Diet and Biology of Deep-reef Lionfish (*Pterois volitans*) at Curaçao

Sarah Yerrace

A thesis

submitted in partial fulfillment of the

requirements for the degree of

Master of Science

University of Washington

2024

Committee:

Luke Tornabene

Julian Olden

Kerry Naish

Program Authorized to Offer Degree:

School of Aquatic and Fishery Sciences

University of Washington

Abstract

Diet and Biology of Deep-reef Lionfish (*Pterois volitans*) at Curaçao

Sarah Yerrace

Chair of the Supervisory Committee:

Luke Tornabene

School of Aquatic and Fishery Sciences

Indo-Pacific Red Lionfish (*Pterois volitans*) are a well-established invasive species in the western Atlantic Ocean and have been documented at depths of 0-304 m. However, our knowledge of lionfish biology and diet below 40 m is limited due to the constraints of recreational SCUBA diving depth limits. Communities below 40 m are distinct from communities above 40 m, thus it cannot be assumed that lionfish have the same impacts or consume the same species below 40 m. Studies targeting lionfish diet and biology below 40 m are necessary to determine potential impacts on understudied mesophotic communities and consequences for invasion management. Specifically, understanding where on the reef lionfish reach the largest sizes will give insights into what areas of the reef may experience the highest predation pressure. Understanding where lionfish are spawning on the reef may indicate long term effectiveness of removal efforts. The purpose of this study was to describe the diet, size, and reproductive characteristics of lionfish across depth in Curaçao, Southern Caribbean. Using a combination of SCUBA and manned submersible diving, 136 lionfish off the southern coast of Curaçao were collected along the reef slope down to 189 m. Lionfish below 40 m were longer and heavier than lionfish above 40 m but did not have better

body condition (Fulton's condition factor). Significantly more males than females were collected above 40 m. Fertile females were present along the entire depth range, however no actively spawning females, based on gross morphology of gonads, were found below 126 m. Using DNA metabarcoding of the mitochondrial COI gene of gut contents, 598 exact sequence variants from prey species were recovered representing 51 species of fishes from 18 families and an additional 13 families of invertebrates. Across all individuals, the four most commonly occurring teleost families were Pomacentridae, Apogonidae, Gobiidae, and Scorpaenidae. Gobiidae was frequently consumed above and below 40m, while Apogonidae and Serranidae were more frequently consumed below 40m. No dietary trends were detected with increasing lionfish length or mass. Eighteen teleost taxa were recorded for the first time from lionfish guts. Overall, species of fishes identified in lionfish guts closely reflect prey that is available at the depth at which the lionfish were collected, suggesting that lionfish on mesophotic and rariphotic reefs are consuming mesophotic and rariphotic prey. These deep-reef taxa may be especially vulnerable to lionfish predation given their naturally low abundances coupled with the larger sizes and lack of top-down control of lionfish on deep reefs. Given that culling has been successful in controlling local shallow populations, developing an effective means of culling lionfish below 40 m may be critical reducing the impact of lionfish predation on vulnerable deep-reef fishes.

Acknowledgements

I would like to thank my committee members for their comments and feedback on this manuscript as well as their thought-provoking and stimulating questions during in-person discussions. Sincere thank you to my advisor, Dr. Luke Tornabene, for his unwavering support.

Thank you to all those who provided assistance in the field with collections. A special thank you to the Substation Curacao crew for their efforts, in particular Tico Christiaan and Bruce Brandt. Thank you to Aly Liu for her company and assistance in the genetics lab. Immense gratitude to Kalena Kattil-deBrum, Courtney Skalley, Laurel Nave-Powers, and the entire Fish Systematics and Biodiversity lab. You are all brilliant students and scientists that inspire me daily. Thank you to my dearest friends and family who kept me going.

This project was funded by the Hall Conservation Genetics Grant, the William W. and Dorothy T. Gilbert Ichthyology Research Fund, and through a fellowship from the University of Washington School of Aquatic and Fishery Sciences.

Table of Contents

List of Figures

List of Tables

List of Supplementary Material

Introduction

Invasive species are generally defined as nonresident species introduced to a new geographic region, either intentionally or accidentally, and are able to spread and reproduce (Blackburn et al. 2011). Records of invasive species have been increasing exponentially over the past century (Mormul et al. 2022). These introductions are usually associated with negative impacts on the invaded ecosystems and associated socio-economic systems (Marbuah et al. 2014; Gallardo et al. 2016). Predation is an important mechanism through which invaders affect the receiving ecosystem; therefore, studying the diet of an invasive species in its nonnative range is a valuable way to understand potential impacts on native ecosystems. For example, studying the diet of invasive ship rats (*Rattus rattus*) in New Zealand revealed predation on both of New Zealand's only endemic frogs species, one of which is critically endangered (Egeter et al. 2019). An introduced tree snake (*Boiga irregularis*) significantly reduced native bird populations in Guam through predation (Savidge 1987). Continuous monitoring and study of invasive species is necessary to understand what impacts these species have on the invaded environment as well as how those impacts change as the invasion progresses.

Indo-Pacific Red Lionfish (*Pterois volitans*; hereafter lionfish) are a well-established invasive species in the western Atlantic Ocean that were first observed in the mid 1980's (Morris and Akins 2009) or the early 1990's (Courtenay 1995). They are now widespread throughout the tropical and subtropical western Atlantic Ocean and have been found as far north as New York (Schofield 2009) and as far south as the southern coast of Brazil (Ferreira et al. 2015; Luiz et al. 2021). Lionfish are associated with decreases in native fish recruitment, biomass, species richness, and density (Albins and Hixon 2008; Green et al. 2012a; Albins 2015). Exact magnitude of these decreases varies by specific study site and species. For example, lionfish predation had a larger impact on piscivores (~98% reduction in biomass) than herbivorous fishes (~34% reduction in biomass)(Albins 2015). One experiment indicated that lionfish can contribute to local extirpation of prey species (Ingeman 2016) however it is unknown how widely applicable these results are given the small scale and short term nature of the study. Regardless, Lionfish are listed as a major contributor to the status of 25% of threatened species (Linardich et al. 2019). Accurately describing lionfish diet is key to understanding which reef fish species are at risk of decline due to predation.

Most of our knowledge of lionfish is based on studies conducted in the top 30 m of the ocean, however, in the past decade, lionfish have been observed on deep reefs to a maximum depth of 304 m (Gress et al. 2017). The faunal zones of reef ecosystems can be classified into the altiphotic (0 to 40 m), the mesophotic (~40 m to ~130 m), and the rariphotic (~130 m to ~300 m)(Baldwin et al. 2018). Studying deep-reef communities below the altiphotic zone, which coincides with the recreational diving limit of 40 m, is difficult due to the increase in time and money required for necessary training, such as mixed gas diving, or specialized equipment, such as remote operated vehicles or human-occupied submersibles. As a result, these communities are poorly understood and likely harbor significant undescribed biodiversity (Pyle 2000). Preliminary studies indicate that mesophotic and rariphotic reefs have fish communities that are distinct from shallow reefs in abundance, biomass, and species composition (Bejarano et al. 2014; Pinheiro et al. 2016). Lionfish occurring on Caribbean mesophotic reefs is not unexpected, as lionfish occur on mesophotic reefs down to at least 80 m in their native range (Brokovich et al. 2008; Kulbicki et al. 2012). However,

in the western Atlantic, lionfish have been seen as deep as 247 m off the coast of Curaçao (Tornabene and Baldwin 2017) and as deep as 304 m off of Roatan (Gress et al. 2017).

Previous studies found that Atlantic lionfish grow larger and faster than in their native range (Darling et al. 2011; Pusack et al. 2016) which has implications for diet and reproduction. Larger females can produce greater numbers of oocytes (Gardner et al. 2015) and some studies suggest larger lionfish consume a higher abundance and diversity of prey (Muñoz et al. 2011; Mizrahi et al. 2017). Larger individuals and a higher proportion of actively spawning females have been found on mesophotic coral reefs around Utila Island (Andradi-Brown et al. 2017b, 2017a). These trends of larger lionfish on mesophotic reefs could be an artifact from a lack of spearfishing in the mesophotic or evidence of a continuation of an ontogenetic migration from shallow to deep. Regardless of the mechanism, Lionfish presence on mesophotic and rariphotic reefs is concerning given their negative impacts on native shallow-reef fish biodiversity, biomass, and recruitment, coupled with the refuge deep reefs provide to potentially reproducing lionfish from spearfishing-based control programs (Albins and Hixon 2008; Green et al. 2012a; Albins 2015).

Despite observations of invasive lionfish on deep reefs, it remains unclear how much lionfish diets are composed of deep-reef versus shallow-reef fishes, as most diet studies have limited sampling depths to 30 m or shallower. Those studies show that lionfish are generalist predators limited by the size of their gape that feed on a wide variety of reef fishes and invertebrates (Morris and Akins 2009; Muñoz et al. 2011) (Table 1). Larger lionfish with larger gape may have more theoretical prey options. Some studies have found an increase in mean prey size with increasing lionfish body size (Morris and Akins 2009; Peake et al. 2018) while others have not (Río et al. 2022). Lionfish diet is also known to shift from primarily invertebrates to primarily teleosts as lionfish grow in size (Morris and Akins 2009; Dahl and Peterson III 2014; Arredondo-Chávez et al. 2016; Mizrahi et al. 2017; Sancho et al. 2018; Río et al. 2022). While shrimp and fish are consistent contributors to lionfish diet, the exact species composition varies significantly across the entire Caribbean (Peake et al. 2018). Given this variation across the region, further study of lionfish diets at the local scale may provide critical information regarding which prey species may be locally threatened.

There are several approaches to study fish diets, each with benefits and drawbacks regarding sensitivity, sample size, cost, precision, and other considerations. Direct observation of predatory events and visual identification of gut or fecal contents rely on undigested prey material and taxonomic identification skills. Stable isotope analysis does not require taxonomic skills and is useful for determining general dietary patterns or trophic level but does not reveal species-specific prey items (de Sousa et al. 2019). DNA based methods utilize the DNA in partially digested material to identify prey items that would otherwise be unidentifiable but does not provide abundance information. Depending on the methods used and the number of individuals sampled, between 14 (Albins and Hixon 2008) and 47 prey teleost species (Arredondo-Chávez et al. 2016) have been identified from stomach contents of lionfish collected above 40 m (supplementary material 1). Diet studies that use visual identification methods often rely on high sample sizes—in some cases, more than 1,000 individuals (Morris and Akins 2009; Eddy et al. 2016; Mizrahi et al. 2017)— due to limited information gathered from empty stomachs or highly digested prey material.

Using molecular data for diet analysis (DNA barcoding or metabarcoding) can facilitate more complete descriptions of diet by identifying prey items from DNA in partially digested material that would otherwise be unidentifiable and thereby reducing sampling effort. The mitochondrial gene cytochrome *c* oxidase I (COI) is a commonly used DNA barcoding marker that can reliably return species level assignments given an appropriate reference sequence database (Hebert et al. 2003a). For example, one study was able to nearly double the number of lionfish prey species identified using DNA barcoding (Dahl et al. 2017). However, sequencing and identifying one prey item at a time is slow and costly. With high-throughput sequencing, identification of multiple prey items within digested material using DNA barcodes has become more accessible through DNA metabarcoding. Metabarcoding still utilizes DNA in unidentifiable material but enables identification of short DNA fragments representing multiple taxa within one sample and thus gives a more efficient and comprehensive analysis of stomach contents. While DNA methods offer advantages in sampling effort and taxonomic resolution for dietary studies, it's important to be aware of biases associated with selected primers and DNA amplification and the inability evaluate prey abundance and biomass. Only one previous study used DNA metabarcoding methods to explore lionfish diet, where a total of 39 prey species were found in 63 lionfish stomachs all collected from ≤ 30 m (Harms-Tuohy et al. 2016).

Studies on the diet of invasive deep-reef lionfish are fewer in number and more limited in scope than those on shallow lionfish. A few studies examined lionfish diet below 30 m (maximum sampled depth 72 m) using visual identification of prey and or stable isotopes (Muñoz et al. 2011; Eddy et al. 2016; Andradi-Brown et al. 2017a; Aguilar-Medrano and Vega-Cendejas 2020). One study used DNA barcoding with a maximum sampled depth of 50 m and reported 46 teleost species, 28 species of shrimp, and 31 species of crab (Sancho et al. 2018). This represents the only study using molecular data that sampled below 40 m. There are currently no studies using molecular data to describe the diet of lionfish below 50 m with high taxonomic resolution despite observations of lionfish on deep reefs down to ~300 m (Gress et al. 2017). There are records of lionfish predation on critically endangered species (Rocha et al. 2015) and direct observations of lionfish predation on undescribed deep-reef species (Tornabene and Baldwin 2017). Understanding lionfish diet, particularly diet below 40 m, is critical to understanding the threat lionfish predation poses to these poorly understood communities.

