

# Diet Analysis of Mahimahi (*Coryphaena* spp.) Caught on O'ahu, Hawai'i, Using DNA Barcoding

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U.S. Department of Commerce

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# **Executive Summary**

Mahimahi (*Coryphaena hippurus* and *C. equiselis*) are epipelagic predatory fishes that inhabit tropical and subtropical waters globally. They are an important component of insular non-commercial and small-scale commercial fisheries on the island of O'ahu in Hawai'i. Previous research targeting morphological identifications of stomach contents found mahimahi to feed on a variety of fishes, cephalopods, and crustaceans. However, the degraded nature of stomach contents limits species-level identifications. In this study, we examine mahimahi diets from 200 stomachs collected around O'ahu from 2019–2022 using a DNA barcoding approach to increase the taxonomic resolution of prey items. Stomachs were comprehensively dissected and all individual prey items were measured (to investigate the life stages of prey), weighed, and DNA barcoded. We found that mahimahi consume a diversity of prey types, with pelagic juvenile stages of reef-associated fishes representing over half of the numerical proportion and biomass of identified prey items. This study provides evidence that mahimahi consume a wide variety of prey taxa that spans multiple habitats and taxonomic guilds.

### Introduction

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The family Coryphaenidae contains two species, *Coryphaena hippurus* (Linnaeus, 1758) and *Coryphaena equiselis* (Linnaeus, 1758), which are often referred to as the common dolphinfish or dorado, and the pompano dolphinfish, respectively. In the Hawaiian Language, *Coryphaena* spp., are aptly named mahimahi, meaning strong and energetic ('Ōlelo Hawai'i ref. 'mahi' vs. strong, energetic; Pukui & Elbert, 2003). Best known for their green-blue-gold coloring, fast growth, catchability, and great table fare, mahimahi are highly migratory predatory fishes (Kojima, 1965; Merten et al. 2014) that are distributed circumtropically throughout the epipelagic zone (Palko et al. 1982; Gibbs & Colette, 1959). They typically inhabit the upper 30 m above the thermocline (Tripp-Valdez et al., 2010; Varela, 2017) where they are observed foraging in subsurface waters and under floating objects such as fish aggregation devices or sargassum mats (Kojima, 1965; Beardsley, 1967; Manooch et al., 1984; Velasco-Tarelao, 2003; Castriota et al., 2007; Brewton et al., 2016). Mahimahi undergo rapid growth and development, with body sizes ranging from 47.5–117.5 cm fork length (FL) by 1 year old (Palko et al., 1982; Uchiyama et al., 1986).

In Hawai'i, mahimahi play an important role in both the insular non-commercial and commercial longline fisheries. From 2018–2022, mahimahi averaged 16% of all non-commercial landings of migratory pelagic fishes by weight each year. During this 5-year period, an estimated 4,880 metric tons of mahimahi were caught by non-commercial anglers (WPRFMC SAFE Report, 2023; NOAA FOSS, 2024) and an estimated 1,770 metric tons were landed by commercial operations [including non-insular longline vessels] valued at approximately \$15.3 million USD (NOAA FOSS, 2024). Due to their recreational and commercial value, it is important to understand the feeding ecology, diet composition, and life history of mahimahi in Hawai'i.

Previous diet studies indicate mahimahi feed on a wide variety of fishes. Exocoetidae (flyingfishes) were reported as the dominant prey family in the Pacific Ocean (Tester & Nakamura, 1957; Rothschild, 1964; Hida, 1973; Campos et al., 1993; Olson & Galván-Magaña, 2002; Varela et al., 2017), Atlantic Ocean (Schuck, 1951; Rose & Hasslet, 1974; Rudershausen et al., 2010), Caribbean Sea (Lewis & Axelsen, 1967; Oxenford & Hunte, 1999), Arabian Sea (Varghese et al., 2013), and Mediterranean Sea (Massutí et al., 1998) highlighting the ability of mahimahi to chase and capture highly mobile prey. When Exocoetidae did not dominate the diet, the top consumed taxa reported were pelagic juveniles from common coral reef fish families such as Balistidae (triggerfishes), Monacanthidae (filefishes), and Tetraodontidae (puffers; Ronquillo, 1953; Manooch et al., 1984). Although studies have documented evidence of reef fish juveniles contributing to mahimahi diet, no studies have provided detailed descriptions of the role that these cross-habitat prey play in mahimahi diet.

All previously published studies relied exclusively on morphological identifications of digested prey items (**Table A1**). As a result, diagnostic features needed to identify prey to species were not always present due to prey degradation and fragmentation. Thus, many prey items were identified to family level or labeled as "unidentified" (Saroj et al., 2018; Castriota et al., 2017; Brewton et al., 2016; Rudershausen et al., 2010; Bannister, 1976; Ronquillo, 1953). Taxa identified at a low resolution or omitted entirely limit the accuracy of diet studies and our understanding of the ecology of mahimahi. DNA barcoding can overcome such constraints rendering it as a powerful tool to identify digested biological materials and generate high resolution diet information. This approach has been applied successfully in diet studies of marine taxa around Oʻahu (Oyafuso et al., 2016) and when paired with a comprehensive and curated database, DNA barcoding provides species-specific information from digested prey items that might otherwise be identified only to class or family level.

The main Hawaiian Islands (MHI) provide a unique environment given the proximity of the open-ocean pelagic realm to nearshore coral reef ecosystems. Around O'ahu, the steep bathymetric gradient and lack of extended coastal shelf foster cross-habitat interspecific interactions among organisms. In order to better describe mahimahi diet, this study aims to analyze the trophic contribution of individual prey species and functional prey groups of mahimahi using DNA barcoding techniques that identify prey at a high taxonomic resolution.

# **Methods**

#### **Sample Collection**

Two hundred mahimahi stomachs were collected from 83 unique fishing events between February 2019 and August 2022. All mahimahi were caught by rod and reel with either lure or bait, within 25 nautical miles of O'ahu (**Figure 1**). Once caught, stomachs were removed from each fish and kept on ice until frozen individually at -20 °C. Additional information such as catch location, method of catch (i.e., lure or bait type used), and size and sex of fish was provided by each angler for all stomachs collected.





