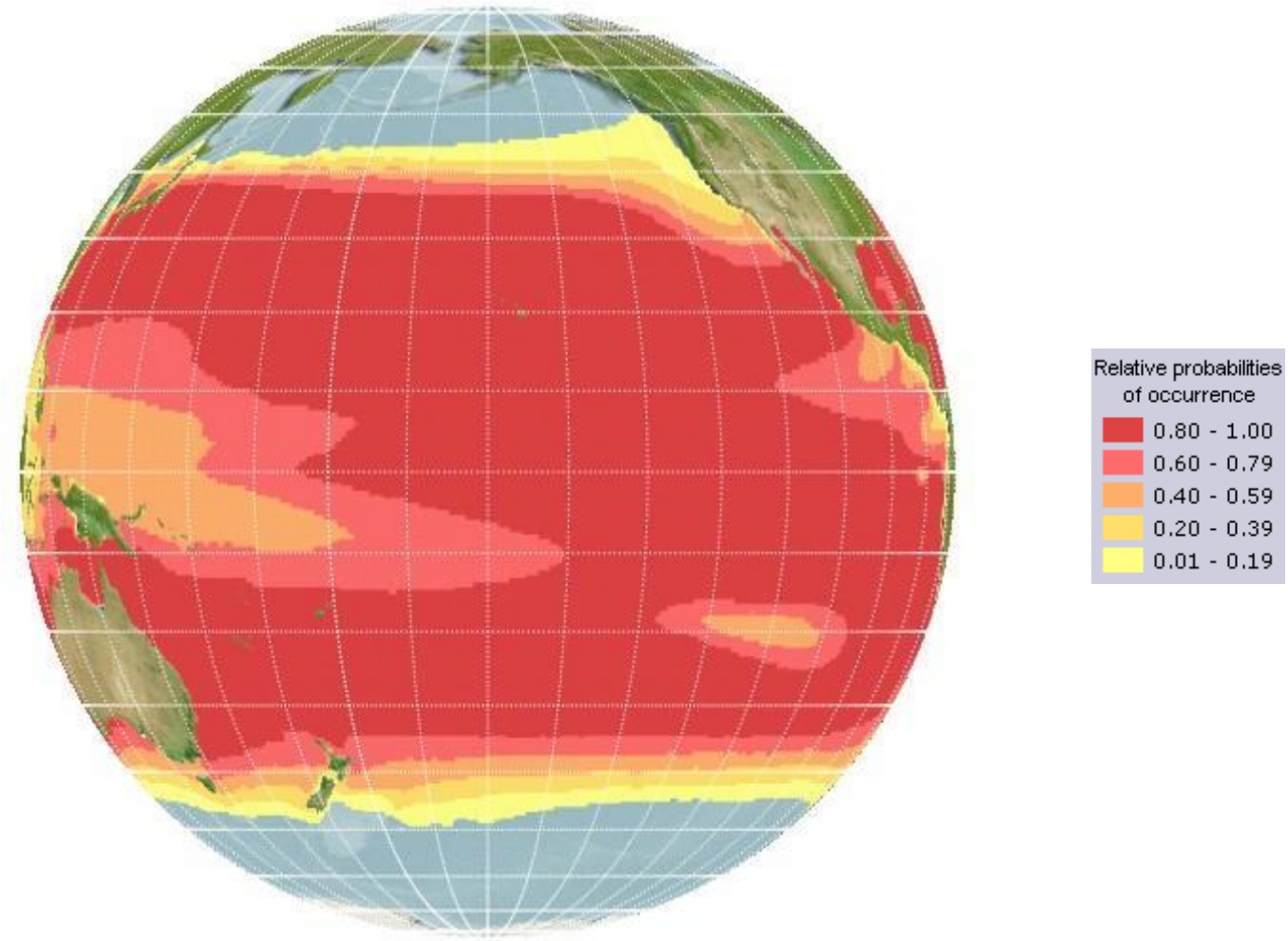


Figure 1. This is a map of the current distribution range for skipjack tuna in the Pacific Ocean, produced in aquamaps (AquaMaps, 2019). The color bar (shown in the legend) represents the relative probability of skipjack occurring across the Pacific Ocean.