To understand what threat lionfish pose to understudied deep-reef communities as well as how deeper lionfish may impact control efforts, we first need a better understanding of the diet and life history traits of lionfish below 40 m. This study examines trends in lionfish diet and biology across their depth range in Curaçao. I first describe the general patterns of lionfish size and maturity with depth. Specifically, I investigate how standard length, mass, body condition, sex ratio, and spawning status changes with depth. Second, I describe and compare the diet of lionfish collected above 40m (using SCUBA) and of lionfish collected below 40 m (using a manned submersible) using gut content DNA metabarcoding of the mitochondrial COI gene. Finally, I investigate how diet may change with size as well as with changes in prey availability with depth. This study is the first to use lionfish collected from below 100 m coupled with DNA metabarcoding. Collectively, these data will give us a more comprehensive understanding of the lionfish population in Curacao and unique insights into lionfish beyond the reach of recreational diving.

Methods

Collection

Red lionfish (*Pterois volitans*) were collected by spearfishing on SCUBA (0 to 40 m) and by using a human-occupied submersible (below 40 m; Substation Curaçao's *Curasub*, http://www.substation-curacao.com, accessed 21 June 2024) off the southern coast of Curaçao in May and November of 2019 and in April and December of 2022. A pole spear was affixed to the front of the submersible and was fired and reloaded using a hydraulic arm. Speared lionfish were anesthetized with quinaldine solution before being deposited in a basket on the front of the submersible. SCUBA and submersible dives consisted of roving surveys not restricted to a specific depth and were conducted during the day. Depth of collection was recorded for each individual. Stomachs were removed and preserved in 95% ethanol in the field and kept on ice or at -20°C until the time of DNA extraction. To prevent cross contamination, dissection utensils were sterilized with flame followed by a 95% ethanol rinse. Standard length, total length, mass, gonad mass, and sex of each individual were recorded. Female reproductive stages based on gross morphology of the ovaries, including egg size and hydration level, were assessed as "developing", "spawning capable" or "actively spawning" following Green et al. (2012b).

Native Biodiversity

To compare diet diversity with species diversity along the reef slope, visual surveys were conducted on SCUBA at 5 m, 10 m, 20 m, and 30 m. Divers recorded all species observed in a 2 m width transect for 30 m. Below 40 m, observations were made from the human-occupied submersible, the *Curasub*. Submersible dives, which consisted of roving surveys moving at 2 knots or less, typically lasted 4 hours and reached a maximum depth of 310 m. For additional details of the observational methods from below 40 m, see Baldwin et al. (2018). All biodiversity surveys were conducted during daylight hours therefore nocturnal or crepuscular species are not represented in the data.

Genetics

To process gut contents, the stomachs were cut open and any items were visually identified to the lowest taxonomic level where possible. A tissue sample of the individually identified items, consisting of either fin clips or muscle depending on the state of the prey item, and all the remaining contents were scraped into a mortar and crushed with a pestle. Subsampled tissues from larger prey items were no bigger than 0.5 cm^3. Tissue lysis buffer from DNEasy Blood and Tissue Kit (Qiagen, Hilden, Germany) was added to the mortar and homogenized with the gut contents. DNA was extracted from the homogenized gut contents following the DNEasy Blood and Tissue Kit manufacturer's protocol (Qiagen). If there was an exceptional volume of gut contents following homogenization, a 1.5 mL subsample was taken for DNA extraction. To prevent cross contamination between samples, dissecting tools were exposed to an open flame and rinsed with 95% ethanol solution. The mortar and pestle were washed with soap, briefly soaked in a 10% bleach solution followed by a deionized water bath. All surfaces were wiped down with a 10% bleach solution.

Extracted DNA samples were sent to JonahVentures (https://jonahventures.com/, accessed 21 June 2024) for sequencing a 313 bp segment of the mitochondrial Cytochrome *c* Oxidase I gene (COI). This gene is a commonly used DNA barcode (e.g. Harms-Tuohy et al. 2016; Marques et al. 2022; Coker et al. 2023) that undergoes rapid evolution which enables distinction between related taxa (Hebert et al. 2003b) while also having high coverage of Caribbean fishes (Weigt et al. 2012) and other marine metazoan taxa (Bucklin et al. 2011) in online databases. Primers mlCOIintF (Leray et al. 2013) combined with jgHCO2198 (Geller et al. 2013) have been shown to perform well across phylogenetic diversity including Arthropoda, Mollusca, and Chordata (Leray et al. 2013). Predator specific blocking primers were not included to prevent unintentional blocking of prey DNA.

Both forward and reverse primers contained a 5' adaptor sequence to allow for subsequent indexing and Illumina sequencing. Each 25 µL PCR reaction was mixed according to the Promega PCR Master Mix specifications (Promega catalog # M5133, Madison, WI) which included 12.5ul Master Mix, 0.5 µM of each primer, 1.0 µl of gDNA, and 10.5 µl DNase/RNase-free H2O. DNA was PCR amplified using the following conditions: initial denaturation at 94 °C for 2 minutes, followed by 45 cycles of 15 seconds at 94 °C, 30 seconds at 50 °C, and 1 minute at 72 °C, and a final elongation at 72 °C for 10 minutes. To determine amplicon size and PCR efficiency, each reaction was visually inspected using a 2% agarose gel with 5µl of each sample as input. Amplicons were then cleaned by incubating amplicons with Exo1/SAP for 30 minutes at 37 °C following by inactivation at 95 °C for 5 minutes and stored at -20 °C. A second round of PCR was performed to complete the sequencing library construct, appending with the final Illumina sequencing adapters and integrating a sample-specific,12-nucleotide index sequence. The indexing PCR included Promega Master mix, 0.5 µM of each primer and 2 µl of template DNA (cleaned amplicon from the first PCR reaction) and consisted of an initial denaturation of 95 °C for 3 minutes followed by 8 cycles of 95 °C for 30 sec, 55 °C for 30 seconds and 72 °C for 30 seconds. Final indexed amplicons from each sample were cleaned and normalized using SequalPrep Normalization Plates (Life Technologies, Carlsbad, CA). 25µl of PCR amplicon is purified and normalize using the Life Technologies SequalPrep Normalization kit (cat#A10510-01) according to the manufacturer's protocol. Samples are then pooled together by adding 5µl of each normalized sample to the pool. Sample library pools were sent for sequencing on an Illumina MiSeq (San Diego, CA) at the Texas A&M Agrilife Genomics and Bioinformatics Sequencing Core facility using the v2 500-cycle kit (cat# MS-102-2003).

Bioinformatics

To describe diet at the species level and compare across depths, exact sequence variants (ESVs) were used as a proxy for species occurrence. ESVs capture all sequence variation (including intraspecific variation) and high-resolution diversity by retaining unique sequences as opposed to operational taxonomic units (OTUs) which cluster sequences by a similarity threshold (Callahan et al. 2017).

To assemble ESVs, first raw sequence data were demultiplexed using Pheniqs v2.1.0 (Galanti et al. 2021), enforcing strict matching of sample barcode indices (i.e, no errors). Cutadapt v3.4 (Martin 2011) was then used remove gene primers from the forward and reverse reads, discarding any read pairs where one or both primers were not found at the expected location (5') with an error

rate below 0.15. Read pairs were then merged using VSEARCH v2.15.2 (Rognes et al. 2016), discarding resulting sequences with a length less than 298 bp, more than 328 bp, or with a maximum expected error rate more than 0.5 bp (Edgar and Flyvbjerg 2015). For each sample, reads were then denoised using the UNOISE3 denoising algorithm (Edgar 2016) as implemented in VSEARCH, using an alpha value of five and discarding unique raw sequences observed less than eight times. Counts of the resulting ESVs were then compiled and putative chimeras were removed using the Uchime3 algorithm, as implemented in VSEARCH.

A library of DNA sequences from fishes of the tropical western Atlantic (including previously unpublished sequences), each with corresponding voucher specimens cataloged at the Smithsonian National Museum of Natural History, was used for comparison to gut content sequences. The specimens associated with these sequences were collected by the Smithsonian from 2011-2017 and include all deep-reef fishes collected by the Smithsonian Deep Reef Observation Project (DROP) (Robertson et al. 2022), including all fishes observed at the study site in Curaçao (Baldwin et al. 2018). These sequences were combined with the MARES database (Arranz et al. 2020), which contains all COI sequences available in GenBank (NCBI Resource Coordinators 2017) and Barcode of Life Data System (Ratnasingham and Hebert 2007) for marine taxa, to create a custom database.

ESVs were queried against the custom combined database for taxon identification using BLASTn. Previous work on Caribbean reef fish taxonomy has shown that many closely related species differ in COI by 2-5% or more (Tornabene et al. 2010; Weigt et al. 2012; Smith-Vaniz et al. 2018) thus sequencies were confidently identified to species at ≥ 98% sequence similarity. Remaining sequences were identified to genus at ≥ 95% sequence similarity, to family at ≥ 90% sequence similarity, and to order at ≥ 85% sequence similarity. Phylum level taxonomy was assigned below 85% sequence similarity. ESVs that match to the genus *Pterois* were removed under the assumption of sequencing host tissue rather than capturing cannibalism.

Data Analysis

General additive models (GAMs) were used to test the relationship between depth and mass, condition, gonadosomatic index (GSI), and standard length of lionfish. The relationship between these factors was assumed to be non-linear and sampling year was included as a random effect. General liner models (GLMs) were used to investigate the relationships between size, total ESVs, percent invertebrate ESVs, as well as between prey mass, number of prey items in the stomach, raw reads recovered, and ESVs recovered. GLMs were also used to examine the relationship between teleost ESVs in the stomach, teleost prey species in the stomach, and native biodiversity across depth. Fish condition was determined by Fulton's Condition factor : $M \times 100/TL^3$ where M = mass in grams and TL = total length in centimeters (Froese 2006). The GSI for females was calculated as the gonad weight divided by the body weight * 100. Pearson's Chi-squared test was used to test if the ratio between males and females above and below 40m deviated from expected frequencies. A Z-test was used to compare the proportion of empty stomachs above and below 40 meters.

Prevalence of prey taxa in lionfish diet was determined by percentage frequency of occurrence, defined as the number of samples in which that taxon occurred expressed as a proportion of the

total number of samples, including empty stomachs. The total percentage frequency of occurrence may therefore exceed 100% as multiple taxa can be present in each sample. A permutational multivariate analysis of variance (PERMANOVA) based on the Jaccard distance metric was used to test for differences in the composition of diet between lionfish collected in the altiphotic (0-39 m), mesophotic (40-130 m), or rariphotic zone (below 130 m).

Statistical analysis was performed with the statistical software RStudio (Posit team 2024) with the following packages: dplyr (Wickham et al. 2023) and tidyverse (Wickham et al. 2019) for data manipulation; vegan (Oksanen et al. 2024) for multivariate analysis; mgcv (Wood 2011) for general linear and additive models; and ggplot2 (Wickham 2016), ggpmisc (Aphalo 2024), and treemap (Tennekes 2023) for data visualization.

Results

Size and Maturity

A total of 136 lionfish were captured between five and 189 m. Sixty-five lionfish were collected from above 40 m and 71 were collected from below 40 m. Standard length ranged between 70 and 360 mm (mean 207.6 ± 60.2 mm) and mass ranged between 9 and 1033 g (mean 309.6 ± 220.5 g). Condition of individuals varied between 0.302 and 2.551 (mean 1.261 ± 0.242). GAM analysis showed a significant positive relationship between depth and SL of all individuals as well as for males and females separately (Table 1; Figure 1A). Mass also significantly increased across depth for all individuals and both sexes (Table 1; Figure 1C). Collection year was a significant variable for the SL and mass of all individuals but not when considering individual sexes: The length and mass of all individuals decreased from 2019 to 2022. Body condition decreased with depth for all individuals as well as both sexes (Table 1; Figure 1B).

There were 54 males, 61 females, and 21 individuals that were not assigned to either sex (Figure 2). Pearson's Chi-squared test with Yates' continuity correction showed that males and females were not evenly distributed above and below 40 m (X-squared = 7.9761, df = 1, p-value = 0.00474). More females were collected below 40 m and more males were collected above 40 m. GSI of females ranged between 0.47 and 12.48 (mean 3.594 ± 2.714). There was no significant trend of GSI with depth (Figure 1D). Females capable of spawning were found across the entire depth range, including the deepest individual collected (Figure 2). The deepest actively spawning female was collected at 126 m.

Diet

Visual Identification

Of 136 stomachs, 26 (19.1%) were empty (20 from ≤ 40 m, 6 from > 40 m). There were significantly more visually empty stomachs below 40 m than there were above (Z statistic: 2.08, p-value: 0.005). An additional 14 stomachs contained only highly digested material from which no taxa or individual items could be visually identified or counted (5 from ≤ 40 m, 9 from > 40 m). Collectively, there were 40 stomachs (29.4%) where no information on prey items was visually gathered. A total of 253 items were identified from the remaining 96 stomachs. Shrimp were

identified in 39 (28.6%) stomachs, while 138 teleosts were identified in 73 (53.6%) of stomachs. Of the 138 teleost prey items, 44 were visually identified to a more specific taxonomic rank. Ten teleost families were identified: Holocentridae, Apogonidae, Priacanthidae, Chaetodonitdae, Gobidae, Epigonidae, Scorpeanidae, Pomacentridae, Grammatidae, and Serranidae. Six teleost species were identified: *Stegastes partitus* (n=3 individuals), *Gramma loreto* (n=3), *Sphyraenops bairdianus* (n=3), *Prognathodes guyanensis* (n=1), *Azurina multilineata* (n=1), and *Coryphopterus personatus/hyalinus* (n=10) (Supplementary material 2). Two octopuses and one crab were identified. The most prey items identified in one stomach was 18: one Serranidae, four unidentified teleosts, and 13 unidentified shrimp from a lionfish collected at 121 m. While most prey items were less than 30 mm long, a few prey items were notably larger. One fish from the family Apogonidae was 66.5 mm SL and 30% of the lionfish's SL collected at 89 m. One prawn was 89 mm total length and 32% of the lionfish's SL collected at 150 m. Three *Sphyraenops bairdianus* from one lionfish collected at 157 m were approximately 55 to 60 mm SL, 23 – 25% of the lionfish's SL. Two octopus with a mantle length of 22 mm and arm length up to 90 mm were from one lionfish (300 mm SL) collected from 107 m.