#### **Stomach Dissection**

All stomach processing, DNA extraction, and amplification were conducted at the NOAA Pacific Islands Fisheries Science Center in Honolulu, Hawai'i. Stomachs were thawed

for the minimum amount of time needed for dissection. All stomach dissections proceeded on ice. After thawing, each stomach was cut open and contents from the main stomach cavity (esophagus to pylorus) were examined. All prey items were separated, assigned an identification number, measured (greatest linear dimension), weighed, assigned to a broad taxonomic category (fishes, crustaceans, or cephalopods), and preserved individually in 95% ethanol. To aid in distinguishing individual prey items from digested materials, a unique prey item was defined as any organism that was (1) intact (i.e., both head and tail present) or (2) any biological tissue greater than 2 cm in length with a distinct start or end (i.e., either head or tail) present. All prey were examined as a group and prey items that likely came from a single organism (i.e., a head separate from a tail but morphologically similar) were both assigned different prey identification numbers, but upon genetic confirmation that both prey pieces were identified as the same species, were noted as a single individual in subsequent analyses.

#### **DNA Barcoding**

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Genomic DNA from each preserved prey item was extracted using a Qiagen DNeasy Blood and Tissue spin column extraction kit following the manufacturer's protocol (Qiagen, Hilden, Germany). Each extraction was amplified via polymerase chain reaction (PCR) using universal (COI) and taxa specific primers (**Table A2**). All PCR reactions were carried out in 20 µl volumes consisting of 1.0 µl of forward primer, 1.0 µl of reverse primer, 10.0 µl Immomix Red (Bioline), 0.5 µl of bovine serum albumin (4 mg/ml), 5.5 µl of molecular grade water, and 2.0 µl of extracted DNA template. Thermocycling conditions varied by primer set used (**Table A3**).

PCR products were cleaned either with ExoSap-IT (Thermo-Fisher) or using Ampure XP (Beckman-Coulter) magnetic beads in a 1:1.2 ratio of PCR product to magnetic beads. Bead clean-ups followed manufacturer's instructions to eliminate any extraneous sequences smaller than 150 bp that were unintentionally amplified during PCR. Cleaned products were randomly selected for visualization using gel electrophoresis (1% agarose gel) to confirm successful amplification and cleaning.

Cleaned PCR products were Sanger sequenced (Applied Biosystems 3730XL DNA Analyzer) in the 5'-3' direction at the Advanced Studies in Genomics, Proteomics, and Bioinformatics (ASGPB) Core Facility at University of Hawai'i at Mānoa. A taxon was assigned to each annotated sequence (Geneious Prime 2023.0.4, Auckland, New Zealand) using BLAST+ (Basic Local Alignment Search Tool; Altschul et al., 1990; Camacho et al., 2009). All sequences that targeted the COI region were locally BLAST against a custom regional database using sequences from the NCBI (National Center for Biotechnology Information) non-redundant nucleotide (nt) database. Prey were identified to species, genus, or family level when percent identity was  $\geq$  99%,  $\geq$  97%, or  $\geq$  95%, respectively. Low quality sequences (e.g., percent identity < 95%) or sequences that were identified as *Coryphaena* spp. (and thus may be a result of host contamination) were extracted a second time and/or re-amplified and sequenced again in an attempt to identify the prey to species level.

#### **Data Analysis**

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All non-relevant taxa were removed from the data set prior to analysis. First, taxa were filtered to remove all known bait species that do not exist naturally in Hawai'i (i.e., *Engraulis mordax, Doryteuthis opalescens*). Second, possible bait species (i.e., *Encrasicholina punctifer, Decapterus macrosoma, Decapterus macarellus, Selar crumenophthalmus, Scomber australasicus, Euleptorhamphus viridis, Katsuwonus pelamis, Thunnus albacares*) were cross referenced with the bait type information provided by anglers for each stomach collected. If the bait type matched any prey in that specific stomach, it was marked as bait and removed from subsequent analyses. Lastly, all taxa that were identified as mahimahi (i.e., *C. hippurus, C. equiselis*) were reassigned as "unidentified."

All prey were categorized into one of three habitat groups (i.e., reef-associated, coastal pelagic, pelagic) determined by the amount of time allocated in a specific environment during their life. Reef-associated organisms were defined as those known to inhabit coral reef systems as adults but that have a pelagic juvenile and/or pelagic larval stage. Coastal pelagic organisms were defined as those often associated with nearshore environments but are also frequently observed in the open ocean. Pelagic organisms were defined as those that exist entirely in the pelagic realm throughout all life stages.

For each prey taxa, three metrics were calculated to summarize all stomachs analyzed: percent by count, percent by weight, and frequency of occurrence. Numeric percent (%N) was calculated as [100\*(N<sub>x</sub>/(N<sub>Tn</sub>)], where N<sub>x</sub> = number of individual prey belonging to "x" species and N<sub>Tn</sub> = the total number of identified prey species. Gravimetric percent (%W) was calculated as [100\*(W<sub>x</sub>/(W<sub>Tw</sub>)], where W<sub>x</sub> = total weight of individual prey belonging to "x" species and W<sub>Tw</sub> = the total weight of all identified prey species. Frequency of occurrence (%F) was calculated as [100\*(F<sub>x</sub>/F<sub>Tf</sub>)] where F<sub>x</sub> = the number of stomachs containing "x" species, and F<sub>Tf</sub> = total number of prey-containing stomachs. The index of relative importance (IRI) combines all three summary values and was calculated as IRI = [(%N + %W)\*(%F)]/100. The same calculations were done at the family and the broad taxa level (i.e., crustaceans, cephalopods, fishes).

Sampling effort was assessed by plotting a prey species accumulation curve to visualize whether or not the stomachs sampled adequately represent mahimahi prey community. Ninety-five percent confidence intervals were calculated using the spaccum function in the Vegan package in R (Oksanen et al., 2022). After determining the habitat group

data had a non-normal distribution via Shapiro-Wilk normality tests, statistical significance among habitat groups was tested using Kruskal-Wallis and Dunn tests, without correction. Analyses were completed using the rstatix package in R (Kassambara, 2023).

