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Diversity of larval tuna diets across the central equatorial Pacific

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Thesis

DIVERSITY OF LARVAL TUNA DIETS ACROSS THE CENTRAL EQUATORIAL PACIFIC

by

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To all the baby tunas born—past, present, and future—whose tiny lives carry vast meaning.

DEDICATION

I sincerely dedicate this thesis to all of my friends and family who helped shape who I am and supported me in reaching where I am today. I've navigated treacherous waters and battled perilous waves to make this happen, and I wouldn't be here without you. Special thanks to all of the members of team Tuna whose name originates from this very research project– I'm finally doing the name justice through my results. To my dad—Dr. Ho Joong Kim; thank you for your unwavering support throughout my five long years in school, patient endurance during the contactless stretches while I was at sea, and unshakable faith in me throughout this whole journey—even if 80% of this thesis won't quite make sense to you. 아빠 사랑해요, 곧 봐요! Lastly, I would like to show my utmost appreciation to my Eric: the sweet to my salty, the moon to my sun, and the 東 to my 西. Thank you for enduring my grumpy, sleepless days and nights, being my secret tech guy behind the scenes, and making sure I didn't pass out mid-writing. True dedication goes to you—as this thesis truly wouldn't have been possible without you.

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ABSTRACT

Understanding the feeding ecology of larval tuna is essential for predicting their recruitment and survival success, as this directly supports the management of tuna populations across the changing ocean. To provide new insights into early-stage foraging dynamics, we investigated larval tuna gut contents collected from the Phoenix Islands Protected Area (PIPA) during its no-take period between 2016 and 2019. Using 18S sequencing, we characterized prey biodiversity, composition, and selectivity across years and tuna genera. We found that larval tunas consumed a taxonomically diverse range of zooplankton, with *Katsuwonus* exhibiting opportunistic foraging characterized by one or two dominant prey taxa that shifted across years, while *Thunnus*, despite limited sampling–consumed a broader range of prey taxa within a given year. Prey composition and selectivity varied by year and tuna genus, reflecting possible responses to shifting plankton communities and environmental conditions, as well as highlighting genus-specific trophic strategies and the potential for niche partitioning that may reduce interspecific competition for prey. This study sheds light on the ecological flexibility of larval tuna and underscores the importance of considering early-life foraging behavior in efforts to conserve and sustainably manage tuna stocks in the Western and Central Pacific Ocean, particularly in the context of marine protected areas (MPAs) and no-take zones.

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INTRODUCTION

Tuna are large, fast-swimming pelagic fish found throughout the world's oceans, playing a vital ecological and economic role in marine ecosystems and human livelihoods. Belonging to the family Scombridae, tuna species such as skipjack (*Katsuwonus pelamis*), yellowfin (*Thunnus albacares*), bigeye (*Thunnus obesus*), albacore (*Thunnus alalunga*), and bluefin (*Thunnus thynnus*) are highly migratory and occupy a wide range of oceanic environments (Collette & Nauen, 1983; Block et al., 2005). As apex predators, they play a critical role in pelagic ecosystems by regulating prey populations and maintaining ecological balance (Olson et al., 2016). Their physiological adaptations, including regional endothermy and streamlined body shapes, make them efficient apex or mesopredators capable of long-distance migrations across ocean basins (Bernal et al., 2001; Block et al., 2011).

Beyond their ecological importance, tuna support some of the largest and most economically valuable fisheries in the world. In 2018, global capture fisheries production reached the highest ever recorded at 96.4 million tonnes– 7.9 million tonnes of which were catches of tuna and tuna-like species. Of the catch, skipjack tuna (*K. pelamis*) was ranked third for the ninth consecutive year at 3.2 million tonnes (FAO, 2020). Many Pacific Island countries and territories rely heavily on oceanic tuna fisheries as a cornerstone of both economic development and food security (Bell et al., 2009). Furthermore, rising global demand—particularly for canned and sashimi-grade products—has driven the expansion of industrial fishing efforts. However, these efforts present complex management challenges due to the highly migratory and transoceanic

nature of tuna stocks (FAO, 2020). In response, tuna fisheries are governed collaboratively through Regional Fisheries Management Organizations (RFMOs), which aim to ensure sustainability while navigating geopolitical and ecological concerns (Lodge et al., 2007; Gilman et al., 2014).

One increasingly prominent tool for addressing the challenges of tuna conservation and fisheries management is the designation of Marine Protected Areas (MPAs)—clearly defined geographical spaces in the ocean that have been reserved by federal, state, territorial, tribal, or local laws and regulations to provide lasting protection for part or all of the natural and cultural resources therein (NOAA, n.d.). MPAs seek to conserve marine biodiversity, rebuild fish stocks, and mitigate the impacts of industrial fishing. Although their effectiveness for highly migratory species like tuna remains an area of active debate, MPAs can serve as important spatial management tools when strategically located in ecologically significant regions (Game et al., 2009; Sibert et al., 2012).

No-take MPAs are ideal systems to observe larval tuna ecology, as they are not as disturbed by anthropogenic stressors such as fishing, habitat destruction, and pollution, which allow environmental, oceanographic, and temporal factors such as El Niño–Southern Oscillation (ENSO) to be assessed in a relatively more “natural” state. This is particularly important in the study of larval ecology, as fish larvae are known to be more vulnerable to local anthropogenic influences such as top-down pressures from fishing (Hoover, 2018). Despite the significance of early life survival for population dynamics, relatively little is known about the diet and feeding ecology of larval tuna,

particularly in the equatorial Pacific. Previous dietary studies have been conducted in the Gulf of California, the Straits of Florida, and the Western Indian Ocean near Northwest Australia, revealing selective feeding and niche partitioning behaviors among different tuna species (Llopiz & Hobday, 2015). However, the Western and Central Pacific Ocean (WCPO), which account for more than half of the global tuna catch and sales of $17.5 billion a year (Macfadyen et al., 2016), has not been thoroughly studied in this context. As a result, data from this region are uniquely valuable for filling a critical knowledge gap in larval tuna ecology. Given the WCPO's central role in global tuna production, understanding larval feeding patterns in this region is essential for refining recruitment models and managing one of the world's most economically significant fisheries.

A prominent example of large-scale spatial management is the Phoenix Islands Protected Area (PIPA), located in the central equatorial Pacific within Kiribati's exclusive economic zone. Established in 2006, operationalized in 2008, and designated a UNESCO World Heritage Site in 2010 (Rotjan et al., 2014), PIPA functioned as one of the world's largest no-take MPAs until 2021, when commercial fishing was reopened (Kittinger et al., 2024). Its closure to industrial fishing in 2015 encompassed a significant portion of the western and central Pacific which was one of the most heavily exploited tuna habitats globally (PIPA Management Committee, 2019). PIPA is particularly strategic for larval studies because it is influenced by ENSO cycles and is recognized as a spawning ground for tropical tuna (Hernandez et al., 2019). Building on this context, larval tuna studies in and around PIPA offer critical insight into how MPAs may influence population trajectories through early life stages. Analyses of larval distribution, abundance, growth

rates, and oceanographic backtracking simulations are essential because early life stages have a disproportionate influence on recruitment and long-term stock productivity (Hernandez et al., 2019). Recruitment—the transition from larval to juvenile and eventually adult stages—remains a key yet poorly understood process in tuna ecology. Mortality during the egg, larval, and juvenile phases is a major driver of interannual variability, especially in pelagic broadcast spawners like tuna (Bailey & Houde, 1989). Two primary overlapping factors influence larval survival: starvation and predation (Paradis et al., 1996). Of these, starvation is considered the dominant cause of mortality during the larval stage, as on top of larvae simply starving to death, those experiencing poor nutritional conditions are also more susceptible to additional threats such as disease, predation, and reduced foraging efficiency (Amara & Galois, 2004). Larval fish must begin feeding soon after hatching due to the limited nutrients available in their yolk sacs (Prince & Pulos, 1982). Empirical studies have demonstrated that gut fullness in larval fishes directly correlates with prey abundance, indicating that when suitable prey are available, larvae are capable of rapidly responding to these favorable foraging conditions (Sponaugle et al., 2009). Thus, quantifying larval tuna abundance and their diet can provide critical insights into spawning patterns, recruitment dynamics, and the role of MPAs like PIPA in sustaining tuna populations (Hernandez et al., 2019).

This study investigates larval tuna prey biodiversity, composition, and selectivity in PIPA during its fully no-take years (2015–2019) by analyzing gut content data across multiple years and comparing these with environmental plankton samples to assess patterns of prey availability and foraging behavior. The analysis examines interannual

variation in prey groups, contrasts between tuna genera (*Katsuwonus* and *Thunnus*), and potential indicators of prey specialization or generalist feeding strategies.

Through investigating the temporal and ecological differences between prey biodiversity, composition, and selectivity, this study aims to answer the following:

1. What prey are larval tunas consuming in the PIPA region?
2. Do the dietary patterns reflect generalist feeding strategies, prey switching, or specialization?
3. Is there evidence of dietary competition or niche partitioning between different tuna genera?
4. What do these feeding patterns reveal about larval tuna ecology and recruitment dynamics?
5. What are the implications of these findings for conservation and fisheries management?

By addressing these questions, this study aims to fill a critical gap in our understanding of tuna early life history and contribute to the sustainable management of one of the world's most vital fishery resources.

METHODS

Study Region

PIPA was the largest UNESCO World Heritage Site and marine protected area (MPA) under the jurisdiction of the Republic of Kiribati. Located in the central equatorial Pacific, PIPA was established in 2006, operationalized in 2008, and lasted until 2021 (Rotjan et al., 2014; Kittinger et al., 2024). Its boundaries ranged from -1° to -6.5° latitude and -169.5° to -173.5° longitude. Encompassing 8 volcanic atolls, 2 submerged reefs, and about 14 underwater seamounts, PIPA contains roughly 800 known species, including about 200 species of coral, 500 species of fish, 18 species of marine mammals and 44 species of seabirds (UNESCO, n.d.). Between 2015 and 2021, PIPA was designated as a no-take zone, prohibiting all commercial extraction activities such as fishing, seabed mining, and coral harvesting. Its isolation and preservation efforts had allowed it to become a pristine study site for examining biodiversity and the effects MPAs have on marine ecosystems. Being a spawning and nursery site for numerous fish species including pelagic species such as tuna, PIPA was also an ideal location for studying the abundance and distribution of larval ichthyoplankton as well as examining overall zooplankton community composition (Hernandez et al., 2019). Being close to the equator, PIPA is an unique natural laboratory for studying ecosystem resilience, climate change, and how ENSO events affect larval tuna dispersion and feeding ecology (Witting, 2017; Hernandez et al., 2019).