Metabarcoding assignments

A total of 636 ESVs were recovered from 136 lionfish stomachs which were sequentially filtered to differing levels of taxonomy based on percentage matches to sequences in our custom database (Figure 3). One ESV was not assigned to any level of taxonomy and was removed from further analysis. I removed 37 ESVs that were identified as genus *Pterois* (one *P. miles,* 35 *P. volitans,* one *Pterois sp.*), leaving 598 ESVs remaining. Of those, 128 ESVs matched to a sequence in the database with a confidence of 85% or below and were assigned a phylum level taxonomy. Based on sequence similarity to the reference database, of the remaining 470 ESVs, 34 were assigned an order, 62 ESVs were assigned a family, 88 ESVs were assigned a genus. Finally, 286 ESVs matched to a sequence 98% and above. However, some of the voucher specimens of the sequence were not identified to species. In these cases, the sequence was assigned the lowest taxonomic level assigned to the voucher specimen. Of the 286 ESVs that had a match with a confidence of 98% or above, 52 were not assigned a species: one was assigned to an order, 12 to family, and 38 to genus. The remaining 235 ESVs were confidently assigned to a species. Therefore, after accounting for identification uncertainty in the voucher specimens, total assignments were as follows: 128 were assigned to a phylum, 35 to an order, 74 to a family, 126 to a genus, and 235 to a species (Figure 3). Excluding *Pterois* ESVs, 63% of ESVs were identified as teleost taxa. A tenth of all stomachs (14) returned only lionfish DNA and were void of prey DNA, 12 of which were collected below 40 m. There were significantly more empty stomachs detected by metabarcoding below 40 m than there were above (12 vs two; Z statistic: 2.65, p-value: 0.008).

The diversity of ESVs in our data spanned 58 species, 54 genera, 31 families, and 19 orders. Specifically, there were 51 species of fishes from 44 genera, 18 families, and 10 orders. Despite greater diversity detected in fishes, invertebrates frequently occurred in stomachs (Figure 4). The five most commonly occurring teleost families across all lionfish were Pomacentridae (27.21% frequency of occurrence), Apogonidae (19.85%), Gobiidae (18.38%), Scorpaenidae (17.65%), and Serranidae (11.76%) (supplementary material 3; Figure 5). Remaining families occurred less than 7% of the time. The three most frequently occurring species were *Azurnia multilineata* (14.93%), *Stegastes partitus* (11.11%), and *Coryphopterus hyalinus/ personatus* (9.63%) with 27 teleost

species only having one occurrence (<1%) (supplementary material 4). Number of ESVs in stomachs varied from zero to 35 (mean 6.199 ± 6.121 ESVs ; Figure 6A). Number of teleost taxa detected in stomachs ranged from zero to seven (mean 2.72 ± 1.91 taxa; Figure 6B). There was a positive correlation between the number of ESVs recovered from the stomach and the number of raw reads recovered from sequencing as well as the number of prey items visually observed in the stomach (supplementary material figure 5). There was not a strong correlation between prey mass and raw reads, ESVs, or number of items in the stomach (supplementary material figure 5).

Dietary Trends

The number of unique ESVs and prey species diversity declined across depth (Figure 7 There was a significant positive relationship between number of ESVs in a stomach and number of resulting prey species identified (Figure 8A) but there was no relationship between the number of observed teleost species naturally occurring across depth and the number of ESVs or prey species detected in lionfish stomachs (Figure 8B & 8C).

There was a significant change in the composition of lionfish diets across the reef slope. Specifically, when lionfish were grouped into the altiphotic (0 - 40 m), mesophotic (41 – 129 m), and rariphotic zones (below 130 m), composition was different between all groups (PERMANOVA, 999 permutations, F-statistic = 2.72, df = 2, residueal df = 89, p = 0.001, R^2 = 0.0575).While Gobiidae and Apogonidae made up a significant portion of lionfish diets both above and below 40 m, composition within those families as accompanying prey families differed (Figures 9 & 10). Four families were unique to lionfish above 40 m: Scaridae, Priacanthidae, Blennidae, and Tripterygiidae. Seven families were only detected below 40 m: Acropomatidae, Chaenopsidae, Chaetodontidae, Epigonidae, Paralichthyidae, Symphsanodontidae, and Synodonitdae. Notable and novel rariphotic prey captured in this study include *Syphraenops bairdianus* (Epigonidae), *Citharichtyes cornutus* (Paralichthyidae), *Symphysanodon berryi* (Symphsanodontidae), *Ostichthyes trachypoma* (Holocentridae), *Antilligobius nikkiae* (Gobiidae), *Palatogobius incendius* (Gobiidae), and *Baldwinella c.f. vivanus* (Serranidae).

When comparing the depth of collection to the teleost prey species depth distribution, 93.7% of lionfish were collected within the depth distribution of the prey species (Figure 11). Only a few lionfish were collected outside the range of the prey species within its stomach. For example, a lionfish collected at 171 m had DNA from *Apogon lacheri* detected in its stomach, a species not reported below 106 m (Figure 11).

Discussion

Lionfish are a well-documented invasive species in the western Atlantic, with impacts on native species biomass, richness, density, and recruitment (Albins and Hixon 2008; Green et al. 2012a; Albins 2015). While lionfish in the Caribbean have been observed down to 300 m, our understanding of general biology and diet below 50 m is limited with few insights into potential impacts these fish have on mesophotic and rariphotic communities. This study utilized SCUBA and a manned submersible to capture lionfish on the mesophotic and rariphotic reefs of Curaçao to describe general patterns of size, maturity, and diet across the entire observed depth range in Curaçao. Key findings include changing diet across depth that reflects changes in prey

composition, uneven distribution of males and females along the reef, fertile females along the entire depth range, and increasing length and mass but decreasing body condition with depth.

General Biology: Size and Maturity

On Curaçaoan reefs, lionfish were larger and heavier below 40 m but did not exhibit better body condition (Figure 1). These findings are consistent with Andradi-Brown et al. (2017a) who reported lionfish on Utila mesophotic reefs were also found to be larger and heavier than lionfish on shallow reefs, but with lower proportions of body fat. Mesophotic lionfish on Utila had greater GSI compared to lionfish on shallow reefs, so authors suggested more energy is being allocated to reproductive output rather than growth. However, in lionfish from Curaçao, there was no evidence of an increase in GSI with depth (Figure 1D). Therefore, the decreased body condition in deep lionfish at Curaçao is likely not due to more energy being allocated to reproduction, as indicated by consistent GSI and lack of actively spawning females collected from rariphotic reefs. Decreased body condition is instead consistent with a decline in food availability with depth. Pinheiro et al. (2016) found decreasing richness, abundance, and biomass of reef fishes with depth at Curaçao.

Despite declining food availability with depth, lionfish routinely reach considerable sizes at and below 40 m. Aguilar-Medrano and Vega-Cendejas (2020) sampled 17 lionfish from 45 to 71 m from the southern coast of Veracruz, Mexico and reported a maximum of 402 mm SL and 847.7 g. Aguilar-Perera and Hernández-Landa (2022) collected 92 lionfish from approximately 40 m from off the northern coast of the Yucatan Peninsula, Mexico, between 2014 and 2017. While authors observed some variation between years, lionfish were reaching large sizes (up to 450 mm TL) with a strong correlation between SL and weight of the lionfish and exhibiting good condition (>1.4 mean Fulton's Condition factor) on the sampled mesophotic reef. Lionfish on Curaçao's reefs are also reaching large sizes (up to 470 mm TL) and are in good condition (>1.2 mean Fulton's Condition factor) below 40 m. Male lionfish in this study reached a larger maximum standard length and mass compared to females, which is consistent with previous growth studies (Edwards et al. 2014; Fogg et al. 2017), with the largest individuals collected below 40 m. Given observations of large lionfish that maintain good body condition despite declining food availability with depth may suggest intense predation pressure on fewer prey species.

Some patterns of general lionfish biology across depth may be explained by the removal of lionfish via spearfishing. Spearfishing, which is generally restricted to depths above 40 m, can lead to a shift in size distribution towards smaller individuals and reduces lionfish biomass and density (Frazer et al. 2012; de León et al. 2013; Goodbody-Gringley et al. 2023). Additionally, removal efforts can prompt behavioral changes; lionfish on reefs where there are removal efforts have larger alert distances, were less active, and hid deeper in the reef (Côté et al. 2014). Removal efforts around the study area by spearfishers may artificially create the trend of smaller lionfish on shallow reefs by removing large and or bold lionfish, leaving behind smaller or more cautious individuals hidden in complex reef habitat. If male lionfish do reach larger maximum sizes (as reported here, by Edwards et al. 2014; Fogg et al. 2017, but not by Aguilar-Perera and Hernández-Landa 2022), then mechanical removal efforts may preferentially remove males, leaving more females on the reef. However, the inverse was observed in this study, and Goodbody-Gringley et al. (2023) reported percent females declining over a 6-month recurring culling period. There may be a more complex interaction between culling, behavioral changes, and the resulting changes to size and sex

in a local lionfish population which may also vary between locations. Additionally, mature lionfish on mesophotic and rariphotic reefs (reported here and by Andradi-Brown et al. 2017a), which are out of reach of traditional removal programs, may be replenishing altiphotic lionfish populations across the Caribbean and compromising management efforts.

Diet

Prevalence of empty stomachs can impact diet studies by requiring increased sampling effort to fully capture diet variation and composition. Two meta-analyses looking at percent empty stomachs (PES) across hundreds of species found overall means of 16.2% and 26.4% with large ranges around the mean and piscivores having a higher PES (Arrington et al. 2002; Vinson and Angradi 2011). Using data from eight published and seven unpublished datasets, 27.7% of lionfish sampled had empty stomachs (Peake et al. 2018). One study reported 28.1% empty above 30 m but 61.6% empty below 30 m (overall 36% empty) (Eddy et al. 2016), which may suggest intraspecific variation of hunting success across the reef slope, however Andradi-Brown et al. (2017a) did not find differences in PES across depth. On Curaçaoan reefs, 19.1% of lionfish stomachs were visually empty, lower than previous studies but within the general range reported by meta-analyses. When using metabarcoding, 10.3% of all samples returned only lionfish DNA and were void of prey DNA. Both methods revealed more empty stomachs below 40 m, but metabarcoding captured prey DNA from 12 stomachs that appeared empty and from the 14 stomachs where material was too digested to identify. Metabarcoding is one tool that can decrease sampling effort required to describe species diet by using DNA in unidentified digested material.

DNA metabarcoding greatly increased resolution and quantity of taxa identified over visual identification methods, supporting earlier studies (Harms-Tuohy et al. 2016). Teleost species identified here increased from six species to 51 species (750%) between the two methods. Teleost families detected nearly doubled from ten to 18. While metabarcoding does not provide precise information on the abundance of prey within a gut, metabarcoding captures significantly more diversity with high sensitivity compared to visual identification. Examining frequency of occurrence of taxa can still provide insight into dietary preferences, with the understanding that occurrence metrics can inflate rare taxa (Deagle et al. 2019). It is also important to be aware of biases when choosing genes and primers for barcoding and to choose the most appropriate set for the particular research questions. For example, while COI has high taxonomic resolution, there are some groups where species level discrimination is poor (e.g. Porifera and Anthozoa (Shearer et al. 2002; Huang et al. 2008)) or does not amplify well (e.g. Nematoda (Bhadury et al. 2006) and Platyhelminthes (Andújar et al. 2018)). The biases against those phyla were not a concern for a diet analysis of a predatory reef fish, like lionfish. COI has been shown to outperform other markers specifically in reef piscivore metabarcoding studies (Devloo-Delva et al. 2019) although best practice may indeed be the use of a combination of markers for taxonomic coverage (Berry et al. 2017; Pereira et al. 2019)

The success of metabarcoding is dependent on an appropriate reference database to identify sequences. This study benefitted from a custom database which includes a library of DNA sequences from fishes of the tropical western Atlantic collected by the Smithsonian, including their Deep Reef Observation Project (DROP), some of which are not yet publicly available, combined with MARES (Arranz et al. 2020). After a decade of biodiversity and genetic surveys, DROP has

gathered records of over 90% of the known deep-reef fishes from Curaçao (Robertson et al. 2022) and has amassed extensive data on reef-fish below 40 m at the study location (see Baldwin et al. 2018) as well as other Caribbean sites (Bonaire, St. Eustatius, Roatan, Dominica). As a result, all teleost ESVs (364) were assigned to family or a more specific taxonomy, with approximately two thirds of teleost ESVs (224) assigned to species. The custom database matched 51 teleost species found in lionfish guts. Conversely, invertebrates were not well represented in database with high taxonomic resolution and all the ESVs that were assigned to phylum or order (total 163 ESVs, 27.3% of prey ESVs) were non-chordate taxa (Figure 5). Less than 5% of non-chordate ESVs were assigned to species.