#### Results

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In total, 200 mahimahi stomachs (mean FL = 89.56 cm, range = 41.91-200.66 cm, n = 181) were dissected. Of those 200, 170 (85%) stomachs contained prey (n = 1325 items, excluding bait). Using DNA barcoding, 929 prey items belonging to 75 species and 31 families were identified. Over half (54.8%) of the families identified contained at least two unique species within that family; 76.4% of samples were identified to species, 10.4% to genus, and 6.5% to family. The sequence database for Hawaiian stomatopods (mantis shrimps) is not representative of all documented families; therefore, DNA barcoding did not result in identification to the family level or higher. However, all larval stomatopods (4.8%) were morphologically confirmed as erichthus-type stomatopods, and therefore assigned to the order Stomatopoda (Steck et al., 2022). The remaining 396 prey items (29.8% of all prey samples) produced low quality sequences in which percent identity was < 95% and were marked as "unidentified" fish, crustacean, or cephalopod. A prey accumulation curve for all prey species identified in this study indicates the stomachs sampled did not completely represent the diet of mahimahi near O'ahu as the curve did not reach an asymptote (**Figure 2**) at 75 species.



**Figure 2.** Prey accumulation curve (with 95% confidence interval) for all prey species identified (n = 75) via DNA barcoding in 141 stomachs. Items classified as bait are not included

#### **Prey Composition**

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Each non-empty stomach (n = 170) contained an average of 7.8 prey items ( $\pm$  7.7 SD, range 1–48). Of all prey species identified, over three-quarters (77%) occurred in more than one stomach. Prey were pooled across stomachs by organism type (i.e., fish, crustacean, cephalopod) to examine diet composition by broad taxonomic group. Overall, fish dominated the diet. On average, each stomach's prey community was numerically composed of 88% fishes, 8% crustaceans, and 4% cephalopods (**Figure 3**).





Numerically, Mullidae (goatfishes) was the most abundant prey family (27.3 %N), more than double that of the next most abundant family, Carangidae (jacks; 11.6 %N). Within Mullidae, four species were identified, but *Parupeneus pleurostigma* (sidespot goatfish 'malu') accounted for the majority (76.1%) of identified individuals. Conversely, five species were identified within the Caragindae family; however, the most abundant species, *Decapterus macrosoma* (mackerel scad "ōpelu'), accounted for less than half (42.5%) of the identified individuals. While the three most abundant families (Mullidae, Carangidae, and Ostraciidae) were fishes, the fourth and fifth most abundant families

were crustaceans, Carpiliidae (reef crabs; 5.5 %N) and Stomatopoda (mantis shrimps; 4.8 %N), respectively.

Gravimetrically, the family Exocoetidae contributed the greatest mass (19.1 %W), followed by Hemiramphidae (halfbeaks; 13.7 %W), and Mullidae (12.4 %W). Eight individual species were identified within Exocoetidae; however, one taxon, *Paraexocoetus brachypterus* (sailfin flyingfish 'mālolo'), accounted for over one-third of the overall gravimetric percent of the family (7.4 %W). Overall, fishes made up 95% of the total prey weight, followed by cephalopods (4%), and crustaceans (1%) (**Table 1**).

**Table 1**.Summary table of all prey taxa identified in this study. Total percent contributed by number (%N), weight (%W), and frequency of occurrence (%F) have been calculated for each prey species, family, and taxon group. The index of relative importance (IRI) has also been calculated for all groups as  $IRI = [(%N + %W)^*(%F)]/100$ . Habitat group (reef-associated, coastal pelagic, or pelagic) is noted for each species.

Taxon	Family	Species	%N	%W	%F	IRI	Habitat Group
Crustaceans			10.39	1.20	7.65	0.88	
Carpiliidae			5.51	0.45	4.08	0.24	
		Carpilius convexus	5.03	0.43	2.04	0.11	reef-associated
		<i>Carpilius</i> sp.	0.55	0.02	3.40	0.02	reef-associated
	Ocypodinae	Ocypode ceratophthalmus	0.11	0.01	0.68	0.00	reef-associated
	Stomatopoda		4.81	0.92	3.40	0.19	reef-associated
Unidentifi	ed crustaceans		4.00	0.36	15.88	0.69	
Cephalopod			3.14	4.50	8.82	0.67	
	Argonautidae	Argonauta argo	0.11	0.05	0.68	0.00	pelagic
	Ommastrephidae		2.59	2.43	6.80	0.34	
		<i>Eucleoteuthis</i> sp.	0.33	0.08	1.36	0.01	pelagic
		Hyaloteuthis pelagica	0.44	0.60	2.72	0.03	pelagic
		<i>Hyaloteuthis</i> sp.	0.55	0.14	2.72	0.02	pelagic
		Ommastrephidae	0.22	0.00	0.68	0.00	pelagic
		Sthenoteuthis oualaniensis	0.66	1.42	2.04	0.04	pelagic
		Sthenoteuthis sp.	0.44	0.19	1.36	0.01	pelagic
Unidentifie	ed cephalopods		2.25	1.70	9.41	0.37	
Fishes			86.47	94.30	86.47	156.31	
	Acanthuridae		3.78	4.05	17.01	1.33	
		Acanthurus dussumieri	0.98	0.21	4.76	0.06	reef-associated
		Acanthurus nigrofuscus	0.66	0.21	3.40	0.03	reef-associated
		Acanthurus olivaceus	0.22	0.02	1.36	0.00	reef-associated
		<i>Acanthurus</i> sp.	0.98	0.20	6.12	0.07	reef-associated
		Acanthurus thompsoni	0.55	0.27	2.72	0.02	reef-associated
		Acanthurus triostegus	0.22	0.03	1.36	0.00	reef-associated