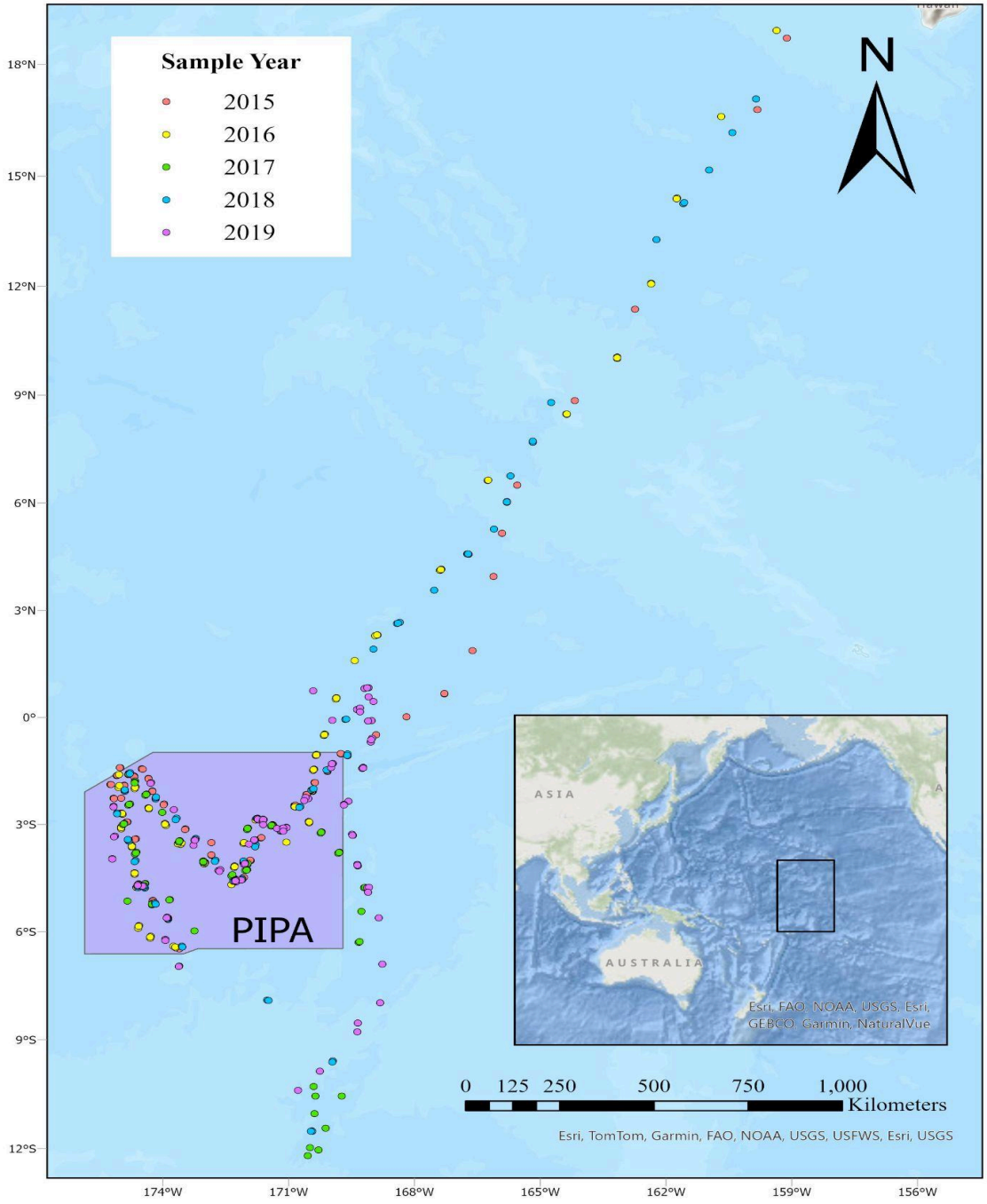
Plankton Collection

Plankton samples were collected by the Sea Education Association aboard the SSV *Robert C. Seamans* within PIPA and its surrounding areas in July and August from 2015 to 2019 (Table 1). Sampling took place over the entire cruise tracks, and 147 of the total 272 sampling stations occurred within the PIPA boundary (Figure 1).

Table 1. **Summary of the PIPA Cruise Tracks.** Summary of cruise year, start and end dates, ship route (start and end positions), and total number of sampling stations per expedition. The number of stations sampled within PIPA is also indicated.

Cruise	*Year*	*Start Position*	*End Position*	*Stations within PIPA* / *Total Stations*	*Sampling Start Date*	*Sampling End Date*
S268	2016	18°54.3' N x 159°22.0' W **Honolulu, Hawaii**	6°25.3' S x 173°42.2' W **Pago Pago, American Samoa**	28 / 56	July 5th	August 6th
S274	2017	12°2.0' S x 170°17.3' W **Pago Pago, American Samoa**	12°11.8' S x 170°32.7' W **Pago Pago, American Samoa**	23 / 47	July 11th	August 9th
S281	2018	17°4.8' N x 159°51.0' W **Honolulu, Hawaii**	11°31.3' S x 170°28.4' W **Pago Pago, American Samoa**	31 / 47	July 8th	August 10th
S287	2019	10°23.9' S x 170°46.7' W **Pago Pago, American Samoa**	6°58.1' S x 173°37.3' W **Pago Pago, American Samoa**	24 / 54	July 7th	August 5th

Figure 1. **Map of the PIPA Study Region and Sample Stations.** Different colors of station markers indicate different sampling years between 2016 and 2019. PIPA boundaries are marked by the purple box.



Plankton nets were towed at each station at approximately 2 knots (≈1 m/s) for 30 minutes, targeting a total tow distance of 1,800 meters. Each station included up to three different tows: a Neuston Tow (NT), which skimmed the upper 0.25 m of the surface layer, and two oblique subsurface tows—a Tucker Trawl Shallow (TTS) sampling the upper 50 m where tuna larvae are usually most abundant (Boehlert & Mundy, 1994), and a Tucker Trawl Deep (TTD) sampling the upper 150 m of the water column which corresponds to the depth of the deep chlorophyll maximum (Witting, 2017). The Neuston net had a 1.0 m × 0.5 m rectangular frame, while the Tucker trawl had a 1 m² mouth opening. Each tow was deployed around 0000 and 1200 local time except for the TTD, which was only deployed at night.

All tows used 333 μm mesh filters, and a General Oceanics flow meter was attached to the Tucker trawl to measure total water volume filtered. For Neuston net deployments, tow volumes were estimated by multiplying tow distance by half the net's area (0.25 m²) to account for the partial submersion. After towing, the nets were rinsed into a sample bucket and the contents were sorted to remove plant matter, halobates, microplastics, and nekton (organisms larger than 2 cm). Remaining samples were preserved in 95% ethanol solutions and shipped to the Rotjan Lab at Boston University (Boston, MA). Over the course of five years, a total of 478 tows were collected, and 1,141 tuna larvae were caught.

Table 2. **Larval Tuna Catch by Tow Type and Year.** Summary of larval tuna collection from 2015 to 2019 across three plankton tow types: Neuston Tow (NT), Tucker Trawl Shallow (TTS), and Tucker Trawl Deep (TTD). The number of tows conducted and the corresponding number of tuna larvae caught are shown per year. Subtotals and overall totals are included for each category.

Year	*Tow Type*	*Tow Number*	*Number of Tuna Caught*
2016	NT	37	39
	TTD	35	137
	TTS	35	173
	Subtotal	107	349
2017	NT	35	7
	TTD	20	52
	TTS	21	48
	Subtotal	76	107
2018	NT	44	5
	TTD	18	45
	TTS	33	287
	Subtotal	95	337
2019	NT	36	2
	TTD	33	53
	TTS	32	93
	Subtotal	101	148
Total		379	941

Plankton Processing

In the Rotjan Lab, the preserved plankton sample jars were each sorted under a Leica M165FC stereomicroscope, and ichthyoplankton larvae were separately pulled for further analysis. Each plankton jar was sorted twice to ensure quality, and each ichthyoplankton sample went through a second round of examination to identify and separate tuna larvae by morphological traits.

Quantification of Community Structure

After the ichthyoplankton were removed, 200 counts were conducted for all plankton jars to create a representative sample and estimate the abundance and biodiversity of the invertebrate zooplankton composition. 200 counts were completed by stirring the jar to thoroughly mix the biomass, removing 1 mL of biomass, and morphologically identifying the first 200 random plankters to the finest taxonomic level possible. If there was insufficient biomass for a standard 200-count, a 100-count was performed instead to assess the sample's community composition.

Larval Tuna Identification

The separated tuna larvae were identified to genus level based on external morphological characteristics, following the descriptions of identification guides (Nishikawa and Rimmer, 1987; Richards, 2005). Individuals were classified as either skipjack tuna (*K. pelamis*) or *Thunnus* spp., which includes several morphologically similar species that are difficult to distinguish at the larval stage (Richards, 2005). *Thunnus* species sampled presumably included yellowfin (*T. albacares*), bigeye (*T. obesus*), albacore (*T. alalunga*), and bluefin (*T. thynnus*). Diagnostic features primarily included species-specific pigmentation patterns on the head and tail regions (Figure 2). Genus-level identifications were later confirmed using 2bRAD sequencing, but the results are not included within the scope of this thesis.