Cryptobenthic families (Brandl et al. 2018), which include two of the most commonly occurring prey families (Gobiidae and Apogonidae) as well as several infrequently occurring prey families (Grammatidae, Chaenopsidae, Blenniidae, and Tripterygiidae) (Brandl et al. 2018), make up a large portion of lionfish diets. In addition to making up the majority of biomass on coral reefs, cryptobenthic fishes are common prey items due to their small bodies, often compressed, and demersal lifestyle. Despite the abundance and diversity of cryptobenthics on reefs (Ackerman and Bellwood 2000) and in lionfish diets, these fishes are not well represented in public genetic databases (Gómez-Buckley et al. 2023). Nonetheless, new species of cryptobenthic fishes are being described at twice the rate of large taxa (Brandl et al. 2018), and many of those new species are being described from below 40 m (Baldwin and Robertson 2015; Schizas et al. 2015; Tornabene and Baldwin 2017; Shepherd et al. 2020, 2020) with more biodiversity yet to be discovered. Notably, this study adds to existing reports of lionfish eating undescribed species. An undescribed genus and species of Gobiidae (Tornabene et al. 2016) frequently occurred in lionfish stomachs below 40 m as well as an undescribed species of *Baldwinella.* This study also confirms video reports of lionfish feeding on *Palatogobius incendius* which was undescribed at the time of observation (Tornabene and Baldwin 2017). To increase accuracy of diet descriptions of all marine fishes, it's imperative to improve reference databases with sequences from all taxa including recently described species.

Lionfish prey types and patterns from Curacao are consistent with previous diet studies. Lionfish often prey on reef fishes from the families Gobiidae, Pomacentridae, Apogonidae, Labridae, and Serranidae (Morris and Akins 2009)(Dahl and Peterson III 2014; Santamaria et al. 2020), though frequency and proportions vary across the western Atlantic (Peake et al. 2018). In Curaçao, these families were also the most frequently occurring prey, although proportions changed above and below 40 m. This change in diet is likely linked to lionfish's opportunistic feeding behavior and the change in prey availability and composition with depth. Lionfish are known to be generalist predators, however prevalent prey species often share similar characteristics: they are demersal. small and shallow-bodied, with additional risk of predation if nocturnal or crepuscular (Green and Côté 2014). The novel deep-reef species reported here, including *Syphraenops bairdianus, Citharichtyes cornutus, Symphysanodon berryi, Ostichthyes trachypoma, Palatogobius incendius, Baldwinella c.f. vivanus,* and an undescribed genus and species of Gobiidae (Tornabene et al. 2016) exhibit some or all those traits. This study did not see evidence of an ontogenetic diet shift from invertebrates to teleosts as reported by numerous previous studies (supplementary material 6), however this could be due to lack of smaller individuals included (ten individuals smaller than 120 mm SL were included), due to metabarcoding providing only frequency data and not abundance data, or a combination of both factors.

Overall, lionfish gut contents reflect faunal assemblages of the different reef zones (Baldwin et al. 2018). Specifically, in Curaçao, Baldwin et al. (2018) describe four dissimilar reef zones based on distinct faunal assemblages: upper mesophotic from 40–79 m, lower mesophotic from 80–129 m, upper rariphotic from 130–189 m, and lower rariphotic from 190–309 m. Each of these reef zones can be characterized by key species, some of which occupy narrow depth bands, however there is some overlap in the depths occupied by taxa. In addition to the changing communities with depth, richness, abundance, and biomass decreases along the reef slope (Pinheiro et al. 2016). Decreasing diet diversity (unique ESVs and prey species) that parallels decreasing community diversity with depth (Figure 4) may indicate more intense predation pressure on a fewer number of species. The overlap between the depth ranges of prey species and the depths at which they appeared in lionfish guts suggests that lionfish were recently feeding near the depth range they were occupying when collected (Figure 11). Specifically, lionfish on mesophotic and rariphotic reefs have diets that consist predominately of mesophotic and rariphotic species while altiphotic lionfish have diets consisting of altiphotic prey. There are only a few cases where a lionfish was caught with prey in its stomach outside the prey species range (Figure 11). In these cases, short vertical migrations are one possible explanation, or alternatively the maximum depth range of the prey species could be underestimated. There is no evidence to support large vertical migrations within the timeframe of digestion, as indicated by the lack of altiphotic prey in the guts of deep lionfish, and vice versa. However, it is possible that large vertical migrations may be occurring at timescales beyond the time of digestion (four to five hours; Harms-Tuohy 2016).

In addition to changing prey composition and availability with depth, abiotic factors also influence lionfish diet and consequently lionfish condition. Water temperatures, light, and habitat complexity decrease with depth. In one experiment, habitat complexity was not shown to have significant impact on the functional feeding response of juvenile lionfish (South et al. 2017) while other experiments did show variable impacts of habitat complexity on lionfish predation efficiency (Kimball et al. 2004). How habitat complexity affects lionfish feeding behavior and efficiency does not seem to be well resolved, but the relationship could be influenced by the size of the lionfish, degree of complexity, and type and behavior of prey. Lionfish around Curacao have been observed down to approximately 250 m, the depth that corresponds to the limits of their thermal tolerance. Depending on seasonal variation, the temperature at 250 m reaches approximately 16°C (Baldwin et al. 2018), the temperature at which lionfish stop feeding (Kimball et al. 2004). Lionfish digest slower and feed less frequently at cooler temperatures (Cerino et al. 2013; South et al. 2017; Steell et al. 2019). Additionally, laboratory experiments indicate lionfish have the lowest attack rates under red light, simulating darkness, but higher attack rates under blue light, simulating crepuscular conditions.(South et al. 2017). Given the effect of light and temperature on lionfish feeding behavior and how both properties change across the zones of the reef, these factors may also explain observations of decreased body condition with depth and higher proportions of empty stomachs below 40 m.

Overall diet can also be variable due to the characteristics of the individual lionfish. For example, despite observations of increasing gape size with body length (Costello et al. 2012; Río et al. 2022), there is mixed evidence regarding the relationship between lionfish body size and prey size. A positive relationship between prey size and lionfish size is reported by Peake et al. (2018) and by Morris and Akins (2009), but no relationship was reported by Río et al. (2022). While lionfish can

eat prey items up to 48% of their total length, they regularly eat prey items much smaller than the maximum size possible by gape limits (Morris and Akins 2009; Muñoz et al. 2011). Lionfish also exhibit prey preferences that don't necessarily correspond with the most abundant prey items on the reef and are more likely to exhibit prey choice when in good body condition (Ritger et al. 2020). Though this study did not investigate choice, given the condition of the lionfish collected for this study, it's possible that many of the individuals were exhibiting prey choice, particularly above 40 meters, where the lionfish were in the best body condition. The selectivity of prey may have decreased with depth as lionfish condition decreased.

Conclusions

This study reports on lionfish general biology and diet from zero to 189 m from the South coast of Curaçao. Lionfish length and weight increase with depth, but body condition and GSI do not. Additionally, males and females are not evenly distributed along the reef slope, with more females found below 40 m. Using DNA metabarcoding, prey DNA was recovered from even the deepest collected lionfish. Nineteen teleost species are reported for the first time in lionfish stomach contents.

Given the observations that lionfish are larger on deeper reefs and the fact that deep lionfish may avoid culling, when compared to lionfish on shallow reefs, deep lionfish may be consuming as many or more prey items overall, with the prey coming from a community that has lower abundance and species richness. This puts greater predation pressure on native fish populations. While sampling bias or culling may artificially create trends of larger lionfish down the reef slope, larger lionfish on deep reefs can have significant impact on local communities regardless of the mechanism behind these trends. Native fish populations on deep reefs are typically small bodied and less abundant, which collectively may increase vulnerability to local extirpation due to lionfish predation. However, despite being larger with depth, it is possible that deep-reef lionfish may not feed as often as shallow-reef lionfish due to the positive correlation between temperature and prey consumption, so it is possible this may offset some of the increased predation pressure on the less diverse and sparse deep-reef communities.

While this study targeted a knowledge gap on Curaçaoan mesophotic reefs, future studies should continue to target mesophotic reefs around the Caribbean to understand variation of lionfish diet. Even with 15 data sets that represent over 8,000 lionfish stomachs from the western Atlantic, the cumulative prey curve analysis from Peake et al. (2018) indicates that the full diversity of lionfish diets have not yet been described. This study adds several new prey species to existing literature, bringing the total number of documented teleost prey species to 229 (supplementary material 7). As the invasion edge continues to expand into Brazil (Soares et al. 2023), more species and ecosystems will be threatened by lionfish predation. Lionfish continue to be observed at mesophotic and rariphotic depths across the Caribbean (Nuttall 2014; Andradi-Brown et al. 2017b; Gress et al. 2017; Tornabene and Baldwin 2017; Soares et al. 2023). While there have been observations of lionfish feeding at depth (Tornabene and Baldwin 2017) and this study supports such observations with gut content DNA metabarcoding, it is still unknown to what degree and frequency lionfish migrate vertically over their lifespan. Tagging or analysis of otolith microchemistry will enable a better understanding of vertical lionfish movement over longer time scales.

As a well-established invasive species, continuous monitoring will be necessary to assess impacts on local ecosystems over time, especially as the invasion edge changes geographically and with depth. Understudied mesophotic and rariphotic communities may be especially vulnerable to lionfish predation. Given the breadth and variation seen in lionfish diets, local management will benefit from local scale diet composition studies. Lionfish and their impact on native communities below 40 m must be considered in control and conservation efforts.

Figures and Tables

Table 1. Summary of General Additive Model (GAM) analysis for SL, mass, and condition of lionfish across the reef slope.

	Group	R^2	Variable	P-value	Effect	Coeff	SE
Standard Length (mm)	All Individuals	0.338	Depth	7.57e-13	+	0.64	0.08
			Year	0.0208	-	-19.75	8.44
	Males	0.412	Depth	1.10e-07	+	0.92	0.15
			Year	> 0.05			
	Females	0.247	Depth	2.87e-05	+	0.37	0.08
			Year	> 0.05			
Mass (g)	All Individuals	0.22	Depth	3.13e-08	+	1.90	0.32
			Year	0.0463	-	-68.04	0.32
	Males	0.395	Depth	2.82e-07	+	3.54	0.60
			Year	> 0.05			
	Females	0.098	Depth	0.01063	+	0.85	0.32
			Year	> 0.05			
Fulton's Condition Factor	All Individuals	0.0904	Depth	0.000163	-	-0.001	0.0004
			Year	> 0.05			
	Males	0.172	Depth	0.0016	-	-0.002	0.006
			Year	> 0.05			
	Females	0.299	Depth	4.57e-06	-	-0.002	0.0004
			Year	> 0.05			