Taxon	Family	Species	%N	%W	%F	IRI	Habitat Group
		Naso brevirostris	0.11	0.29	0.68	0.00	reef-associated
		Naso hexacanthus	0.11	2.83	0.68	0.02	reef-associated
	Balistidae		2.81	2.79	12.24	0.69	
		Sufflamen bursa	0.11	0.01	0.68	0.00	reef-associated
		Xanthichthys	1.64	1.65	6.80	0.22	reef-associated
		auromarginatus					
		Xanthichthys sp.	1.09	1.15	6.80	0.15	reet-associated
	Belonidae		2.27	5.36	3.40	0.26	
		Ablennes hians	1.97	4.81	2.72	0.18	coastal pelagic
		Ablennes sp.	0.33	0.57	2.04	0.02	coastal pelagic
	Carangidae		11.56	11.27	25.17	5.75	
		Caranx melampygus	0.44	0.21	2.04	0.01	reef-associated
		Decapterus macarellus	2.73	6.32	7.48	0.68	coastal pelagic
		Decapterus macrosoma	4.92	1.93	10.20	0.70	coastal pelagic
		<i>Decapterus</i> sp.	1.09	0.21	4.76	0.06	coastal pelagic
		Selar crumenophthalmus	1.86	2.30	7.48	0.31	coastal pelagic
		<i>Selar</i> sp.	0.44	0.34	1.36	0.01	coastal pelagic
		Seriola rivoliana	0.11	0.01	0.68	0.00	reef-associated
	Chaetodontidae	Chaetodon kleinii	0.22	0.04	1.36	0.00	reef-associated
	Dactylopteridae	Dactyloptena orientalis	4.59	2.80	14.29	1.06	reef-associated
	Diodontidae		1.73	1.43	4.76	0.15	
		Chilomycterus reticulatus	0.11	0.05	0.68	0.00	reef-associated
		Diodon holocanthus	1.31	1.30	2.72	0.07	reef-associated
		Diodon hystrix	0.22	0.05	1.36	0.00	reef-associated
		<i>Diodon</i> sp.	0.11	0.04	0.68	0.00	reef-associated
	Exocoetidae		4.10	19.16	14.97	3.48	
		Cheilopogon atrisignis	0.11	1.51	0.68	0.01	pelagic
		Cheilopogon furcatus	0.11	1.22	0.68	0.01	pelagic
		Cheilopogon sp.	0.33	1.52	0.68	0.01	pelagic
		Cypselurus poecilopterus	0.11	0.07	0.68	0.00	pelagic

Taxon	Family	Species	%N	%W	%F	IRI	Habitat Group
		<i>Cypselurus</i> sp.	0.11	0.08	0.68	0.00	pelagic
		Exocoetus monocirrhus	0.33	2.36	1.36	0.04	pelagic
		<i>Exocoetus</i> sp.	0.11	0.03	0.68	0.00	pelagic
		Exocoetus volitans	0.33	0.11	1.36	0.01	pelagic
		<i>Hirundichthys</i> sp.	0.22	0.15	1.36	0.01	pelagic
		Hirundichthys speculiger	0.11	0.02	0.68	0.00	pelagic
		Parexocoetus brachypterus	1.64	7.44	5.44	0.49	pelagic
		<i>Parexocoetus</i> sp.	0.22	0.55	1.36	0.01	pelagic
		Prognichthys sealei	0.33	4.11	1.36	0.06	pelagic
	Fistulariidae	Fistularia commersonii	0.22	0.02	1.36	0.00	reef-associated
	Gempylidae		2.48	2.14	9.52	0.44	
		Gempylus serpens	1.97	1.92	8.16	0.32	pelagic
		<i>Gempylus</i> sp.	0.55	0.23	2.72	0.02	pelagic
	Hemiramphidae		3.02	16.44	8.16	1.59	
		Euleptorhamphus viridis	2.51	13.66	7.48	1.21	coastal pelagic
		<i>Euleptorhamphus</i> sp.	0.55	2.84	2.72	0.09	coastal pelagic
	Holocentridae		2.27	0.77	6.80	0.21	
		Myripristis berndti	0.11	0.01	0.68	0.00	reef-associated
		Myripristis chryseres	0.11	0.11	0.68	0.00	reef-associated
		Myripristis kuntee	0.22	0.09	0.68	0.00	reef-associated
		Neoniphon sammara	0.11	0.00	0.68	0.00	reef-associated
		<i>Plectrypops</i> sp.	0.11	0.05	0.68	0.00	reef-associated
		Sargocentron punctatissimum	0.11	0.04	0.68	0.00	reef-associated
		Sargocentron xantherythrum	1.42	0.43	3.40	0.06	reef-associated
		Sargocentron sp.	0.11	0.04	0.68	0.00	reef-associated
	Lutjanidae	Lutjanus kasmira	0.22	0.02	0.68	0.00	reef-associated
	Microdesmidae	Ptereleotris heteroptera	0.33	0.02	1.36	0.00	reef-associated
	Molidae	Ranzania laevis	0.33	0.90	1.36	0.02	pelagic