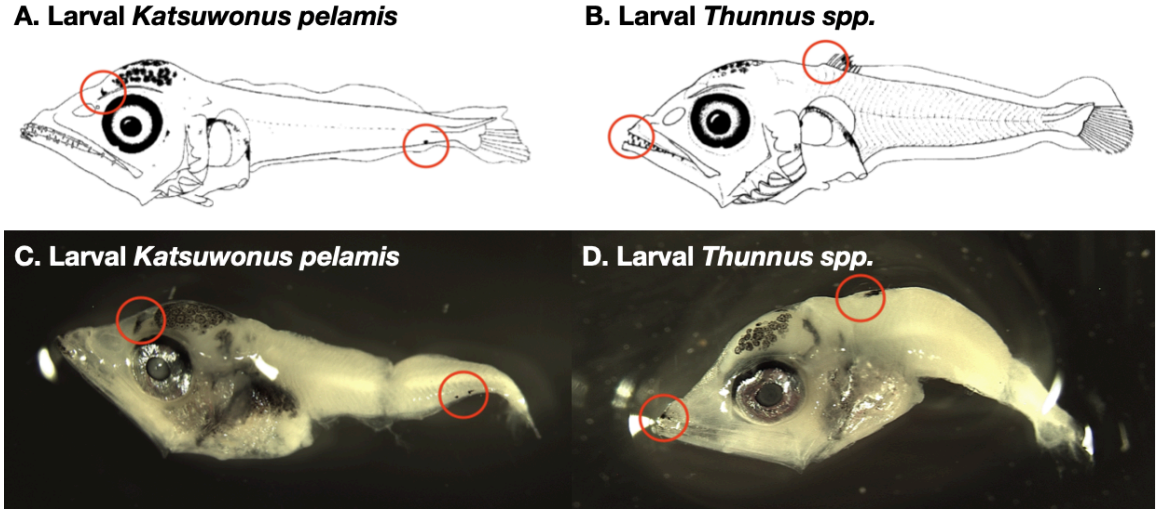


Figure 2: **Larval Tuna Morphological Identifications.** Larvae were identified to genus level based on pigmentation patterns (Richards, 2005). Panels A and B are schematic illustrations from the identification guide (Richards, 2005), while panels C and D are larval specimens collected in 2016. Skipjack (*K. pelamis*) larvae (A, C) exhibit pigment on the forebrain and ventral tail, while *Thunnus* spp. larvae (B, D) show pigment on the jaw tip and dorsal fin (Nishikawa & Rimmer, 1987; Richards, 2005). Red circles indicate diagnostic pigment locations.

Larval Tuna Gut Processing

Tuna larvae were identified to genus and photographed using a Leica DFC550 digital camera with Leica Application Suite V4.9.0. Gut excision was performed under a Leica M165FC stereo microscope using a rounded scalpel. Two incisions were made– one from bottom of jaw to mid-spine, other from anal point to mid-spine to make a triangular clean cut of the gut (Figure 3). Dissection tools were wiped with 95% EtOH between each sample. The excised guts were stored in a separate vial from the remaining head and tail, which were also saved for future DNA analysis. Larvae that were too small (<4 mm) or damaged were not used for gut sequencing.

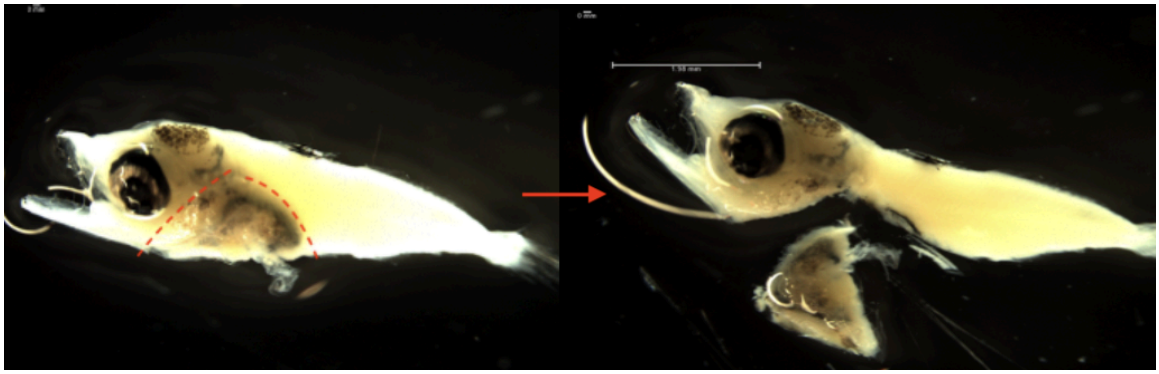


Figure 3: **Larval Tuna Gut Dissection Method**. Photograph of a larval tuna specimen (*Thunnus* spp.) under a stereo microscope, illustrating the gut excision method. Two incisions were made—one from the lower jaw to the mid-spine and another from the anal point to the mid-spine—to extract the full digestive tract for DNA analysis while minimizing contamination from surrounding tissue.

18S Sequencing

DNA was extracted from the larval tuna gut samples using the Qiagen DNeasy Blood & Tissue Kit (Hilden, Germany) following the manufacturer's protocol, except that a reduced volume of elution buffer was used to increase DNA concentration. Prior to extraction, absolute ethanol was removed from the gut sample as much as possible. The quantity and quality of extracted DNA were assessed using a DeNovix DS-11+ Spectrophotometer. To prevent cross-contamination, new disposable pipette tips were used for each sample.

To ensure consistent input concentrations, all DNA samples exceeding 50 ng/µL were diluted to 50 ng/µL using Buffer AE. Dilutions were calculated to obtain a total of 1000 ng of DNA in a final volume of 20 µL. Samples with concentrations ≤ 50 ng/µL were used directly without further dilution or the addition of Buffer AE. Out of the 220 gut samples processed for 18S sequencing, the 120 highest-quality samples–favoring

those with an A260/A280 ratio ≥ 1.8 and concentration ≥ 18; were selected for sequencing. No technical replicates were included. DNA samples were frozen and shipped on dry ice to Argonne National Laboratory (IL, USA) for sequencing.

Metagenomic DNA was PCR-amplified targeting the 18S rRNA gene (V9 region) using barcoded primers compatible with the Illumina MiSeq platform, following a barcoded amplicon sequencing protocol adapted from Caporaso et al. (2012). Each reverse primer included a unique 12 bp barcode to enable multiplexing.

PCR reactions were performed in 25 µL volumes, consisting of 9.5 µL certified DNA-free water, 12.5 µL LongAmp® Hot Start Taq 2X Master Mix (New England BioLabs), 1 µL reverse-barcoded primer (5 µM), 1 µL forward primer (5 µM), and 1 µL template DNA (~10–100 ng). Thermocycling conditions were: 94 °C for 3 minutes, followed by 35 cycles of 94 °C for 45 seconds, 50 °C for 60 seconds, and 72 °C for 90 seconds, with a final extension at 72 °C for 10 minutes.

Amplicons were quantified using PicoGreen, pooled in equimolar concentrations, and purified using AMPure XP beads. The pooled library was quantified with a Qubit fluorometer, normalized to 2 nM, denatured, and diluted for sequencing. Final libraries were sequenced on an Illumina MiSeq using a 2 × 150 bp paired-end run with a 30–50% PhiX spike-in to enhance sequence diversity. Although all 120 samples were successfully extracted and sent to Argonne for sequencing, 8 samples were excluded (2 samples failed sequencing, 4 samples from Palau and 2 from outside of PIPA 2021) and only 112 were retained for downstream analysis given the scope of this thesis (Table 3).

Table 3. **Distribution of 18S Gut Content Samples by Year and Tuna Genus.** *Katsuwonus pelamis* samples were present across all four years, while *Thunnus* spp. samples were only collected in 2018 and 2019. A total of 112 samples were included in the final gut content sequencing dataset.

Year	*Katsuwonus pelamis*	*Thunnus* spp.	*Number of Gut Samples*
2016	21	0	21
2017	13	0	13
2018	41	20	61
2019	11	5	16
Total	86	25	112

Initial Processing

Sequencing data from 18S rRNA gene amplicons were processed in R (v4.3.0) following a pipeline adapted from Wright et al. (2021; https://github.com/rachelwright8/plankto nCommunities). Demultiplexed paired-end reads were processed using the *DADA2* package (Callahan *et al.*, 2016), including quality filtering, trimming, dereplication, error correction, and amplicon sequence variant (ASV) inference. Forward and reverse reads were merged, and chimeric sequences were removed. Taxonomic assignment was performed using the PR^2 database (Guillou et al., 2013; version 5.1.0). ASVs were subsequently clustered into Operational Taxonomic Units (OTUs) based on sequence similarity to facilitate ecological comparisons. The resulting OTU table, representing the abundance of each taxon per sample, was imported into the *phyloseq* package (McMurdie & Holmes, 2013) for ecological and diversity analyses. A total of 205 taxa were assigned across nine taxonomic ranks to 112 samples.

Prey Biodiversity

Biodiversity was assessed using both the Shannon and Simpson diversity indices to characterize within-sample (alpha) diversity. The Shannon index (H′) was used to quantify species evenness and overall diversity, while the Simpson index (D) served as a complementary measure of dominance (1–D).

The Shannon diversity index (H′) is defined as:

$$H' = -\sum_{i=1}^{s} p_i \ln p_i$$

where $p_i = \frac{n_i}{N}$ is the proportion of individuals belonging to species i. Higher values of H' indicate greater diversity and more even distribution of taxa.

The Simpson index is expressed as:

$$D = \sum_{i=1}^{S} \frac{n_i\,(n_i - 1)}{N\,(N - 1)}$$

where n_i is the number of individuals of species i, N is the total number of individuals in the sample, and S is the total number of species observed.

Diversity metrics were calculated using the *vegan* package (Oksanen et al., 2025) in R (v4.3.0). Diversity values were then compared across tuna genera (*Katsuwonus, Thunnus*) and across sampling years. Statistical testing was performed using one-way ANOVA to evaluate whether mean diversity differed significantly among groups.

Prey Composition

Proportional prey abundance was calculated by summing raw ASV read counts assigned to each prey taxon across all gut content samples. Taxonomic abundances were extracted from the *phyloseq* object and aggregated either by sampling year or tuna genus to evaluate temporal and taxonomic variation in prey composition. Stacked bar plots were constructed to visualize the contribution of individual gut-derived prey groups to relative read abundance within each category, providing a qualitative overview of dietary patterns. To ensure statistical validity and consistency across visualizations, prey taxa with fewer than five total read counts across all samples were all aggregated into a single category labeled “other”. This filtering step reduced noise from extremely rare taxa and ensured that only ecologically meaningful prey groups were retained for comparison. In addition to stacked bar graphs and statistical testing, relative abundance pie charts of the top four (plus others) most abundant prey genera were generated to visually summarize the four most dominant prey taxa within each group. It is important to note that these results reflect proportional DNA representation and not absolute prey counts or biomass, due to methodological limitations inherent to metabarcoding approaches.