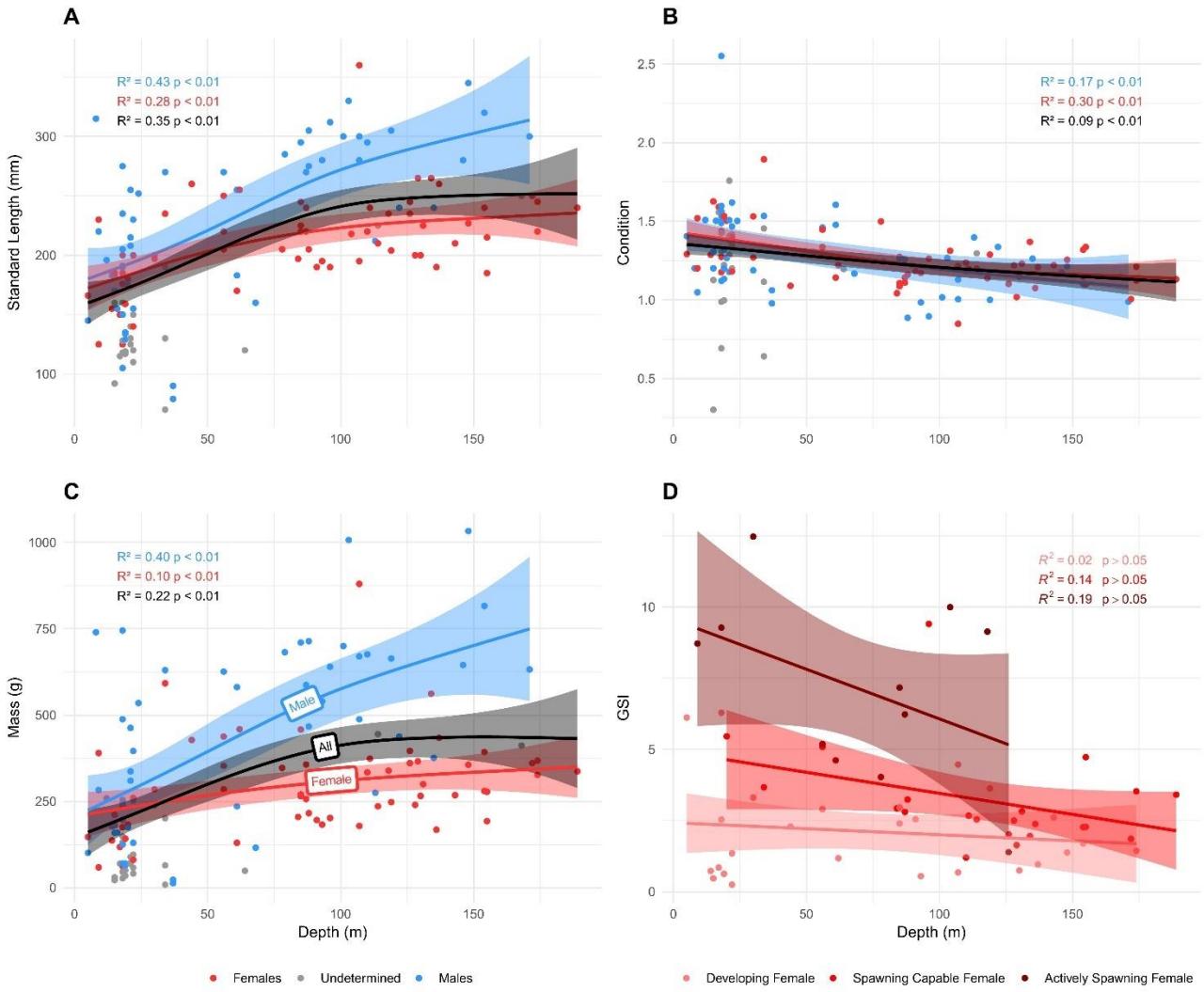


Figure 1. Lionfish standard length (A), condition (B), mass (C), and GSI (D) with depth. Condition was calculated using Fulton condition factor: $M \times 100/TL^3$ where M = mass in grams and TL = total length in centimeters. GSI is gonad weight as a percentage of body weight. Each point represents one individual lionfish. Trend lines represent the fitted Generalized Additive Model for males (blue), females (red), and all individuals (black) with a 95% confidence interval.

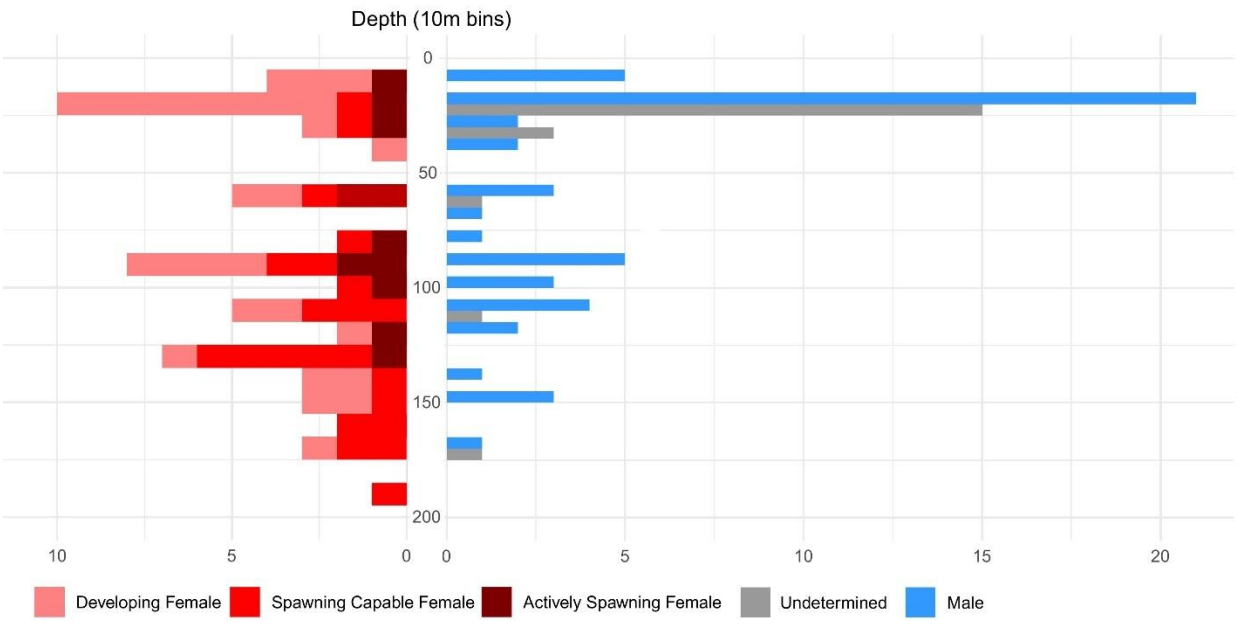


Figure 2. Number of females (left, reds) and males and unsexed individuals (right, blue and grey respectively) across depth, grouped by 10 m depth bins. Color of bars represent different spawning stages for females from developing, to spawning capable, to actively spawning.

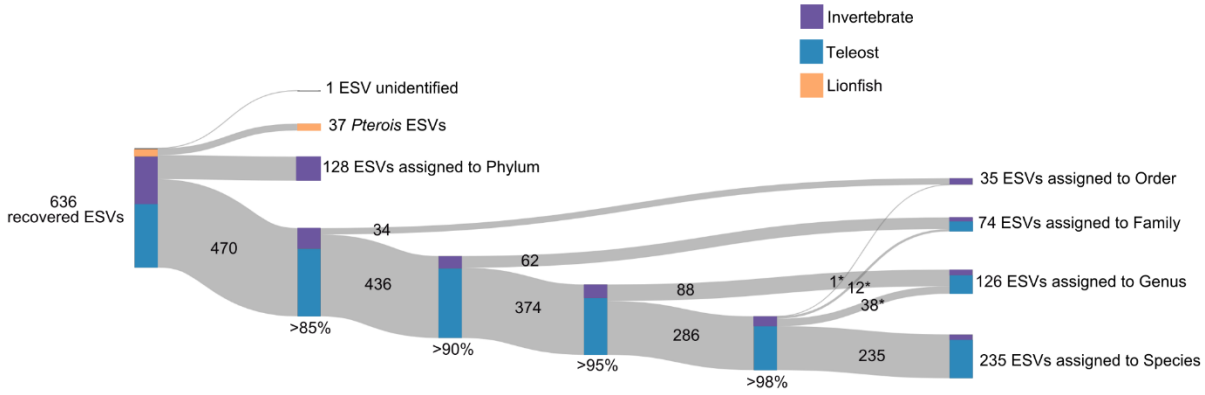


Figure 3. The process (left to right) of assigning taxonomic identifications of ESVs based on sequency similarity to samples in the database. Colored bars represent the portion of teleost and invertebrate ESVs at each node. The percentages below bars represent the arbitrary cutoffs used to classify ESVs into different taxonomic ranks. Other numbers represent the number of ESVs at each step. Asterisks indicate ESVs that matched to incompletely identified vouchers.

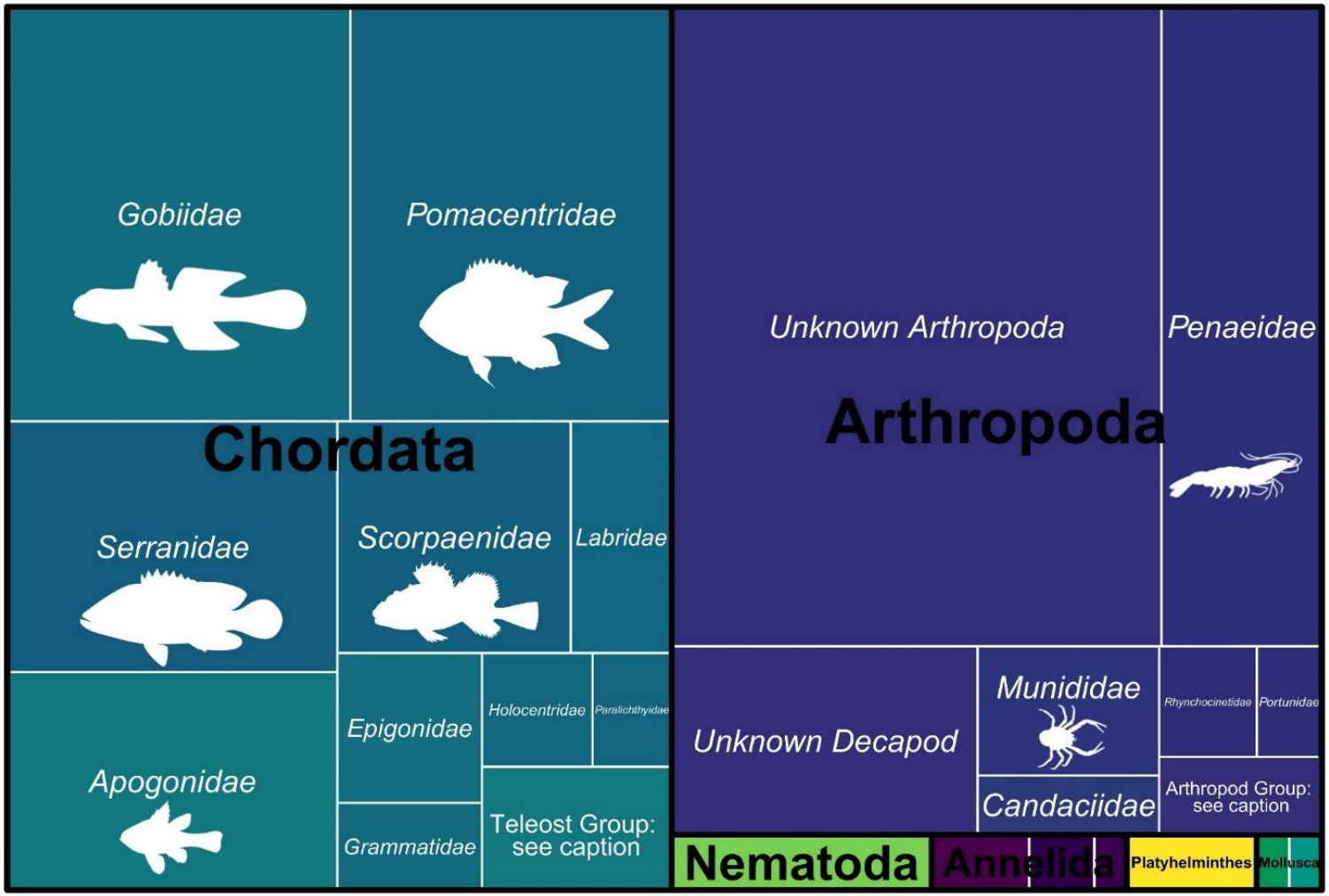


Figure 4. Taxa detected by gut content metabarcoding from all lionfish. The size of the boxes is proportional to the frequency of occurrence for that group. “Teleost group” contains the families Acropomatidae, Blennidae, Chaenopsidae, Chaetodontidae, Priacanthidae, Symphysanodontidae, Synodonitae, and Tripterygiidae. “Arthropod group” contains the families Calappidae, Gonodactyloidae, Hippolytidae, Palaemonidae, and Xanthidae.