Taxon	Family	Species	%N	%W	%F	IRI	Habitat Group
	Monacanthidae		1.51	1.00	4.76	0.12	
		Cantherhines dumerilii	0.11	0.50	0.68	0.00	reef-associated
		Cantherhines	1.31	0.44	3.40	0.06	reef-associated
		sandwichiensis					· · · · ·
		Pervagor aspricaudus	0.11	0.06	0.68	0.00	reef-associated
	Mullidae		27.54	12.42	38.10	14.54	
		Mulloidichthys vanicolensis	0.11	0.19	0.68	0.00	reet-associated
		Mulloidichthys sp.	0.55	0.47	3.40	0.03	reef-associated
		Parupeneus cyclostomus	0.11	0.05	0.68	0.00	reef-associated
		Parupeneus multifasciatus	3.93	1.65	10.88	0.61	reef-associated
		Parupeneus pleurostigma	20.98	9.16	29.25	8.82	reef-associated
		<i>Parupeneus</i> sp.	1.64	0.59	8.84	0.20	reef-associated
	Nomeidae		4.54	3.98	11.56	0.99	
		Cubiceps pauciradiatus	0.22	0.15	1.36	0.00	pelagic
		<i>Cubiceps</i> sp.	0.11	0.04	0.68	0.00	pelagic
		Nomeus gronovii	0.22	0.03	0.68	0.00	pelagic
		Psenes cyanophrys	3.61	3.35	8.84	0.62	pelagic
		Psenes pellucidus	0.11	0.10	0.68	0.00	pelagic
		<i>Psenes</i> sp.	0.33	0.34	1.36	0.01	pelagic
	Ostraciidae		8.10	1.67	19.73	1.93	
		Lactoria fornasini	2.62	0.72	7.48	0.25	reef-associated
		<i>Lactoria</i> sp.	4.59	0.73	10.20	0.54	reef-associated
		Ostracion meleagris	0.22	0.12	1.36	0.00	reef-associated
	Priacanthidae	Heteropriacanthus	0.11	0.14	0.68	0.00	reef-associated
		cruentatus					
	Scombridae		2.16	3.01	7.48	0.39	
		Auxis thazard	0.22	0.22	1.36	0.01	pelagic
		Katsuwonus pelamis	1.53	1.07	4.76	0.12	pelagic
		<i>Katsuwonus</i> sp.	0.33	0.69	1.36	0.01	pelagic
		Scomber sp.	0.11	1.05	0.68	0.01	pelagic

Taxon	Family	Species	%N	%W	%F	IRI	Habitat Group
	Syngnathidae	<i>Hippocampus</i> sp.	0.11	0.02	0.68	0.00	pelagic
	Synodontidae		1.08	0.26	2.04	0.03	reef-associated
	Tetraodontidae		0.86	6.05	5.44	0.38	
		Arothron hispidus	0.11	0.01	0.68	0.00	reef-associated
		Lagocephalus Iagocephalus	0.66	2.66	4.08	0.14	pelagic
		Lagocephalus sp.	0.11	3.40	0.68	0.02	pelagic
	Zanclidae		1.19	0.48	4.08	0.07	
		Zanclus cornutus	1.09	0.41	3.40	0.05	reef-associated
		Zanclus sp.	0.11	0.08	0.68	0.00	reef-associated
Unid	entified fishes		28.65	48.74	72.94	56.45	

#### **Prey Proportion**

In all subsequent analyses, only stomachs in which at least 50% of all prey were identified are considered (n = 118, **Figure B1**).

At the family level, Mullidae and Carangidae represent the highest and second highest mean proportion per stomach, respectively, whether measured numerically or gravimetrically (**Figure 4**. A, B). Across both metrics, Mullidae, Carangidae, Balistidae, Acanthuridae, Nomeidae, Ostraciidae, Exocoetidae, and Hemiraphidae appear in the top 10 families; however, the relative order differed by metric.

At the species level, *P. pleurostigma* (sidespot goatfish 'malu') represented the highest mean proportion numerically and gravimetrically (**Figure 4**. C, D). Both the mean numeric and gravimetric proportion of *P. pleurostigma* was more than double that of the next highest species. Additionally, *P. pleurostigma* appeared in 33% of all non-empty stomachs, followed by the second and third most frequently occurring species, *Lactoria fornasini* (thornback cowfish, 22%) and *P. multifasciatus* (manybar goatfish 'moano,' 16%, **Table A4.**).



**Figure 4.** Mean numeric proportion  $\pm 1$  standard error and mean gravimetric proportion  $\pm 1$  standard error by prey family and prey species. Prey families and species with a mean proportion less than 1% are omitted. Bars are colored based on habitat group: reef-associated prey are tan, coastal pelagic prey are orange, and pelagic prey are brown. Panels A and B show mean proportion at the family level, and panels C and D show mean proportion at the species level.

#### **Habitat Group Proportion**

Reef-associated organisms represented the highest mean numeric proportion (58.0%), mean gravimetric proportion (51.5%), and frequency of occurrence (78.6%) compared to coastal pelagic and pelagic prey. The mean numeric and mean gravimetric proportions of reef-associated prey were re-calculated under alternate criteria of data quality in which only stomachs where > 0%, > 25%, > 75%, and = 100% of prey items were identified were analyzed. In these re-analyses, the mean proportion of reefassociated prey was robust (Figure B2). A Kruskal-Wallis test [and Dunn test] showed the median proportion for reef-associated prey was significantly higher than the coastal pelagic and pelagic group, both numerically (median = 66.7%, H(2) = 68.51, p < 0.001) and gravimetrically (median = 55.8%, H(2) = 50.39, p < 0.001). However, the coastal pelagic and pelagic groups were not statistically different from one another for both metrics (p > 0.05). The frequency of occurrence of reef-associated prey items was greater than both the coastal pelagic and pelagic groups, indicating that over two-thirds (78.6%) of all non-empty stomachs contained at least one reef-associated species. Pelagic prey were observed in 44.4% of stomachs and coastal pelagic prey were observed in 38.1% of all non-empty stomachs (Figure 5).



**Figure 5.** Mean numeric proportion (blue) and mean gravimetric proportion (pink) by habitat group (reef-associated, coastal pelagic, pelagic) ± 1standard error. Frequency of occurrence

(white) tabulates the fraction of stomachs that included a prey item from each respective habitat group.

A majority of the prey families and species with the greatest numeric proportion were identified as reef-associated taxa (**Figure 4**. A, B). The dominance of reef-associated prey is less pronounced when ranked by gravimetric proportion; however, the family Mullidae and species *P. pleurostigma* have the greatest mean gravimetric proportion of all identified prey (**Figure 4**. C, D). Similarly, a large sum of the most frequently occurring prey families and prey species were categorized as reef-associated (**Table 1**). Although reef-associated prey were most often present, many stomachs contained prey from at least two different habitat groups (**Figure 6**) and the proportion of habitat groups represented varied substantially across stomachs (**Figure 7**).



**Figure 6.** Percent of stomachs containing at least one prey item from each habitat group, or a combination of multiple habitat groups.



**Figure 7.** Violin plot of the distribution of reef-associated, coastal pelagic, and pelagic prey items by numeric and gravimetric proportion for each habitat group. Each point represents one stomach.