Prey Selectivity

Prey selectivity in larval tuna was evaluated using Chesson’s alpha (α), which quantifies dietary preference by comparing the relative abundance of prey in gut contents to their environmental availability. This analysis leveraged two datasets: larval tuna gut content data generated via 18S metabarcoding and environmental prey availability data

derived from concurrent 200-count morphological plankton surveys. Because molecular and morphological methods inherently differ in taxonomic resolution, prey taxa were aggregated into broader, ecologically relevant categories to facilitate direct comparison (e.g., “calanoid copepods,” “appendicularians,” etc). For example, the genera *Neocalanus* and *Clausocalanus* were grouped as “calanoid copepods” based on their shared classification within the order Calanoida, and their shared characteristics of possessing elongated antennules, specialized thoracic appendages for filter feeding, and primarily herbivorous diets (Mauchline, 1998; Boxshall & Halsey, 2004). Similarly, *Oikopleura* and *Megalocercus* were grouped as “appendicularians” due to their shared classification within the tunicate class Appendicularia and their use of mucous structures for particle filtration (Deibel, 1998; Hopcroft & Roff, 1995). Functional categories were cross-referenced using the World Register of Marine Species (WoRMS Editorial Board, 2025) and the Integrated Taxonomic Information System (ITIS, 2025). Only categories confidently identified in both datasets were retained for final analysis and taxa that could not be confidently aligned across datasets were excluded (Table 4). However, it is important to note that this approach assumes environmental plankton abundance is an unbiased proxy for prey availability and does not account for localized depletion due to larval tuna foraging. As such, the calculated selectivity indices may underestimate the true preference for highly targeted prey items that are already reduced in the environment due to predation pressure.

Table 4: **Mapping of Gut Content Taxa to Environmental Prey Taxa in functional Groups for Prey Selectivity Analysis (Chesson's α)**. Each genus identified in the tuna gut contents (left column) was matched to a corresponding prey category in the 200-count morphological plankton dataset, based on shared taxonomic classification and ecological function. The "Matching Taxonomy" column indicates the closest shared taxonomic rank used to justify grouping, and the "Functional Group" column denotes the broader ecological category used in Chesson's alpha calculations. Highlighted rows indicate instances where multiple gut taxa were aggregated into the same functional group. The the yellow highlights indicate the genera grouped into the functional group of "appendicularians", blue highlights indicate the genera grouped into the functional group of "calanoid copepods," and the green highlights indicate the genera grouped into the functional group "colonial cnidarians." The red highlights indicate the prey genera that were unmatched.

Tuna Gut Content	200 Count Group	Matching Taxonomy	Functional Group
Oikopleura	Appendicularian	Class (Appendicularia)	Appendicularians
Megalocercus	Appendicularian	Class (Appendicularia)	Appendicularians
Neocalanus	Calanoid Copepod	Order (Calanoida)	Calanoid Copepods
Clausocalanus	Calanoid Copepod	Order (Calanoida)	Calanoid Copepods
Sulculeolaria	Siphonophore	Order (Siphonophorae)	Colonial Cnidarians
Cordagalma	Siphonophore	Order (Siphonophorae)	Colonial Cnidarians
Corycaeus	*Corycaeus* Copepod	Genus (Copepoda)	Corycaeus Copepods
Collozoum	Radiolarian	Order (Radiolaria)	Protists
Maganyetiphanes	Euphausid Shrimp	Order (Euphausiacea)	Euphausiids
Amphithyrus	Mysid Shrimp	Order (Mysida)	Mysids
Creseis	Pteropod	Class (Gastropoda)	Pteropods
Sapphirina	*Sapphirina* Copepod	Genus (Copepoda)	Sapphirina Copepods
Delibus	Unmatched	Unmatched	Unmatched / Excluded
Mayetiola	Unmatched	Unmatched	Unmatched / Excluded
Liriope	Unmatched	Unmatched	Unmatched / Excluded

Chesson's alpha (α) is calculated as:

$$a_i = \frac{r_i / p_i}{\sum_{j=1}^{n} \left(r_j / p_j \right)}$$

Where r_i is the proportion of prey type *i* in the gut content data, p_i is its relative abundance in the environment, and n is the number of matched prey groups within each sampling year.

To evaluate prey selectivity in larval tuna, gut content and environmental data were grouped by year to avoid conflating temporal shifts in prey availability and normalized to proportional abundances, ensuring that the total abundance of all prey groups within each year summed to one. This normalization allowed for direct comparison of diet composition to prey availability, regardless of differences in sample size or sequencing depth. Chesson's alpha (α) was then computed independently for each year to account for interannual variation in prey availability, using grouped operations in the *dplyr* package (Wickham et al., 2023).

To assess taxon-specific foraging patterns, gut content data were also grouped by tuna genus (e.g., *Thunnus*, *Katsuwonus*), and α-values were computed separately for each genus. For this genus-level analysis, all environmental prey abundances were aggregated across years to create a unified environmental baseline, allowing long-term differences in prey preference between genera to be evaluated. While environmental prey abundances may vary across years, aggregating these data provided a robust, long-term baseline that

reflects the average prey field accessible to larvae across surveys. This approach was chosen to highlight persistent genus-level differences in prey selectivity rather than year-specific fluctuations, which were addressed separately in year-by-year analyses. Although environmental samples were not individually paired with larval tuna, they were collected within the same temporal and spatial framework of each survey and were assumed to represent a shared foraging environment accessible to all larvae.

Final α-values were compiled into summary tables and visualized using *ggplot2* (Wickham, 2016) as grouped bar charts. Two complementary figure sets were produced: one displaying α-values across years (aggregated across all genera) to capture temporal trends, and another separating α-values by tuna genus (aggregated across years) to compare taxon-specific prey selectivity. In all cases, a red dashed line was used to indicate the neutral selection threshold ($1/n$), where n is the number of prey groups observed in a given comparison. This threshold represents the expected α-value under random (neutral) selection, where each prey type is consumed in direct proportion to its environmental availability. Prey groups with α-values greater than $1/n$ were interpreted as positively selected, values approximately equal to $1/n$ as neutrally selected, those less than $1/n$ as negatively selected, and values near zero as indicative of complete avoidance. Prey genera with an environmental count of zero were excluded from Chesson's alpha calculation but were retained in the visualizations and displayed as zero (i.e., NA in Supplemental Figure 1). The corresponding $1/n$ threshold for each year or genus-level comparison was annotated above the red dashed line for reference.

RESULTS

Prey Biodiversity

Prey diversity in larval tuna guts was assessed using Shannon (H′) and Simpson (1–D) diversity indices across 112 samples for each sampling year. Among the four years analyzed, 2018 exhibited the most variable diversity, with both the median and range of values exceeding those of other years (Figure 4). In contrast, 2016, 2017, and 2019 showed comparatively low median values and minimal variability, suggesting limited variability in prey diversity in those years. However, a one-way ANOVA revealed no statistically significant differences in diversity across years for either the Shannon index (DF = 1, F value = 0.007, p = 0.935) or Simpson index (DF = 1, F value = 1.251, p = 0.602) (Figure 5). No consistent temporal variation in prey diversity was detected across years based on the ANOVA results.

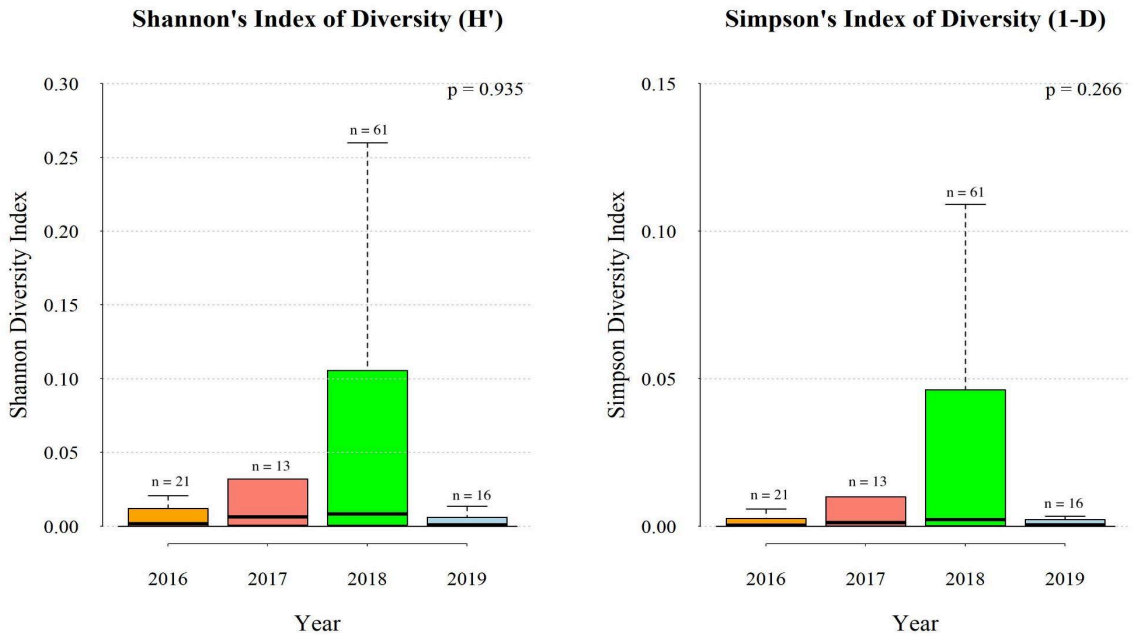


Figure 4: **Prey Diversity by Sampling Year.** Boxplots of Shannon (H′) and Simpson (1–D) diversity indices in larval tuna gut contents across four sampling years (2016–2019) including 2 genera (*Thunnus* and *Katsuwonus*). 2018 exhibited the highest diversity and greatest variability, but no statistically significant differences were found across years (Shannon: p = 0.674; Simpson: p = 0.602).

Prey diversity was also compared between the two dominant tuna genera in the dataset: *Katsuwonus* (n = 88) and *Thunnus* (n = 23). As with the temporal analysis, diversity was quantified using both Shannon and Simpson indices. At a significance level of α = 0.05, differences between genera were not statistically significant for either the Shannon index (DF=1, F value = 2.813, p = 0.096) or Simpson index (DF=1, F value = 2.686, p = 0.104) (Figure 6). Although *Katsuwonus* exhibited slightly higher variability than *Thunnus*, these differences were not statistically significant.