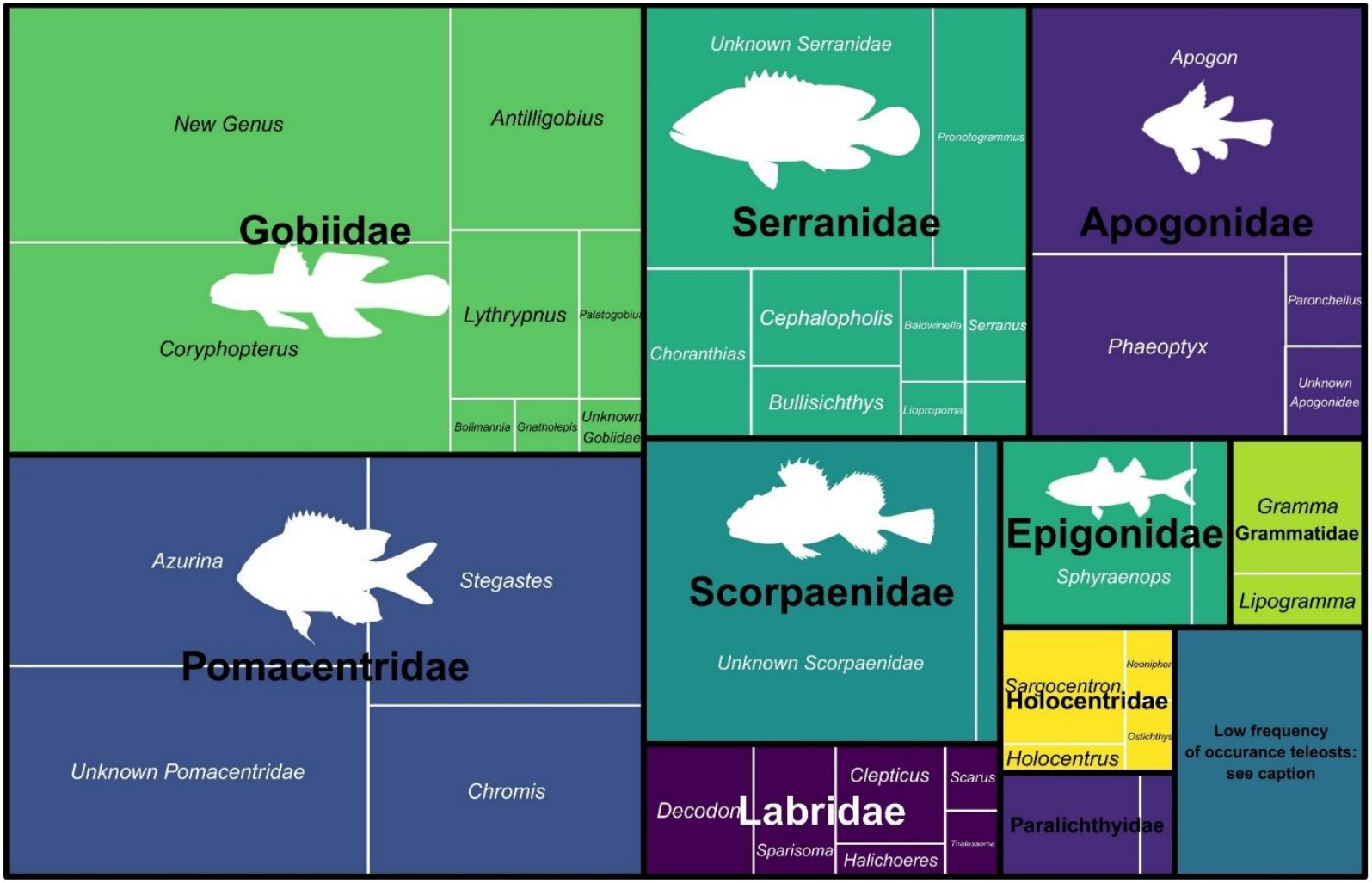


Figure 5. Fishes detected by gut content metabarcoding from all lionfish. The size of the boxes is proportional to the frequency of occurrence for that group. Low frequency of occurrence teleost families include: Acropomatidae, Blennidae, Chaenopsidae, Chaetodontidae, Priacanthidae, Symphysanodontidae, Synodontidae, and Tripterygiidae.

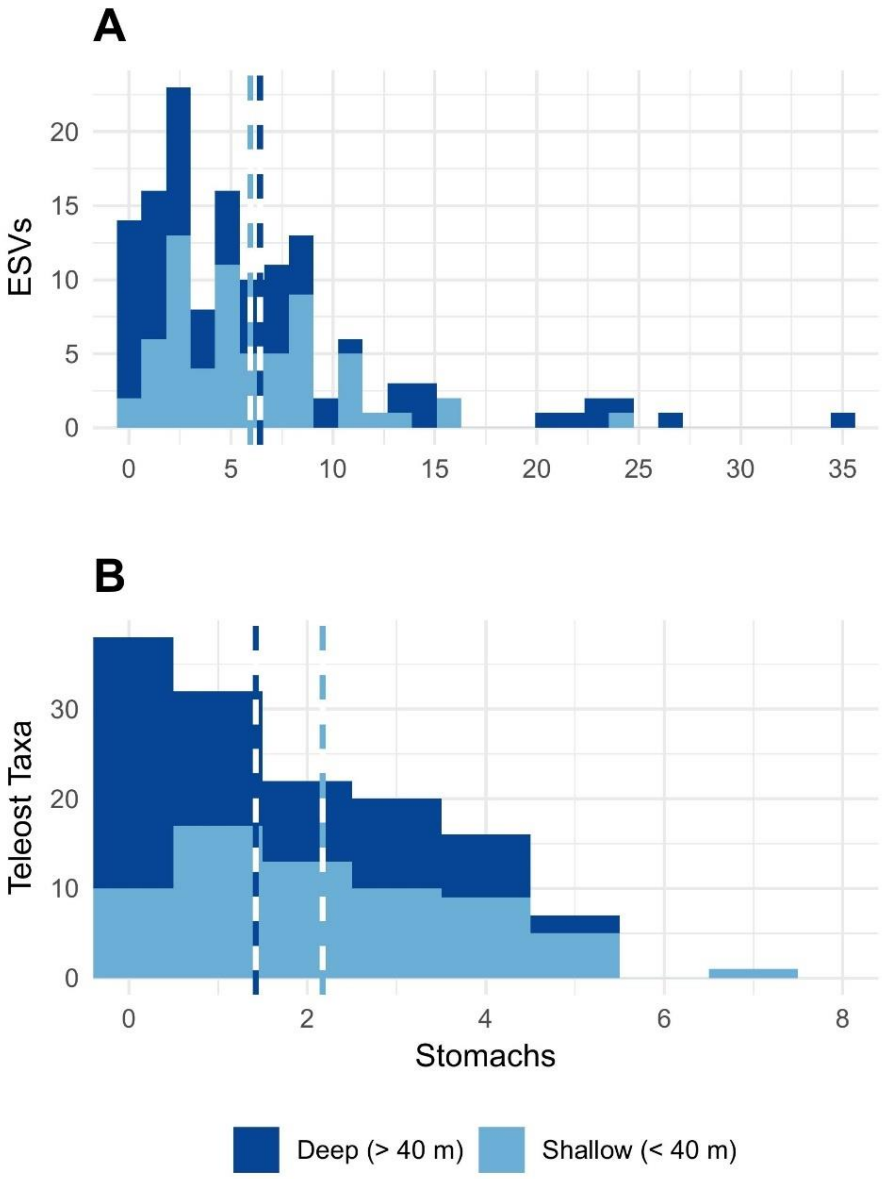


Figure 6. Right-skewed distribution of ESVs (A) and teleost taxa (B) across shallow (< 40 m) and deep (> 40 m) lionfish stomachs. Dashed lines represent the mean of each group.

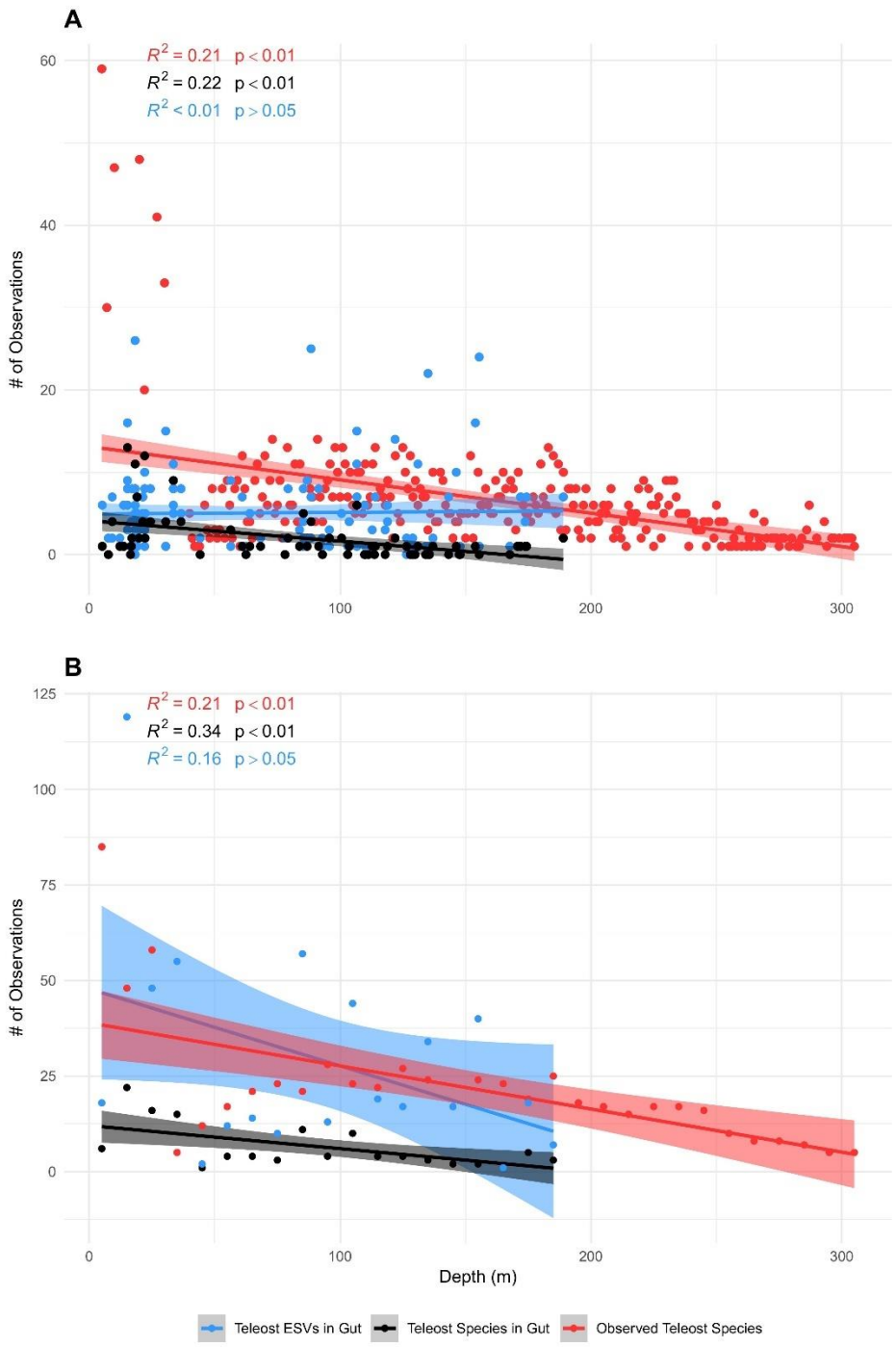


Figure 7. Number of detected ESVs and prey species in lionfish stomachs compared to species observed during visual surveys across the reef slope. (A) Observations are plotted individually (B) Observations are grouped into 10 m depth bins. Visual surveys were conducted on SCUBA between 0 and 30 m and from a manned submersible below 40 m (data from SCUBA unpublished, submersible data from Baldwin et al. 2018).

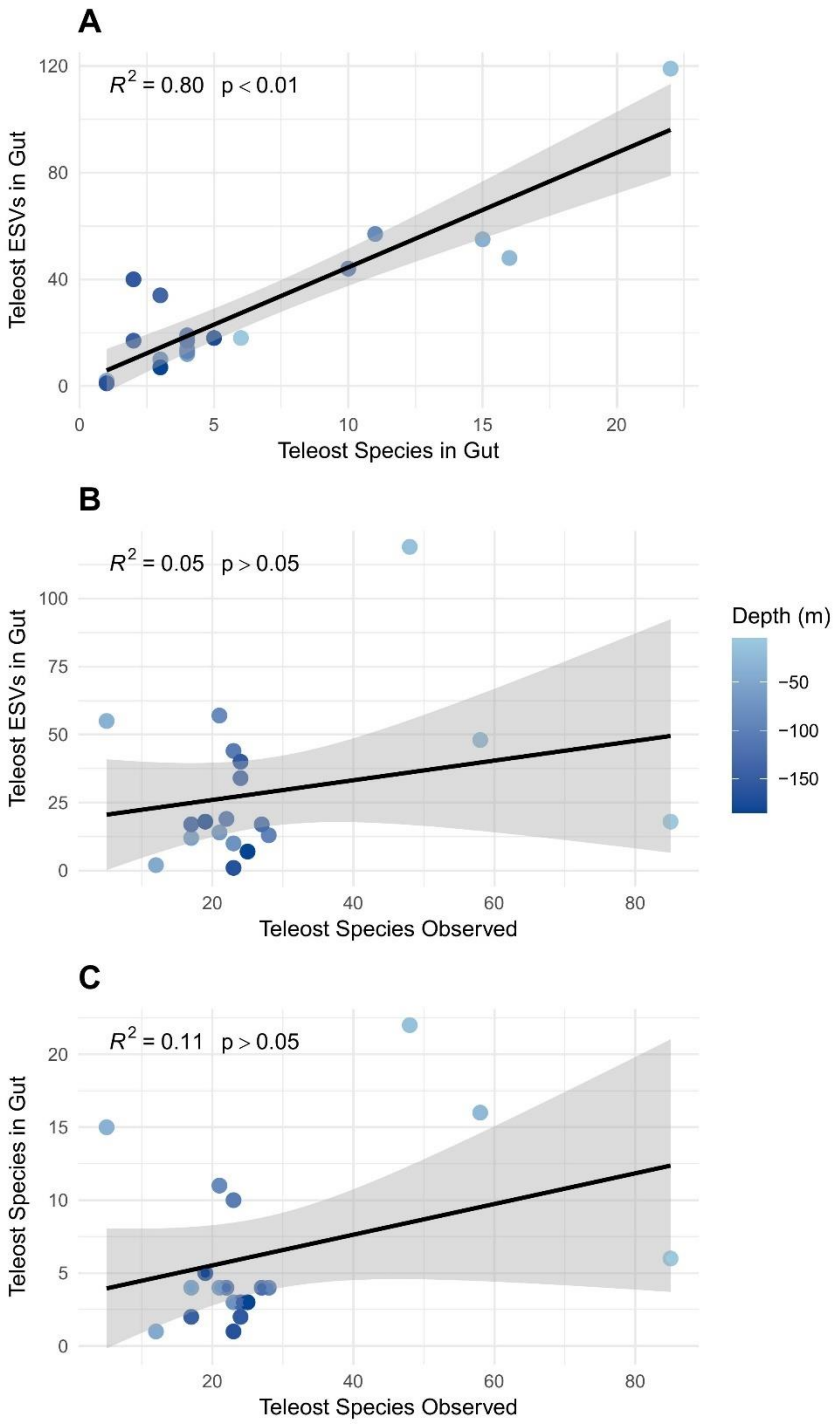


Figure 8. (A) Teleost species detected using metabarcoding vs teleost ESVs detected using metabarcoding in lionfish stomachs. (B) Teleost ESVs detected using metabarcoding compared to teleost species observed during biodiversity surveys. (C) Teleost species detected using metabarcoding compared to teleost species observed during biodiversity surveys. Data points in A though C are grouped into 10-meter bins.