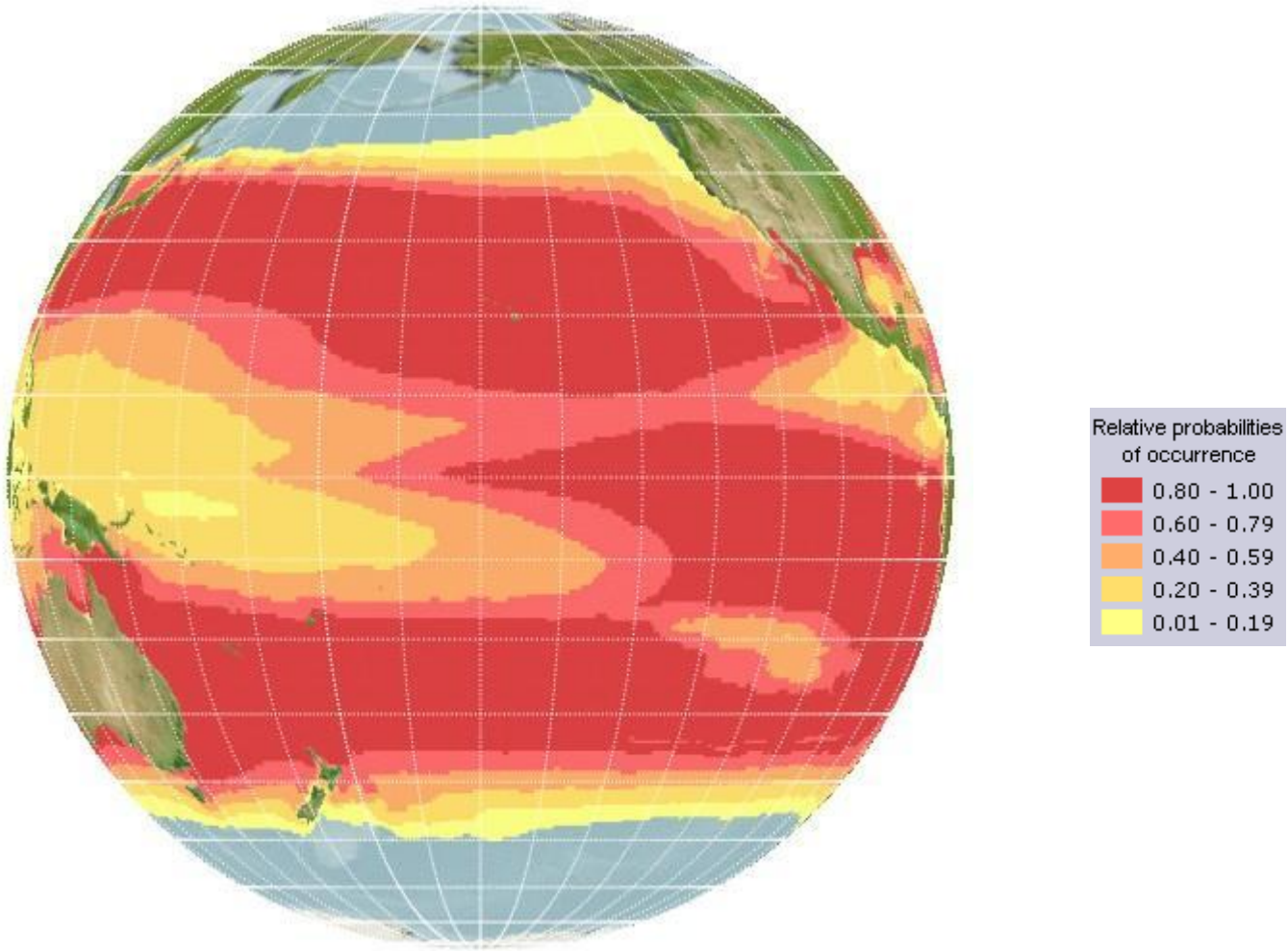


Figure 2. This is a map of the 2050 distribution range for skipjack tuna in the Pacific Ocean, produced in aquamaps (AquaMaps, 2019). The color bar (shown in the legend) represents the relative probability of skipjack occurring across the Pacific Ocean.

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