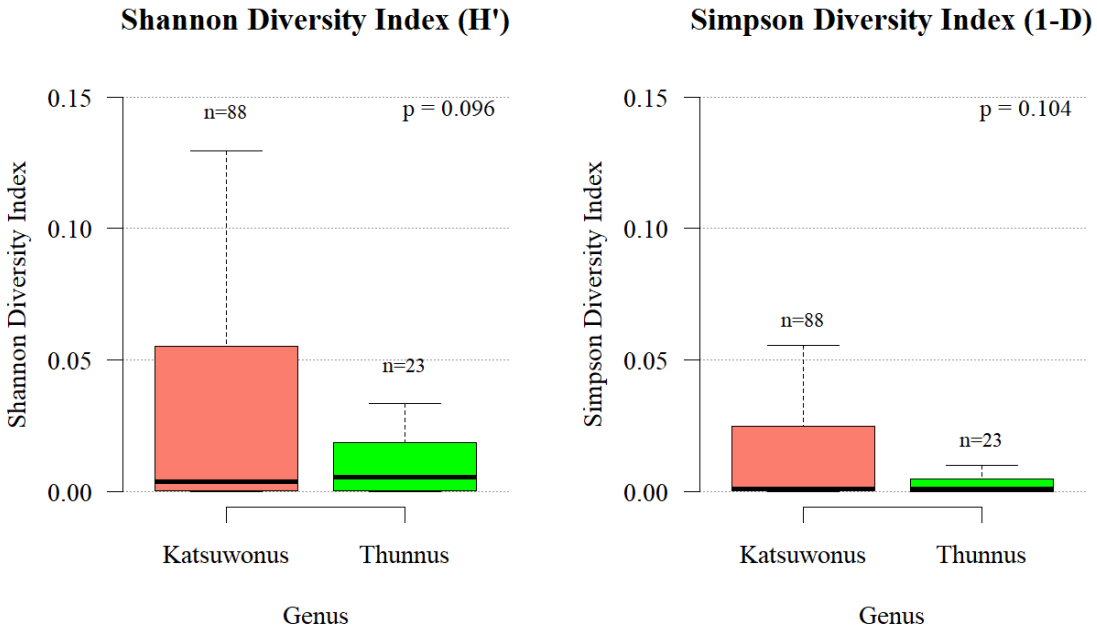


Figure 5: **Prey Diversity by Tuna Genus.** Boxplots comparing Shannon (H′) and Simpson (1–D) diversity indices between *Katsuwonus* and *Thunnus* larvae across four sampling years (2016~2019). *Katsuwonus* showed higher median diversity and

variability, though mean differences were not statistically significant (Shannon: p = 0.096; Simpson: p = 0.104).

Prey Composition

The total number of 18S ASV reads assigned to prey taxa in larval tuna gut contents was compared across four sampling years (2016–2019) by tuna genus (*Katsuwonus* and *Thunnus*), providing insight into broad temporal patterns in prey DNA representation (Figure 6). Prey composition within *Katsuwonus* varied considerably across years based on relative read abundance: *Neocalanus* and *Corycaeus* dominated in 2016, *Creseis* in 2017, and *Sapphirina*, *Sulculeolaria*, and *Meganyctiphanes* in 2018. Interannual comparisons could not be made for *Thunnus* due to limited sample availability; however, 2018 samples revealed a more taxonomically diverse set of gut-associated reads, including *Corycaeus*, *Amphithyrus*, *Sapphirina*, and *Clausocalanus*.

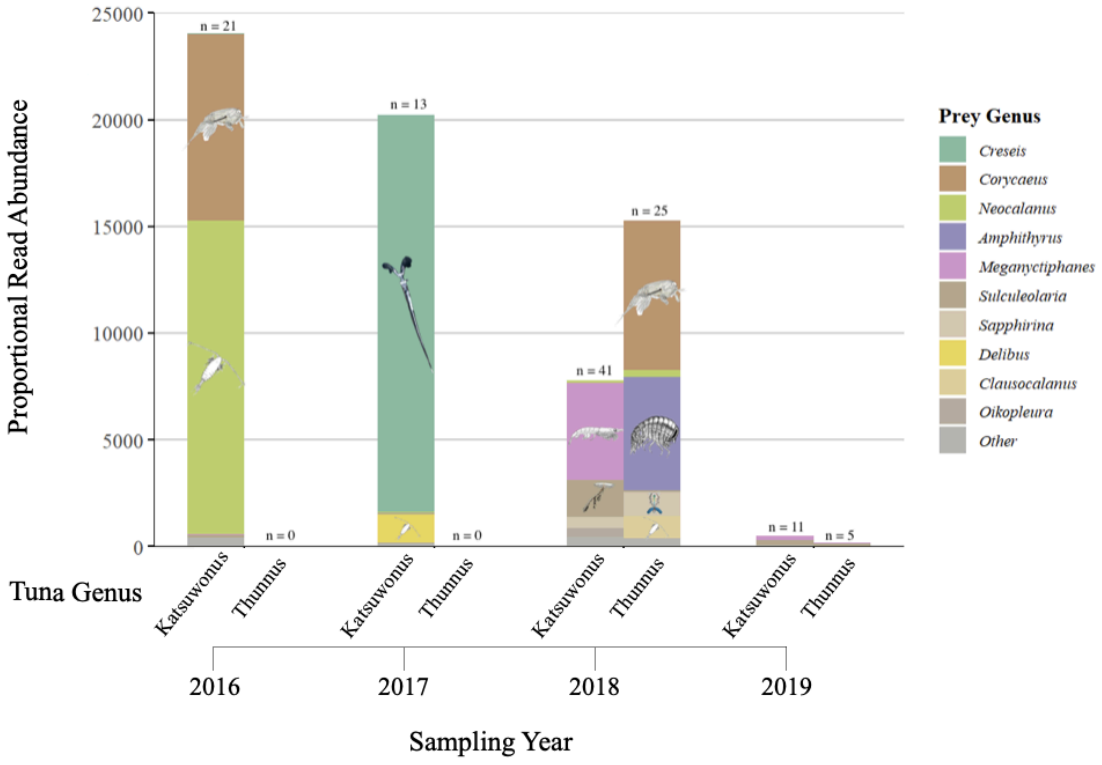


Figure 6: **Relative Sequence Read Abundance of Prey Taxa by Tuna Genus and Year.** Stacked bar chart showing the total number of 18S rRNA sequence reads assigned to each prey genus in larval *Katsuwonus* (left bar) and *Thunnus* (right bar) gut contents across four sampling years (2016–2019). Bar heights represent the sum of raw ASV read counts per genus within each genus-year combination. Sample sizes (*n*) are indicated above each bar. Prey genera with low read counts are grouped under "Other." Note that read counts reflect proportional DNA representation and do not directly indicate prey biomass or individual abundance.

Across all sampling years (2016–2019), larval *Katsuwonus* and *Thunnus* exhibited distinct differences in prey composition based on 18S rRNA gene sequencing (Figure 7). *Katsuwonus* diets were heavily dominated by *Creseis* (36%) and *Neocalamus* (28%). In contrast, *Thunnus* larvae showed a more even distribution of prey taxa, with *Corycaeus* (45%) and *Amphithyrus* (34%) comprising the majority of the diet.

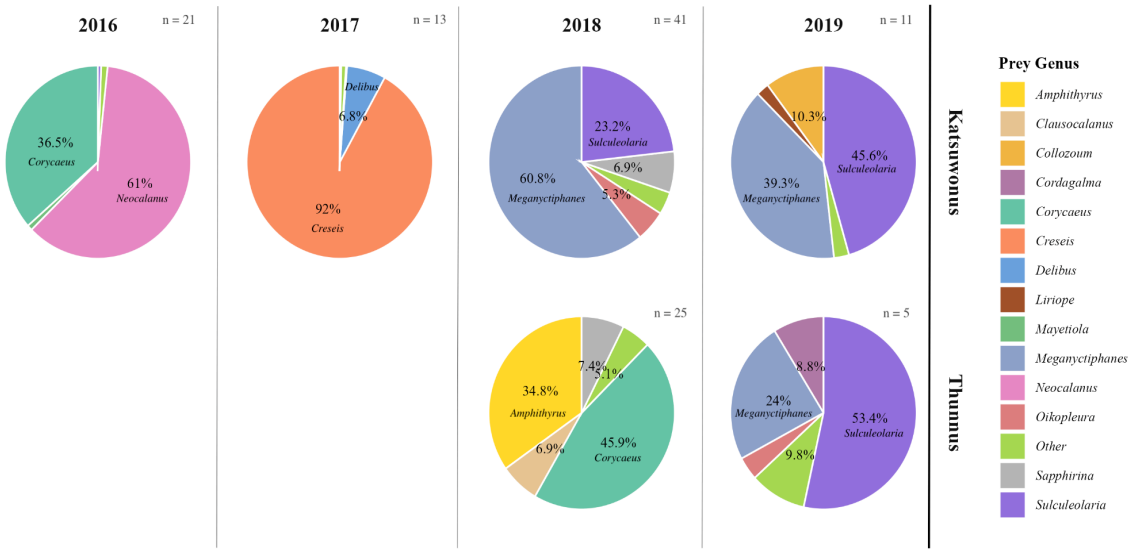


Figure 7: **Top Prey Genera in Larval Tuna Gut Contents by Tuna Genus per Sampling Year.** Pie charts showing the top four most abundant prey genera (by proportional read count) in larval tuna gut contents across four sampling years (2016–2019). All remaining taxa were grouped into the category "Other." The top row shows *Katsuwonus* and the bottom row shows *Thunnus*. Each color represents a distinct prey genus identified through 18S rRNA metabarcoding. Note that values represent relative DNA abundance and may not reflect true prey biomass or individual counts.

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Figure 8 displays the top four most abundant plankton groups identified via the 200-count method for each year from 2015 to 2019. These proportions represent environmental availability based on counts of 200 individual organisms per sample. While a range of taxa were observed, only those that were successfully matched to functional groups present in the gut content dataset (Table 4) were used for prey selectivity comparisons. Calanoid copepods comprised the majority of environmental prey reads each year, with the highest in 2016 and the lowest in 2018. *Corycaeus* copepods were consistently the second most abundant group. The relative abundance of all other taxa, including euphausiid shrimp, mysid shrimp, and "other" taxa, varied across years.