# Discussion

#### **Diet Summary**

Mahimahi have a diverse diet, foraging on a wide range of fishes, crustaceans, and cephalopods in coastal and offshore waters around O'ahu, Hawai'i. Mahimahi predominantly consume fishes from the families Mullidae (goatfishes), Carangidae (jacks), and Exocoetidae (flyingfishes). These findings differ from numerous existing studies that highlight Exocoetidae as the single dominant prey family (Tester & Nakamura, 1957; Rothschild, 1964; Hida, 1973; Campos et al., 1993; Olson & Galván-Magaña, 2002; Varela et al., 2017). Despite being the most important family in this study, Mullidae were rarely documented as prey in previous studies of mahimahi diet. The most important species, *P. pluerostigma*, was not identified in any previous diet studies, regardless of having a circumtropical distribution and suitable habitat near the sites of many previous studies. While Tester and Nakamura (1957) also studied mahimahi from O'ahu and identified prey from the family Mullidae, they were unable to identify prey to species level due to extensive digestion of prey items.

When considering prey species' abundance relative to prey size and weight, it becomes apparent that mahimahi around O'ahu often rely on numerous small prey for sustenance. On occasion, a single large prey item (i.e., flyingfish) was observed to be the only prey in a stomach, but more frequently a suite of many smaller prey taxa were present. Many of the smaller prey items were identified as reef-associated fishes in their pelagic juvenile stage, thus the smaller size was expected compared to adult pelagic fishes.

A majority of the mahimahi in this study consumed a combination of prey belonging to different habitat groups; thus, there is no evidence that mahimahi are targeting prey from one habitat group. While other studies report reef-associated fishes as prey taxa, this study emphasizes the importance of this habitat group to mahimahi caught near O'ahu. The significantly greater contribution of reef-associated prey in this study may be attributed to the proximity of mahimahi foraging habitat to the main Hawaiian Islands, as a direct consequence of the island mass effect (Gove et al., 2016) enhancing productivity near the islands. However, due to limited field-based surveys assessing the density of prey populations around O'ahu, the overall selectivity of mahimahi cannot be determined from these diet data.

#### **DNA Barcoding Summary**

The advantages of DNA barcoding were made obvious in this study. More than half of all prey items were fishes digested beyond recognition (e.g., spine with flesh bits, pieces of fin rays) and, in the absence of DNA barcoding, they would have been categorized as

"unidentified fishes." By utilizing DNA barcoding, a majority of the highly digested prey items have been identified with high resolution to the species level.

DNA barcoding has helped to illuminate the diversity of mahimahi diet by identifying numerous species within each family. Because many of the prey species present in this study are not yet in their adult stage, identifying to species level may be arduous or impossible due a lack defining morphometrics and meristics that are expressed in adult reef-associated fishes. DNA barcoding allowed for high confidence species level identifications of juvenile prey individuals.

In this study, all prey that were identified as *Coryphaena* spp. (*C. hippurus, C. equiselis*) were treated as misidentifications caused by contamination from the host. As mahimahi are documented cannibals (Ronquillo, 1953; Gibbs & Collette, 1959; Lewis & Axelsen, 1967; Rose & Hassler, 1974; Massutí et al., 1998; Oxenford and Hunte, 1999; Vaske-Júnior & Lessa, 2004; Rudershausen et al., 2010; Varghese et al., 2013), we recognize that eliminating all mahimahi identifications from downstream analysis underestimates [does not represent] their cannibalistic nature. In some cases, a fish was identified as mahimahi but was distinctly another species (i.e., triggerfish, goatfish, flyingfish) and minimally digested. However, many other prey in advanced states of digestion were also identified as mahimahi. Considering only minimally digested prey could be visually identified, there were no discernable mahimahi prey observed; thus, no direct observations of cannibalism occurred in this data set. For digested prey, determining true mahimahi identifications from host contamination was difficult and highly subjective; therefore, mahimahi hits were considered a misidentification by default and excluded.

#### Conclusions

Juvenile reef fishes such as Mullidae (goatfishes), Acanthuridae (surgeonfishes), and Balistidae (triggerfishes) make up a substantial portion of mahimahi diet caught in waters far from coral reefs. These results highlight the significant food web links between coral reefs and mahimahi thereby bridging nearshore and pelagic habitats. Similarly, ono (*Acanthocybium solandri*) caught off O'ahu preyed predominantly on juvenile reef fishes in summer months (Oyafuso et al., 2016), further reinforcing these cross-habitat linkages to pelagic predators. The trophic links identified in this study contribute to our understanding of the life history of mahimahi caught near O'ahu and are important to ecosystem-based management efforts. Future work should further explore the relationship between healthy nearshore coral reef habitats and mahimahi growth, reproduction, and catchability near O'ahu.

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# **Appendix A–Tables**

**Table A 1.** Summary of publicly available diet focused studies on *Coryphaena hippurus* and *C. equiselis*, in descending order by sampling year(s). Sample size indicates the number of stomachs included in the study. ID method "M" indicates morphological identification was the primary method of identification, "SI" indicates stable isotope analyses were paired with morphological identification.

Sampling Year(s)	Species	Basin	Location	Sample Size	ID Method	Reference
2017–2019	C. hippurus	Indian Ocean	Bay Bengal Sea	1150	Μ	Ghosh et al. (2021)
2015–2016	C. hippurus	Indian Ocean	Arabian Sea	128	Μ	Saroj et al. (2018)
2014–2015	C. hippurus	Pacific Ocean	-	320	Μ	Varela (2017)
2015	C. hippurus	Pacific Ocean	South Sea of Korea	174	М	Jeong et al. (2017)
2000–2001	C. hippurus	Atlantic Ocean	-	28	Μ	Sinopoli et al. (2017)
2013–2015	C. hippurus	Indian Ocean	Arabian Sea	256	Μ	Rajesh et al. (2016)
2010–2011	C. hippurus	Atlantic Ocean	Gulf of Mexico	357	Μ	Brewton et al. (2016)
2000–2003	C. hippurus	Pacific Ocean	-	445	M, SI	Tripp-Valdez et al. (2014)
2005–2007	C. hippurus	Pacific Ocean	-	418	M, SI	Torres-Rojas et al. (2014)
2003–2009	C. hippurus	Atlantic Ocean	Brazil	409	Μ	Pimenta et al. (2014)
2006–2009	C. hippurus	Indian Ocean	Arabian Sea	238	Μ	Varghese et al. (2013)