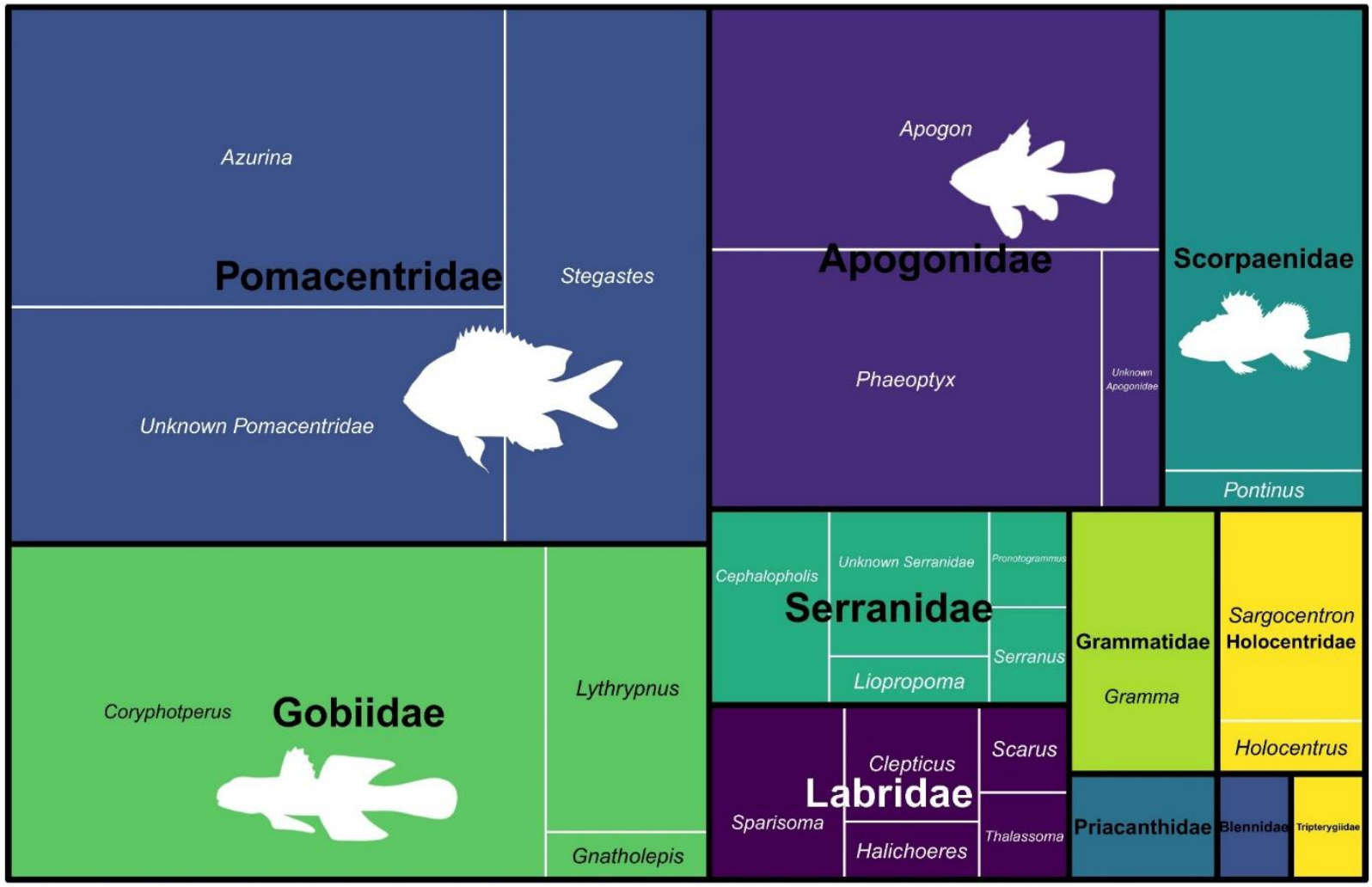


Figure 9. Fishes detected by gut content metabarcoding from lionfish collected from 40 m and shallower. The size of the boxes is proportional to the frequency of occurrence for that group.

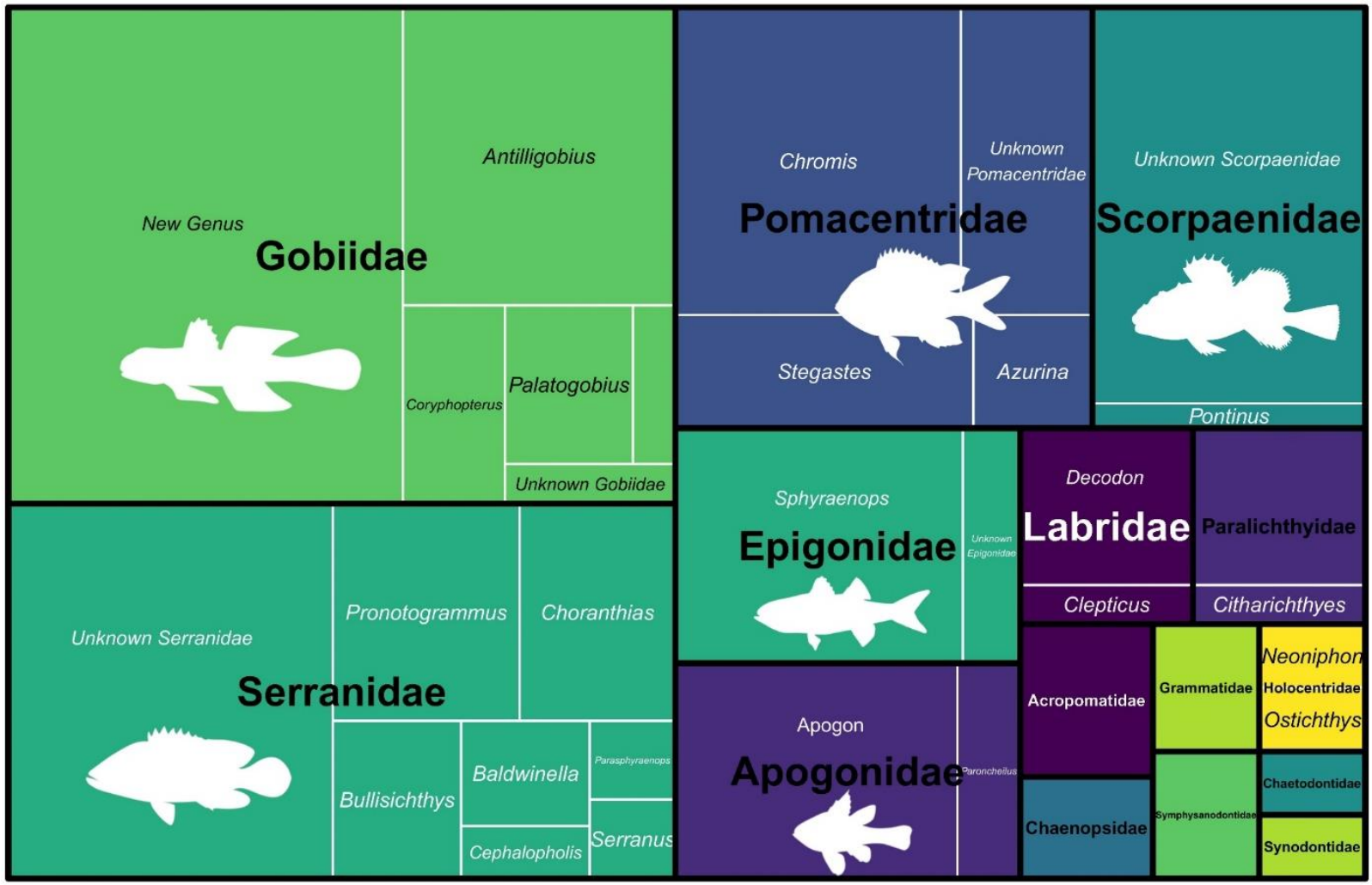


Figure 10. Fishes detected by gut content metabarcoding from lionfish collected below 40 m. The size of the boxes is proportional to the frequency of occurrence for that group.

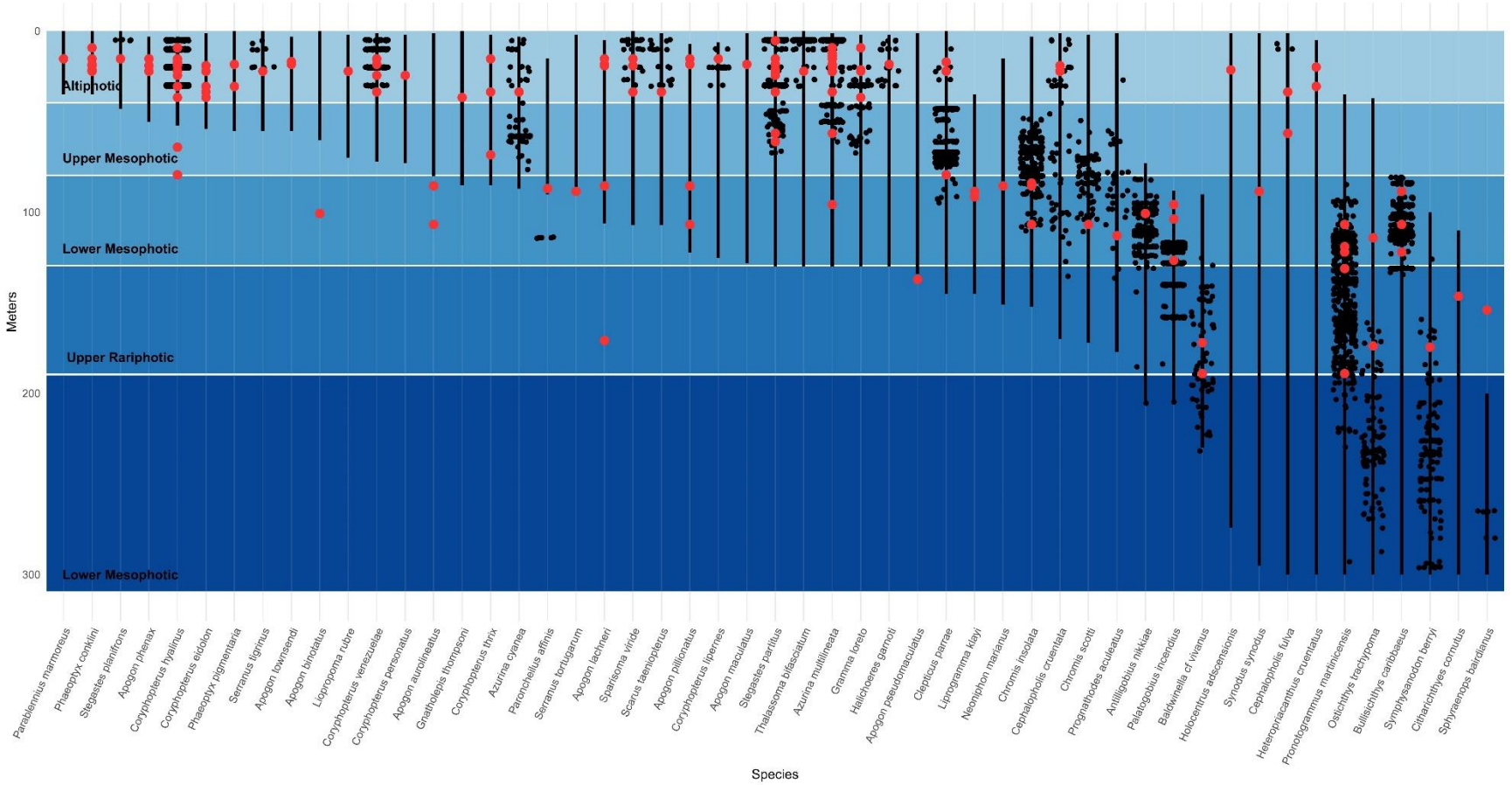


Figure 11. Distribution of teleost prey species detected across depth compared to known species depth of occurrence. Red dots represent an occurrence of that species from a lionfish gut collected at a specific depth. Black bars are depth ranges of a prey species across its entire geographic range as reported by Robertson and Van Tassell (2023). Black dots represent observations from surveys on SCUBA or from the manned submersible, Curasub, along the same slope from which lionfish were collected for this study (data from SCUBA unpublished, submersible data from Baldwin et al. 2018). 93.7% of the red dots fall within the prey species depth distribution.