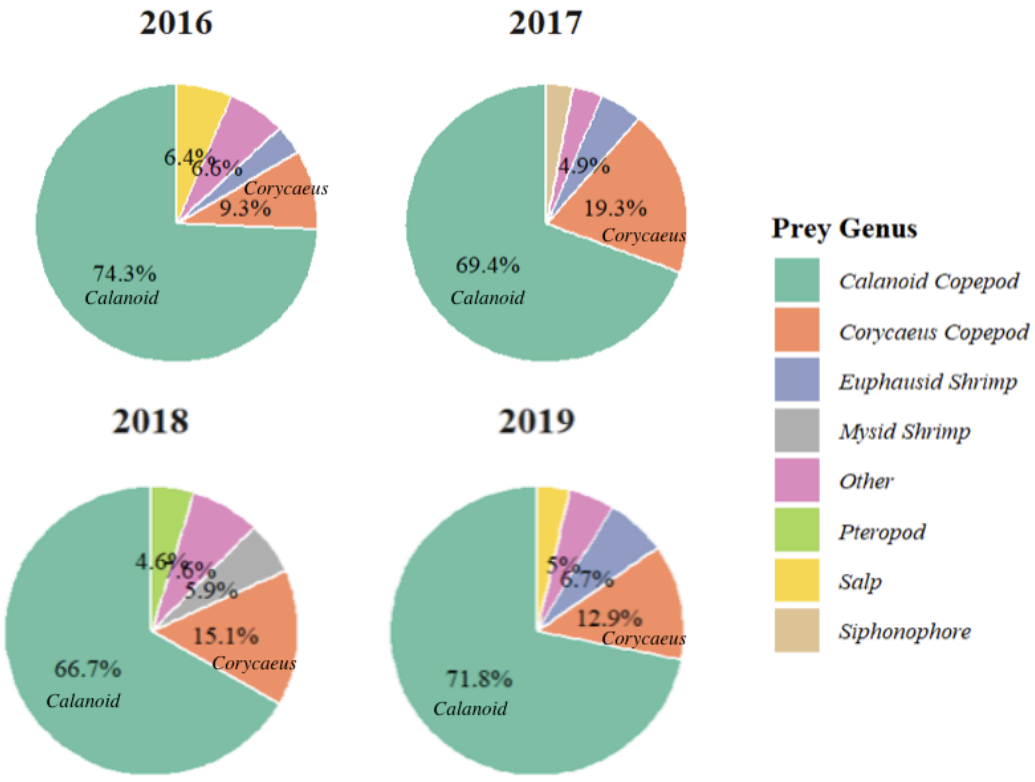


Figure 8: **Top Plankton Groups in Environmental Samples (200 Count) by Sampling Year.** Pie charts showing the top four most abundant plankton groups (by proportional abundance) observed in environmental samples across four sampling years (2016–2019), based on morphological identification using the 200-count method. All remaining taxa were grouped into the category "Other." Each color represents a distinct plankton functional group, categorized by ecological or functional role to match prey types observed in larval gut contents. Note that values represent relative morphological abundance based on fixed-count surveys and may not reflect true biomass or prey availability in the water column.

Prey Selectivity

Chesson's alpha values revealed distinct interannual variability in larval tuna prey selectivity between 2016 and 2019 (Figure 9). In each year, only one to two prey groups dominated gut contents for *Katsuwonus*, but these groups varied markedly year to year. In 2017, pteropods were the sole prey group positively selected, with an α-value of 0.999, indicating near-exclusive dietary contribution relative to environmental availability. In contrast, other years had strongly negative selection for Pteropods, and exhibited variable combinations of other positively selected prey groups, none of which overlapped across years except for colonial cnidarians in 2016 and 2019. Notably, *Sapphirina* copepods consistently exhibited negative selection across all years, indicating they were underrepresented in gut contents relative to their environmental availability (see Supplemental Table 1). Between *Thunnus,* 2018 showed nonoverlapping selections for prey but 2019 showed a slight overlap in selection for colonial cnidarians.

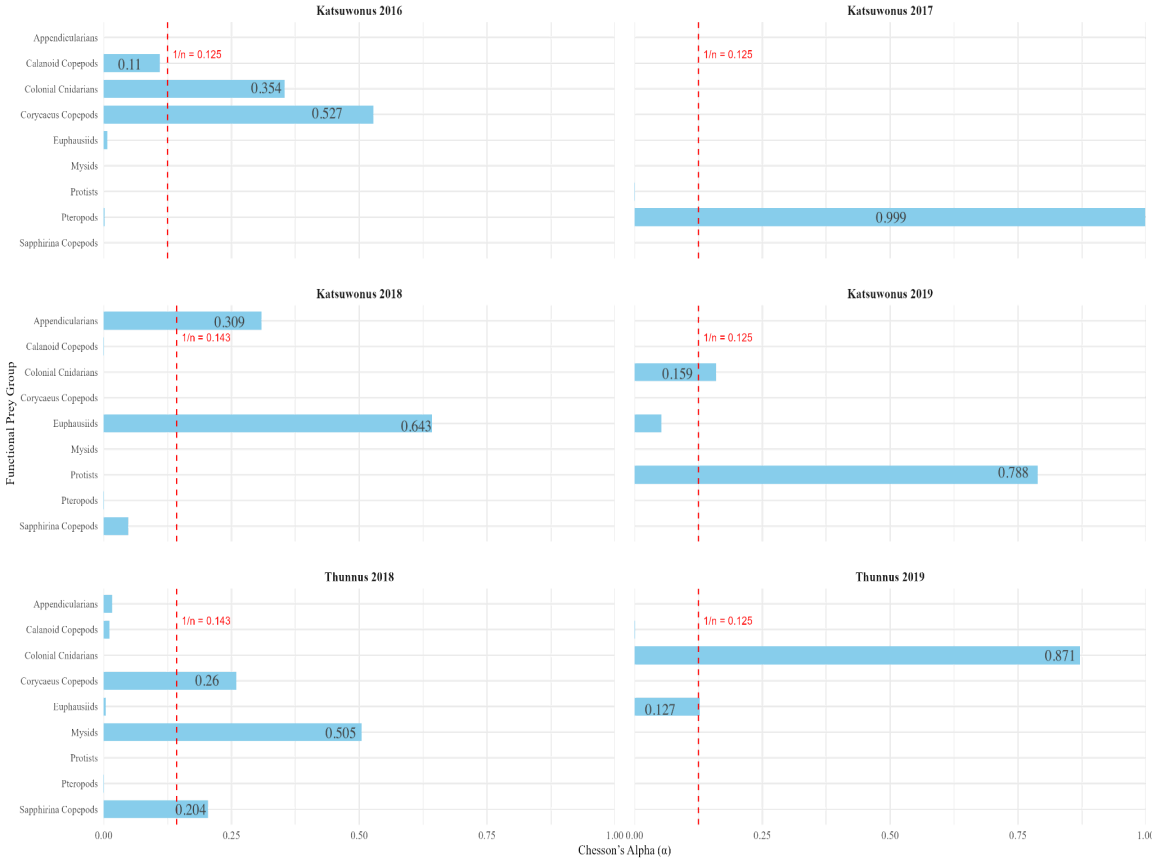
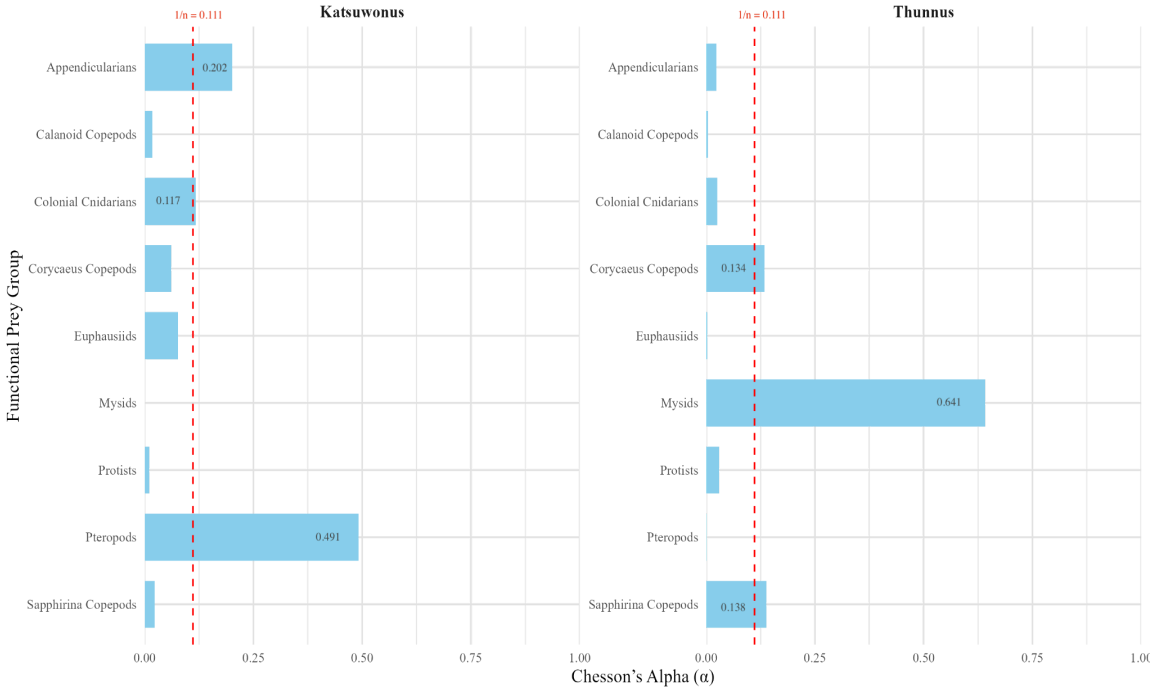


Figure 9: **Prey Selectivity Based on Chesson's Alpha by Genus per Sampling Year.** Bar plots of Chesson's alpha values (α) comparing gut content data per sampling year to environmental prey availability. Top four graphs are *Katsuwonus* and bottom two are *Thunnus*. Gut content data was achieved from 18S DNA sequencing and environmental data was computed from 200 counts. The neutral threshold is indicated with a red dashed line (1/n) and each threshold value is written above each line. These values differ depending on how many Chesson's alpha values were computed- those without environmental counts yield undefined values, and were excluded from the representation. Values above the neutral threshold indicate positive selection, 1/n indicate neutral selection and below the threshold indicate negative selection. Alpha values of significant prey items are indicated in the bars.