Sampling Year(s)	Species	Basin	Location	Sample Size	ID Method	Reference
2000–2003	C. hippurus	Pacific Ocean	Gulf of California	232	Μ	Tripp-Valdez et al. (2010)
1998–2000, 2002–2009	C. hippurus	Atlantic Ocean	-	727	Μ	Rudershausen et al. (2010)
1994–1995	C. hippurus	Atlantic Ocean	Ionian &Tyrrhenian Sea	300	Μ	Castriota et al. (2007)
1992–1999	C. hippurus	Atlantic Ocean	-	272	Μ	Vaske-Júnior & Lessa (2004)
2001–2003	C. hippurus	Indian Ocean	-	179	Μ	Taquet (2004)
2000–2001	C. hippurus	Pacific Ocean	-	354	Μ	Dempster (2004)
1992–1994	C. hippurus	Pacific Ocean	-	545	Μ	Olson & Galván- Magaña (1973)
1995–1997	C. hippurus	Atlantic Ocean	Mediterranean Sea	235	Μ	Duedero <i>et al.</i> (2001)
1994–1996	C. hippurus	Pacific Ocean	-	228	Μ	Lasso & Zapata (1999)
1981–1985	C. hippurus	Atlantic Ocean	Caribbean Sea	397	Μ	Oxenford & Hunte (1999)
1990–1991	C. hippurus	Pacific Ocean	-	500	Μ	Aguilar-Palomino (1998)
1990–1991	C. hippurus	Atlantic Ocean	Mediterranean Sea	316	Μ	Massutí et al. (1998)
1990	C. hippurus	Pacific Ocean	-	-	Μ	Campos et al. (1993)
-	C. hippurus	Atlantic Ocean	-	559	Μ	Zacala-Camin (1986)

Sampling Year(s)	Species	Basin	Location	Sample Size	ID Method	Reference
1980–1981	C. hippurus	Atlantic Ocean	Gulf of Mexico	2632	Μ	Manooch et al. (1984)
1974	C. hippurus	Atlantic Ocean	Mediterranean Sea	20	Μ	Bannister (1976)
1961–1963	C. hippurus	Atlantic Ocean	-	396	Μ	Rose & Hassler (1974)
1969–1970	C. hippurus, C. equiselis	Pacific Ocean	-	7	Μ	Hida (1973)
-	C. hippurus	Atlantic Ocean	Caribbean Sea	70	Μ	Lewis & Axelsen (1967)
-	C. hippurus, C. equiselis	Pacific Ocean	-	144	Μ	Rothschild (1964)
-	C. hippurus	Pacific Ocean	Japan Sea	1103	Μ	Kojima (1961)
1956–1957	C. hippurus,	Atlantic	Gulf of Mexico	46	Μ	Gibbs & Collette
	C. equiselis	Ocean				(1959)
1952–1954	C. hippurus	Indian Ocean	-	36	Μ	Williams (1956)
1947–1949	C. hippurus	Pacific Ocean	-	26	Μ	Ronquillo (1953)
1949	C. hippurus	Atlantic Ocean	-	19	Μ	Schuck (1951)

Primer Set	Sequence (5'-3')	Target Taxa	Reference
Baldwin-F	TCAACYAATCAYAAAGATATYGGCAC	Fish	Baldwin et al. (2009)
Baldwin-R	TAAACTTCAGGGTGACCAAAAAATCA	Fish	Baldwin et al. (2009)
Fish1-Ward	TCAACCAACCACAAAGACATTGGCAC	Fish	Ward et al. (2005)
FishR1-Ward	TAGACTTCTGGGTGGCCAAAGAATCA	Fish	Ward et al. (2005)
MiFishU-F	GTCGGTAAAATCCGTGCCAGC	Fish	(2015) (2015)
MiFishU-R	CATAGTGGGGTATCTAATCCCAGTTTG	Fish	(2015) Miya et al.
mICOlintF	GGWACWGGWTGAACWGTWTAYCCYCC	Eukaryote	Leray et al.
jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	Eukaryote	Geller et al. (2013)

Table A 2. All primer sequences used to DNA barcode prey items.

\*Folmer et al. 1994

**Table A 3.** Thermocycling conditions for primer sets used to amplify extracted DNA fragments.

Target Taxa	Primer Region	Primer Set	Thermocycling Conditions	Reference
Fish	COI	Baldwin	95°C for 5 min; 35 cycles of (95°C for 30 s, 52°C for 30 s, 72°C for 45 s); 72°C for 5 min. Hold at 4°C	Baldwin et al. (2009)
Fish	COI	Ward	95°C for 2 min; 35 cycles of (94°C for 30 s, 54°C for 30 s, 72°C for 1 min); 72°C for 10 min. Hold at 4°C	Ward et al. (2005)
Fish	12S	MiFish	95°C for 5 min; 35 cycles of (95°C for 30 s, 60°C for 30 s, 72°C for 30 s); 72°C for 10 min. Hold at 4°C	Miya et al. (2015)
Eukaryote	COI	Leray	95°C for 3 min; 16 cycles of (95°C for 10s, 62°C for 30 s (-1°C per cycle), 72°C for 1 min); 25 cycles of (95°C for 30 s, 46°C for 45 s, 72°C for 1 min); 72°C for 10 min. Hold at 4°C	Leray et al. (2013), Geller et al. (2013)

**Table A 4**. Summary table of mean numeric, mean gravimetric, and frequency of occurrence values for prey families and species.Data included only from stomachs where  $\geq$ 50% of the total number of prey items have been successfully identified (n = 118). Taxa with values < 0.01 (1%) not shown in Figure 4.