Chesson's alpha values revealed clear differences in prey selectivity between tuna larvae genera –*Thunnus* and *Katsuwonus* (Figure 10). *Thunnus* showed strong positive selection for mysids (α = 0.641), indicating that this prey group made up a

disproportionately large share of the diet relative to its environmental availability. *Corycaeus* copepods (α = 0.134) and *Sapphirina* copepods (α = 0.138) were also weakly selected for but not as prominent. In contrast, *Katsuwonus* larvae exhibited a different range of positively selected prey. *Pteropods* (α = 0.491) were strongly selected, followed by appendicularians (α = 0.202) and colonial cnidarians (α = 0.117) which were also positively selected. Other prey groups showed α-values below the neutral threshold,



indicating negative selection.

Figure 10: **Prey Selectivity Based on Chesson's Alpha per Tuna Genus.** Bar plots of Chesson's alpha values (α) comparing gut content data per tuna genus to environmental prey availability. Gut content data was achieved from 18S DNA sequencing and environmental data was computed from 200 counts. Neutral threshold is indicated with a red dashed line (1/n) and each threshold value is written above each line. Chesson's Alpha for all 9 prey species were computed for both species, yielding a neutral threshold of 1/9 = 0.111. Values above the neutral threshold indicate positive selection, 1/n indicate neutral selection and below the threshold indicate negative selection. Alpha values of significant prey items are indicated in the bars.

DISCUSSION

In this study, we investigated the feeding ecology of larval tunas in PIPA by characterizing their diets, feeding strategies, and patterns of prey selection across tuna genera and years. We found both genus-specific and interannual variation in prey use and selectivity. Notably, *Katsuwonus* diets were more taxonomically restricted within individual years but exhibited greater flexibility across years, whereas *Thunnus* displayed more evenly distributed prey use over time, despite a smaller sample size. There was evidence of interannual prey switching, indicating that larval tuna diets are dynamic and responsive to the changing community composition of the plankton. Using Chesson's alpha, we detected tuna genus-specific differences in prey selectivity, supporting evidence of niche partitioning between tuna genera. These results contribute to a growing body of evidence that larval tunas exhibit trophic plasticity early in life (Catalán et al., 2011), with implications for how they may cope with fluctuating oceanographic conditions. These findings also provide insight into how larval tuna foraging behavior and ecological flexibility may influence early survival and recruitment, with implications for understanding population resilience and informing conservation strategies in the Western and Central Pacific Ocean.

Prey Biodiversity

Neither the Shannon or Simpson biodiversity indices revealed significant temporal shifts in prey biodiversity according to statistical testing, suggesting that the overall diversity of larval tuna diets remained relatively stable between 2016 and 2019.

The elevated prey diversity observed in 2018 may reflect sampling artifacts rather than true ecological differences. Larger sample sizes increase the likelihood of detecting rare or low-abundance prey taxa, a known consideration in metabarcoding and environmental DNA studies (Albaina et al., 2016). This highlights the importance of sample size standardization when interpreting biodiversity metrics in diet analyses. Overall, the lack of significant differences across years indicates that temporal variation in prey diversity was not strong enough to suggest a meaningful ecological trend.

Among genera, *Katsuwonus* exhibited a trend toward higher dietary richness and evenness, suggesting more opportunistic or generalist foraging behavior relative to *Thunnus*. This aligns with previous literature indicating that *K. pelamis* are faster-growing, surface-oriented feeders with broader foraging ranges (Bertrand et al., 2002; Llopiz & Hobday, 2020). However, the absence of statistically significant differences—likely due to the small *Thunnus* sample size (n = 23)—means these observations remain suggestive. Future studies with larger and more balanced sample sizes are needed to further assess interspecific differences in dietary diversity and foraging ecology.

Prey Composition

Gut content analyses revealed a wide range of planktonic prey across larval tuna samples, with notable interannual and genus-specific variability in prey preferences, particularly for *Katsuwonus*. Comparisons of interannual patterns in *Thunnus* were limited due to sampling only in 2018 and 2019, with just five individuals sampled in the

latter year. In *Katsuwonus*, diets were typically dominated by one to two prey groups annually, suggesting that larval tunas capitalize on locally abundant prey rather than maintaining rigid taxonomic preferences, consistent with the view that prey availability shapes feeding diversity during early life stages (Whitney et al., 2021).

In contrast, *Thunnus* diets in 2018 were more evenly distributed among prey such as *Corycaeus*, *Amphithyrus*, *Sapphirina*, and *Clausocalanus*, diverging from the concurrently sampled *Katsuwonus* diets dominated by *Sulculeolaria* and *Meganyctiphanes*. These findings suggest that *Thunnus* may exhibit broader prey selectivity while *Katsuwonus* diets tend to be more specialized. This pattern also indicates genus-specific prey preferences and potential trophic niche partitioning. Although *Katsuwonus* strongly selected for *Corycaeus* in 2016, it switched to other prey in later years despite *Corycaeus*'s continued presence, potentially reflecting interspecific competition or dynamic prey field restructuring. Similar divergence in prey use and vertical habitat preference has been observed between skipjack and *Thunnus* spp. larvae in the Straits of Florida, and interpreted as a strategy to reduce niche overlap (Llopiz et al., 2010).

Interestingly, *Katsuwonus* exhibited higher read counts with fewer species in 2016 and 2017 than in 2018, despite 2018 having the largest sample size (n = 41). This pattern suggests more efficient foraging driven by the greater availability of fewer, but more energy-limited, prey types. This may have reduced larval survival and competition, resulting in more prey available per surviving individual. An additional consideration is that 2018–the year with the highest prey diversity–also corresponded with the highest

observed abundance of tuna larvae across both genera. In that year, larvae appeared to positively select for prey types frequently reported as optimal or preferred, such as *Meganyctiphanes* and *Sulculeolaria*, both of which have been identified as important or selectively consumed prey in larval fish diets (Llopiz & Hobday, 2015). This likely reflects the presence of a particularly favorable and energy-rich prey field, potentially contributing to increased larval survival and reducing the total number of prey items required to meet energetic demands. These observations align with the "match–mismatch hypothesis," which posits that successful recruitment depends on the temporal overlap between fish larvae and peaks in prey availability (Cushing, 1990; Durant et al., 2007).

The patchiness and seasonality of plankton blooms—driven by oceanographic processes such as upwelling, eddies, and thermal fronts—can strongly influence larval access to suitable prey (Bakun, 1990**;** Boyd et al., 2016). Consequently, interannual variability in plankton dynamics plays a critical role in shaping larval feeding success, growth, and survival (Quintanilla et al., 2024). These dynamics are often linked to large-scale climate oscillations, particularly ENSO events, which significantly influence surface productivity, stratification, and plankton assemblages across the Pacific (Fiedler & Talley, 2006). Across all years, the identity of these dominant taxa varied markedly, suggesting that shifts in zooplankton community composition and prey availability–likely driven by ENSO-associated oceanographic changes–shaped larval diet structure. During the 2016 El Niño, altered prey fields, such as an increase in calanoid copepods, could have reshaped the prey field available to larval tunas, potentially explaining prey switching in *Katsuwonus* and divergence between tuna genera. These patterns are

consistent with previous observations that larval fish feeding strategies reflect bottom-up environmental forcing, particularly in open-ocean pelagic ecosystems (Bakun, 1996; Olson et al., 2016). Because of its central location in the Pacific Ocean, the PIPA is particularly susceptible to ENSO variability, as it lies directly beneath the developing warm pool during El Niño events (Rotjan et al., 2014). This, combined with its no-take reserve status during sampling years, makes these tuna samples ideal to investigate the natural drivers of interannual variability in larval fish abundance and planktonic community structure. Future analyses integrating remote sensing and in situ environmental datasets (e.g., SST, chlorophyll-a, and zooplankton biomass) can further help in testing these associations.

Finally, comparisons between gut contents and environmental plankton counts suggest that prey abundance in the water column does not directly predict dietary composition. For example, calanoid copepods were the most abundant taxa in environmental samples across all years but only dominated *Katsuwonus* diets in 2016. Similarly, although *Creseis* dominated *Katsuwonus* gut contents in 2017, pteropods (its functional group) were not highly represented in the environmental data that year. This may reflect intense predation pressure by larval tunas, resulting in local depletion of targeted prey items. These mismatches reinforce the idea that larval tuna are selective foragers, choosing prey based on factors like nutritional value, detectability, and handling efficiency. Such patterns support earlier studies demonstrating that larval tunas feed selectively and do not consume prey in direct proportion to environmental availability (Young et al., 2010).

Prey Selectivity

Our findings reveal that larval tunas exhibit clear and dynamic patterns of prey selectivity that vary by both tuna genus and year, with *Thunnus* spp. displaying more specialized prey preferences and *K. pelamis* showing broader, more opportunistic feeding behavior. These differences suggest trophic niche partitioning between the genera and highlight the importance of both biological and environmental drivers in shaping early life foraging strategies. These genus-specific differences align with prior research showing that skipjack and *Thunnus* species differ in their vertical distributions and foraging behaviors during early ontogeny. It has been previously found that larval skipjack are generally associated with surface waters and exhibit broader, more flexible prey use, showing a more opportunistic generalized foraging strategy (Lopiz & Hobday, 2020). On the other hand, larval *Thunnus* species display evidence of more structured foraging behaviors, preferentially targeting prey with high energetic value but variable catchability (Shiroza et al., 2021). These contrasting strategies suggest that the two genera adopt different larval survival tactics, which may help explain the lower and more variable catches of *Thunnus* larvae in field collections. Our data corroborate this in larval fishes: *Thunnus* selected for mysids and *Sapphirina* copepods—prey that are often more mobile or visually detectable—while *Katsuwonus* targeted a broader range including appendicularians, colonial cnidarians, and pteropods, many of which are gelatinous or soft-bodied and indicative of surface-dwelling prey fields.

Chesson's alpha values further confirm that these patterns of selectivity are not static. Across years, *Katsuwonus* shifted dominant prey preferences over years, but notably, no single prey group was selected consistently across all years. This interannual variability reflects what has been described in other pelagic fish larvae, where prey switching occurs in response to changes in plankton availability, oceanographic conditions, and larval ontogeny (Shiroza et al., 2021). In our study, 2017's strong selection for pteropods may reflect the early La Niña influence, favoring prey types abundant in cooler surface waters (Thibodeau et al., 2019). In contrast, 2016's selection for *Corycaeus* copepods likely reflects residual impacts of the 2015–2016 El Niño, which is known to shift zooplankton community structure and vertical prey distribution (Fiedler & Talley, 2006; Lehodey et al., 2011).

The consistently negative selection against *Sapphirina* copepods in *Katsuwonus* supports previous studies suggesting that larval tuna may avoid prey with low energetic return or high handling costs (Shiroza et al., 2021). Males of the genus *Sapphirina* are known for producing tunable, angle-dependent iridescence via guanine crystal layers embedded in their cells (Kimura et al., 2020). This structural coloration can rapidly disappear depending on the viewing angle and illumination, effectively allowing the animal to vanish from view, a mechanism believed to serve as camouflage or predator evasion. Such optical properties may reduce detectability or increase capture difficulty, explaining consistent avoidance by visually oriented predators like larval tuna. Meanwhile, positive selection for soft-bodied prey like appendicularians and pteropods in *Katsuwonus* likely reflects opportunistic capture of easily digestible prey—a strategy that

may confer resilience in patchy or oligotrophic environments, such as the central Pacific (Bakun, 1996; Olson et al., 2016). Although pteropods are shelled, species like *Limacina* extend delicate muscular lobes used for swimming (parapodia) beyond the shell, which may visually resemble appendicularians and stimulate similar feeding responses (Pirtle & Satterlie, 2004). This morphological convergence may help explain the consistent positive selection observed for both groups.

*B*y contrast, *Thunnus* showed consistently narrower selectivity patterns, favoring mysids and colonial cnidarians in 2018 and 2019. While this specialization may reflect inherent genus-specific foraging strategies, it is also possible that *Thunnus* experienced reduced prey field variability during those years or that smaller sample sizes (particularly in 2019) magnified apparent preference. Nevertheless, the sharp alpha peaks for *Thunnus* even within limited datasets suggest active prey selection and potential vulnerability under shifting prey regimes, as selective feeders may have fewer viable alternatives when conditions change (Young et al., 2010). Unlike *Katsuwonus,* its negative selection against pteropods may suggest its preference for prey items that require less-energy to hunt, as the shell of pteropods could impose higher handling costs and make them less desirable.

Overall, our findings demonstrate that larval tuna prey selection is context-dependent, shaped by both biological traits and external oceanographic variability. This plasticity in *Katsuwonus* may represent an adaptive advantage in fluctuating ecosystems, while the more specialized *Thunnus* strategy may enable efficient foraging under stable conditions but limit flexibility. Understanding these dynamics is

essential for predicting how climate variability and plankton community shifts may impact larval survival and recruitment across tuna species.

Ecological and Management Implications

Overall, this study highlights the ecological flexibility and trophic strategies of larval tunas in PIPA, an area with low anthropogenic influence and relatively pristine conditions. The dynamic and taxon-specific prey selectivity we observed suggests that larval tuna survival may depend on both prey availability and the ability to respond to shifting plankton communities. As climate-driven changes continue to reshape tropical pelagic ecosystems, understanding these early life feeding dynamics is essential for forecasting recruitment success and ensuring sustainable management of tuna stocks in the WCPO.

CONCLUSION

This study provides the very first detailed assessment of larval tuna feeding ecology in the WCPO–offering novel insights into how prey diversity, abundance, and selectivity vary across tuna genera and years. Through 18S analysis of gut contents and comparisons to environmental zooplankton data, we found that larval tunas exhibit both prey switching and trophic niche feeding strategies that are modulated by taxonomic identity and environmental context.

Although overall diet biodiversity was stable across years, shifts in dominant prey—especially within *Katsuwonus*—suggest that larvae respond dynamically to prey fluctuations. Chesson's alpha analysis showed consistent differences in selectivity: *Thunnus* favored mobile prey such as mysids and *Sapphirina*, while *Katsuwonus* displayed broader, opportunistic feeding. These results indicate trophic niche partitioning that may reduce interspecific competition in early stages.

The selective foraging observed—including consistent avoidance of certain prey despite high environmental abundance—highlights the complexity of larval feeding behavior. Our findings suggest that larval tunas do not feed randomly on available prey but instead exhibit preferences likely driven by prey quality, detectability, and ease of capture. The genus-specific flexibility seen in *Katsuwonus* may offer adaptive advantages in fluctuating environments, while the more specialized strategy of *Thunnus* could increase vulnerability under rapidly changing conditions.

Understanding these feeding dynamics is critical for forecasting recruitment variability and resilience in tuna populations, particularly in the face of human-driven shifts in plankton communities across the tropical Pacific. As management efforts in the WCPO increasingly focus on early life history processes, incorporating larval foraging ecology into stock assessment models and ecosystem-based management frameworks will be essential to sustaining tuna fisheries and preserving the ecological integrity of pelagic ecosystems.

APPENDIX

Appendix A: Supplemental Tables

Table A1: **Chesson's α Calculations for Figure 9.**

Year	*Tuna Genus*	*Prey Type*	*Gut Count*	*200 Count*	*Abundance in Gut (r)*	*Abundance in Environment (p)*	*Chesson's α*
2016	*Katsuwonus*	Appendicularians	0	6	0.00%	0.05%	NA
		Calanoid Copepods	14668	9550.5	62.08%	79.77%	0.11
		Colonial Cnidarians	137	27.67	0.58%	0.23%	0.354
		Corycaeus Copepods	8774	1189.67	37.14%	9.94%	0.527
		Euphausiids	43	441.83	0.18%	3.69%	0.007
		Mysids	0	207.33	0.00%	1.73%	0
		Protists	0	185.5	0.00%	1.55%	0
		Pteropods	5	188.5	0.02%	1.57%	0.002
		Sapphirina Copepods	0	175.17	0.00%	1.46%	0
2017	*Katsuwonus*	Appendicularians	0	0	0.10%	0.00%	NA
		Calanoid Copepods	36	4115.58	0.19%	69.63%	0
		Colonial Cnidarians	60	177.33	0.32%	3.00%	0
		Corycaeus Copepods	0	1147.67	0.00%	19.42%	0
		Euphausiids	23	289.5	0.12%	4.90%	0
		Mysids	0	24.5	0.00%	0.41%	0
		Protists	4	6	0.02%	0.10%	0.001
		Pteropods	18593	15.58	99.23%	0.26%	0.999
		Sapphirina	0	134.67	0.00%	2.28%	0

		Copepods					
2018	*Katsuwonus*	Appendicularians	466	53.5	8.26%	0.44%	0.309
		Calanoid Copepods	96	8206.17	1.70%	68.06%	0
		Colonial Cnidarians	0	0	0.00%	15.43%	0
		Corycaeus Copepods	0	251.17	0%	2.08%	0
		Euphausiids	4555	726	80.69%	6.02%	0.643
		Mysids	0	571.67	0.18%	4.74%	0.001
		Protists	0	383.33	0%	3.18%	NA
		Pteropods	10	53.5	0.18%	0.44%	0.001
		Sapphirina Copepods	518	8206.17	9.18%	68.06%	0.048
	Thunnus	Appendicularians	13	53.5	0.09%	0.44%	0.017
		Calanoid Copepods	1359	8206.17	9.14%	68.06%	0.011
		Colonial Cnidarians	0	0	0%	0%	NA
		Corycaeus Copepods	7018	1860.5	47.21%	15.43%	0.26
		Euphausiids	13	251.17	0.09%	2.08%	0.004
		Mysids	5325	726	35.82%	6.02%	0.505
		Protists	0	5	0%	0.04%	NA
		Pteropods	4	571.67	0.03%	4.74%	0
		Sapphirina Copepods	1135	383.33	7.61%	3.18%	0.204
2019	*Katsuwonus*	Appendicularians	0	5	0.00%	0.04%	NA
		Calanoid Copepods	3	9622.5	0.63%	74.50%	0
		Colonial Cnidarians	226	339.5	47.58%	2.63%	0.159
		Corycaeus Copepods	0	1730	0.00%	13.39%	0

		Euphausiids	195	895	41.05%	6.93%	0.052
		Mysids	0	16.5	0.00%	0.13%	0
		Protists	51	15.5	10.74%	0.12%	0.788
		Pteropods	0	62	0.00%	0.48%	0
		Sapphirina Copepods	0	229.5	0.00%	1.78%	0
	Thunnus	Appendicularians	0	5	0.00%	0.04%	NA
		Calanoid Copepods	6	9622.5	3.30%	74.50%	0.001
		Colonial Cnidarians	127	339.5	69.78%	2.63%	0.871
		Corycaeus Copepods	0	1730	0.00%	13.39%	0.000
		Euphausiids	49	895	26.92%	6.93	0.127
		Mysids	0	16.5	0.00%	0.13%	0.000
		Protists	0	15.5	0.00%	0.12%	0.000
		Pteropods	0	62	0.00%	0.48%	0.000
		Sapphirina Copepods	0	229.5	0.00%	1.78%	0.000

Table A2: **Chesson's α Calculations for Figure 10.**

Tuna Genus	*Prey Type*	*Gut Count*	*200 Count*	*Abundance in Gut (r)*	*Abundance in Environment (p)*	*Chesson's α*
Katsuwonus	Appendicularians	497	117.5	0.99%	0.22%	0.202
	Calanoid Copepods	14803	39094.25	29.47%	72.25%	0.018
	Colonial Cnidarians	2167	881.5	4.31%	1.63%	0.117
	Corycaeus	8774	6821.83	17.46%	12.61%	0.061

	Copepods					
	Euphausiids	4816	2996	9.59%	5.54%	0.077
	Mysids	0	1082.33	0.00%	2.00%	0
	Protists	55	241	0.11%	0.45%	0.0109
	Pteropods	18608	1804.25	37.04%	3.33%	0.491
	Sapphirina Copepods	518	1070.17	1.03%	1.98%	0.023
Thunnus	Appendicularians	21	117.5	0.14%	0.22%	0.023
	Calanoid Copepods	1365	39094.25	9.01%	72.25%	0.005
	Colonial Cnidarians	173	881.5	1.14%	1.63%	0.026
	Corycaeus Copepods	7018	6821.83	46.30%	12.61%	0.134
	Euphausiids	62	2996	0.41%	5.54%	0.003
	Mysids	5325	1082.33	35.13%	2.00%	0.641
	Protists	55	241	0.36%	0.45%	0.030
	Pteropods	4	1804.25	0.03%	3.33%	0
	Sapphirina Copepods	1135	1070.17	7.49%	1.98%	0.138

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