Family	Species	Mean	Mean	
-		Numeric	Gravimetric	Frequency of
		Proportion	Proportion	Occurrence
Acanthuridae		0.052	0.041	0.161
	Acanthurus dussumieri	0.023	0.019	0.059
	Acanthurus nigrofuscus	0.009	0.006	0.059
	Acanthurus nigroris	0.001	0.000	0.008
	Acanthurus olivaceus	0.003	0.001	0.008
	Acanthurus thompsoni	0.007	0.010	0.034
	Acanthurus triostegus	0.005	0.002	0.025
	Naso brevirostris	0.004	0.003	0.008
Balistidae		0.057	0.067	0.119
	Sufflamen bursa	0.001	0.001	0.008
	Xanthichthys auromarginatus	0.056	0.066	0.119
Belonidae	Ablennes hians	0.029	0.035	0.042
Carangidae		0.102	0.102	0.254
	Caranx melampygus	0.002	0.000	0.008
	Decapterus macarellus	0.032	0.031	0.076
	Decapterus macrosoma	0.032	0.029	0.119
	Decapterus muroadsi	0.000	0.000	0.008
	Selar crumenophthalmus	0.035	0.041	0.093
	Seriola rivoliana	0.001	0.000	0.008
Carpiliidae	Carpilius convexus	0.026	0.018	0.042
Chaetodontidae	Chaetodon kleinii	0.001	0.001	0.008
Dactylopteridae	Dactyloptena orientalis	0.064	0.064	0.153
Diodontidae		0.009	0.016	0.042
	Chilomycterus reticulatus	0.000	0.001	0.008
	Diodon holocanthus	0.008	0.015	0.034
	Diodon hystrix	0.001	0.000	0.008
Exocoetidae		0.060	0.083	0.153
	Cheilopogon atrisignis	0.002	0.003	0.008
	Cheilopogon furcatus	0.004	0.011	0.017
	Cheilopogon unicolor	0.003	0.000	0.008
	Cypselurus angusticeps	0.002	0.002	0.008
	Cypselurus poecilopterus	0.001	0.002	0.008
	Exocoetus monocirrhus	0.008	0.008	0.008
	Exocoetus volitans	0.011	0.010	0.017
	Hirundichthys speculiger	0.001	0.001	0.008
	Parexocoetus brachypterus	0.027	0.037	0.076
	Prognichthys sealei	0.001	0.008	0.008
Fistulariidae	Fistularia commersonii	0.001	0.000	0.008
Gempylidae	Gempylus serpens	0.047	0.032	0.110
Hemiramphidae	Euleptorhamphus viridis	0.044	0.070	0.102
Holocentridae		0.020	0.012	0.059
	Myripristis chryseres	0.001	0.001	0.008
	Myripristis kuntee	0.008	0.008	0.008
	Neoniphon sammara	0.004	0.000	0.008

Family	Species	Mean	Mean	
	-	Numeric	Gravimetric	Frequency of
		Proportion	Proportion	Occurrence
	Plectrypops lima	0.001	0.000	0.008
	Sargocentron xantherythrum	0.006	0.002	0.025
Lutjanidae	Lutjanus kasmira	0.006	0.007	0.008
Microdesmidae	Ptereleotris heteroptera	0.006	0.002	0.008
Molidae	Ranzania laevis	0.002	0.001	0.008
Monacanthidae		0.016	0.020	0.042
	Cantherhines dumerilii	0.004	0.005	0.008
	Cantherhines sandwichiensis	0.010	0.015	0.025
	Pervagor aspricaudus	0.001	0.000	0.008
Mullidae		0.205	0.182	0.381
	Mulloidichthys flavolineatus	0.004	0.003	0.008
	Mulloidichthys vanicolensis	0.008	0.006	0.017
	Parupeneus cyclostomus	0.002	0.003	0.008
	Parupeneus multifasciatus	0.026	0.026	0.161
	Parupeneus pleurostigma	0.164	0.145	0.331
Nomeidae		0.060	0.063	0.119
	Cubiceps pauciradiatus	0.002	0.002	0.017
	Nomeus gronovii	0.004	0.004	0.008
	Psenes cyanophrys	0.053	0.058	0.102
	Psenes pellucidus	0.002	0.000	0.008
Ocypodinae	Ocypode ceratophthalmus	0.004	0.002	0.008
Ommastrephidae		0.025	0.027	0.085
	Eucleoteuthis luminosa	0.003	0.000	0.017
	Hyaloteuthis pelagica	0.014	0.014	0.059
	Sthenoteuthis oualaniensis	0.008	0.012	0.034
Ostraciidae		0.074	0.051	0.229
	Lactoria fornasini	0.068	0.043	0.220
	Ostracion meleagris	0.006	0.008	0.017
Priacanthidae	Heteropriacanthus cruentatus	0.000	0.001	0.008
Scombridae		0.034	0.039	0.085
	Auxis thazard	0.001	0.002	0.008
	Katsuwonus pelamis	0.024	0.029	0.068
	Scomber australasicus	0.008	0.008	0.008
Stomatopoda		0.018	0.015	0.034
Syngnathidae	Hippocampus fisheri	0.001	0.000	0.008
Synodontidae		0.008	0.008	0.008
Tetraodontidae		0.014	0.026	0.068
	Arothron hispidus	0.001	0.000	0.008
	Lagocephalus lagocephalus	0.013	0.026	0.059
Zanclidae	Zanclus cornutus	0.016	0.014	0.042

# **Appendix B–Figures**



**Figure B 1.** Percent of total prey in a stomach identified successfully to at least family level. Bin width set to [0,25), [25,50), [50,75), [75,100), [100].



**Figure B 2**. Mean gravimetric (pink) and mean numeric (blue) proportion per stomach of reefassociated prey. Proportion was calculated using different cutoff values for inclusion of stomachs based on the percent of prey items that were identified. The most stringent was "all" non-empty stomachs (n = 141) where at least one prey item was identified. Regardless of cutoff value, the mean gravimetric and mean numeric proportion of reef-associated prey hovers around 0.5.