References

Ackerman J, Bellwood D (2000) Reef fish assemblages: a re-evaluation using enclosed rotenone stations. Marine Ecology Progress Series 206:227–237

Aguilar-Medrano R, Vega-Cendejas MaE (2020) Size, weight, and diet of the invasive lionfish Pterois volitans (Linnaeus, 1758) on the southern coast of Veracruz, Gulf of Mexico. Cienc Mar 46:

Aguilar-Perera A, Hernández-Landa RC (2022) Variation in Body Length, Length-Weight Relationship, and Condition Factor of Lionfish (Pterois volitans) Between Years in a Mesophotic Coral Ecosystem in Alacranes Reef, Southern Gulf of Mexico. cjos 52:142–151

Albins M (2015) Invasive Pacific lionfish Pterois volitans reduce abundance and species richness of native Bahamian coral-reef fishes. Mar Ecol Prog Ser 522:231–243

Albins M, Hixon M (2008) Invasive Indo-Pacific lionfish Pterois volitans reduce recruitment of Atlantic coral-reef fishes. Mar Ecol Prog Ser 367:233–238

Andradi-Brown DA, Grey R, Hendrix A, Hitchner D, Hunt CL, Gress E, Madej K, Parry RL, Régnier-McKellar C, Jones OP, Arteaga M, Izaguirre AP, Rogers AD, Exton DA (2017a) Depth-dependent effects of culling—do mesophotic lionfish populations undermine current management? R Soc open sci 4:170027

Andradi-Brown DA, Vermeij MJA, Slattery M, Lesser M, Bejarano I, Appeldoorn R, Goodbody-Gringley G, Chequer AD, Pitt JM, Eddy C, Smith SR, Brokovich E, Pinheiro HT, Jessup ME, Shepherd B, Rocha LA, Curtis-Quick J, Eyal G, Noyes TJ, Rogers AD, Exton DA (2017b) Large-scale invasion of western Atlantic mesophotic reefs by lionfish potentially undermines culling-based management. Biol Invasions 19:939–954

Andújar C, Arribas P, Gray C, Bruce C, Woodward G, Yu DW, Vogler AP (2018) Metabarcoding of freshwater invertebrates to detect the effects of a pesticide spill. Molecular Ecology 27:146–166

Aphalo PJ (2024) ggpmisc: Miscellaneous Extensions to "ggplot2."

Arranz V, Pearman WS, Aguirre JD, Liggins L (2020) MARES, a replicable pipeline and curated reference database for marine eukaryote metabarcoding. Sci Data 7:209

Arredondo-Chávez AT, Sánchez-Jimenez JA, Ávila-Morales OG, Torres-Chávez P, Herrerias-Diego I, Medina-Nava M, Madrigal-Guridi X, Campos-Mendoza A, Domínguez-Domínguez O, Caballero-Vázquez JA (2016) Spatio-temporal variation in the diet composition of red lionfish, Pterois volitans (Actinopterygii: Scorpaeniformes: Scorpaenidae), in the Mexican Caribbean: Insights into the ecological effect of the alien invasion. Acta Ichthyologica et Piscatoria 46:182–200

Arrington DA, Winemiller KO, Loftus WF, Akin S (2002) How Often Do Fishes "Run on Empty"? Ecology 83:2145–2151

Baldwin CC, Robertson DR (2015) A new, mesophotic Coryphopterus goby (Teleostei, Gobiidae) from the southern Caribbean, with comments on relationships and depth distributions within the genus. Zookeys 123–142

Baldwin CC, Tornabene L, Robertson DR (2018) Below the Mesophotic. Sci Rep 8:4920

Bejarano I, Appeldoorn RS, Nemeth M (2014) Fishes associated with mesophotic coral ecosystems in La Parguera, Puerto Rico. Coral Reefs 33:313–328

Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, Stat M, Bejder L, Bunce M (2017) DNA metabarcoding for diet analysis and biodiversity: A case study using the endangered Australian sea lion (Neophoca cinerea). Ecology and Evolution 7:5435–5453

Bhadury P, Austen M, Bilton D, Lambshead P, Rogers A, Smerdon G (2006) Development and evaluation of a DNA-barcoding approach for the rapid identification of nematodes. Mar Ecol Prog Ser 320:1–9

Blackburn TM, Pyšek P, Bacher S, Carlton JT, Duncan RP, Jarošík V, Wilson JRU, Richardson DM (2011) A proposed unified framework for biological invasions. Trends in Ecology & Evolution 26:333–339

Bogdanoff AK, Mostowy J, Peake J, Layman CA, Bermudez AB, Baca CG, Palacios NH, Gonzalez DTM, Del Rocio Bravo Xicoténcatl M, Morris JA (2018) A brief description of invasive lionfish (*Pterois* sp.) diet composition in the Arrecifes de Cozumel National Park. Food Webs 17:e00104

Brandl SJ, Goatley CHR, Bellwood DR, Tornabene L (2018) The hidden half: ecology and evolution of cryptobenthic fishes on coral reefs: Cryptobenthic reef fishes. Biological Reviews 93:1846–1873

Brokovich E, Einbinder S, Shashar N, Kiflawi M, Kark S (2008) Descending to the twilight-zone: changes in coral reef fish assemblages along a depth gradient down to 65 m. Marine Ecology Progress Series 371:253–262

Bucklin A, Steinke D, Blanco-Bercial L (2011) DNA Barcoding of Marine Metazoa. Annual Review of Marine Science 3:471–508

Callahan BJ, McMurdie PJ, Holmes SP (2017) Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal 11:2639–2643

Cerino D, Overton AS, Rice JA, Morris Jr. JA (2013) Bioenergetics and Trophic Impacts of the Invasive Indo-Pacific Lionfish. Transactions of the American Fisheries Society 142:1522–1534

Coker DJ, DiBattista JD, Stat M, Arrigoni R, Reimer J, Terraneo TI, Villalobos R, Nowicki JP, Bunce M, Berumen ML (2023) DNA metabarcoding confirms primary targets and breadth of diet for coral reef butterflyfishes. Coral Reefs 42:1–15

Costello R, Frankel N, Gamble M (2012) Allometric Scaling of Morphological Feeding Adaptations and Extreme Sexual Dimorphism in Energy Allocation in Invasive Lionfish. Dartmouth Undergrad J Sci 39–41

Côté IM, Darling ES, Malpica-Cruz L, Smith NS, Green SJ, Curtis-Quick J, Layman C (2014) What Doesn't Kill You Makes You Wary? Effect of Repeated Culling on the Behaviour of an Invasive Predator. PLoS ONE 9:e94248

Courtenay W (1995) Marine fish introductions in southeastern Florida. American Fisheries Society Introduced Fish Section Newsletter 2–3

Curtis J, Albins M, Peebles E, Stallings C (2020) Stable isotope analysis of eye lenses from invasive lionfish yields record of resource use. Mar Ecol Prog Ser 637:181–194

Curtis J, Wall K, Albins M, Stallings C (2017) Diet shifts in a native mesopredator across a range of invasive lionfish biomass. Mar Ecol Prog Ser 573:215–228

Dahl KA, Patterson WF, Robertson A, Ortmann AC (2017) DNA barcoding significantly improves resolution of invasive lionfish diet in the Northern Gulf of Mexico. Biol Invasions 19:1917–1933

Dahl KA, Peterson III WF (2014) Habitat-Specific Density and Diet of Rapidly Expanding Invasive Red Lionfish, Pterois volitans, Populations in the Northern Gulf of Mexico. PLOS ONE 9:e105852

Darling ES, Green SJ, O'Leary JK, Côté IM (2011) Indo-Pacific lionfish are larger and more abundant on invaded reefs: a comparison of Kenyan and Bahamian lionfish populations. Biol Invasions 13:2045–2051

Deagle BE, Thomas AC, McInnes JC, Clarke LJ, Vesterinen EJ, Clare EL, Kartzinel TR, Eveson JP (2019) Counting with DNA in metabarcoding studies: How should we convert sequence reads to dietary data? Molecular Ecology 28:391–406

Devloo-Delva F, Huerlimann R, Chua G, Matley JK, Heupel MR, Simpfendorfer CA, Maes GE (2019) How does marker choice affect your diet analysis: comparing genetic markers and digestion levels for diet metabarcoding of tropical-reef piscivores. Mar Freshwater Res 70:8

Echevarría LMP, Monteagudo PPC, Guerra DC, Wong RIC, Rojas DC, Aragón HC, Rodríguez AG, Osoria RAF (2017) Superposición de la dieta del pez león Pterois volitans (Teleostei: Scorpaenidae) con la de peces nativos de nivel trófico similar en Cuba. Boletín de Investigaciones Marinas y Costeras 46:

Eddy C, Pitt J, Morris J, Smith S, Goodbody-Gringley G, Bernal D (2016) Diet of invasive lionfish (Pterois volitans and P. miles) in Bermuda. Marine Ecology Progress Series 558:193–206

Eddy C, Pitt JM, Larkum J, Altabet MA, Bernal D (2020) Stable Isotope Ecology of Invasive Lionfish (Pterois volitans and P. miles) in Bermuda. Frontiers in Marine Science 7:

Edgar RC (2016) UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. 081257

Edgar RC, Flyvbjerg H (2015) Error filtering, pair assembly and error correction for next-generation sequencing reads. Bioinformatics 31:3476–3482

Edwards MA, Frazer TK, Jacoby CA (2014) Age and growth of invasive lionfish (Pterois spp.) in the Caribbean Sea, with implications for management. Bulletin of Marine Science 90:953–966

Egeter B, Roe C, Peixoto S, Puppo P, Easton LJ, Pinto J, Bishop PJ, Robertson BC (2019) Using molecular diet analysis to inform invasive species management: A case study of introduced rats consuming endemic New Zealand frogs. Ecology and Evolution 9:5032–5048

Ewing M, Welicky M, Baldwin CC, Tornabene L, Maslenikov K, Robertson DR (In Press) Analysis of bulk stable isotopes and trophic positions of invasive lionfish (Pterois volitans) on deep versus shallow reefs at Curacao. Springer

Ferreira CEL, Luiz OJ, Floeter SR, Lucena MB, Barbosa MC, Rocha CR, Rocha LA (2015) First Record of Invasive Lionfish (Pterois volitans) for the Brazilian Coast. PLOS ONE 10:e0123002

Fogg A, Brown-Peterson N, Peterson M (2017) Reproductive life history characteristics of invasive red lionfish (*Pterois volitans*) in the northern Gulf of Mexico. bms 93:791–813

Frazer TK, Jacoby CA, Edwards MA, Barry SC, Manfrino CM (2012) Coping with the Lionfish Invasion: Can Targeted Removals Yield Beneficial Effects? Reviews in Fisheries Science 20:185–191

Froese R (2006) Cube law, condition factor and weight–length relationships: history, meta-analysis and recommendations. Journal of Applied Ichthyology 22:241–253

Galanti L, Shasha D, Gunsalus KC (2021) Pheniqs 2.0: accurate, high-performance Bayesian decoding and confidence estimation for combinatorial barcode indexing. BMC Bioinformatics 22:359
Gallardo B, Clavero M, Sánchez MI, Vilà M (2016) Global ecological impacts of invasive species in aquatic ecosystems. Global Change Biology 22:151–163
Gardner PG, Frazer TK, Jacoby CA, Yanong RPE (2015) Reproductive biology of invasive lionfish (Pterois spp.). Frontiers in Marine Science 2:
Geller J, Meyer C, Parker M, Hawk H (2013) Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources 13:851–861
Gómez-Buckley MC, Gallego R, Arranz V, Halafihi T, Stone K, Erdmann M, Tornabene LM (2023) Comparing anesthetic stations and environmental DNA sampling to determine community composition of cryptobenthic coral reef fishes of Vava'u, Kingdom of Tonga. Coral Reefs 42:785–797
Goodbody-Gringley G, Chequer A, Grincavitch C, Noyes T, Dowell R, Lundberg A, Corbett E, Smith A (2023) Impacts of recurrent culling of invasive lionfish on mesophotic reefs in Bermuda. Coral Reefs
Green SJ, Akins JL, Maljković A, Côté IM (2012a) Invasive Lionfish Drive Atlantic Coral Reef Fish Declines. PLoS ONE 7:e32596
Green SJ, Côté IM (2014) Trait-based diet selection: prey behaviour and morphology predict vulnerability to predation in reef fish communities. Journal of Animal Ecology 83:1451–1460
Green SJ, Morris JA, Akins JL (2012b) Lionfish dissection : techniques and applications.
Gress E, Andradi-Brown DA, Woodall L, Schofield PJ, Stanley K, Rogers AD (2017) Lionfish (Pterois spp.) invade the upper-bathyal zone in the western Atlantic. PeerJ 5:e3683
Harms-Tuohy C, Schizas N, Appeldoorn R (2016) Use of DNA metabarcoding for stomach content analysis in the invasive lionfish Pterois volitans in Puerto Rico. Mar Ecol Prog Ser 558:181–191
Harms-Tuohy CA (2016) The management and feeding ecology of the invasive lionfish (Pterois volitans) in Puerto Rico.
Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003a) Biological identifications through DNA barcodes. Proceedings of the Royal Society of London Series B: Biological Sciences 270:313–321
Hebert PDN, Ratnasingham S, de Waard JR (2003b) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London Series B: Biological Sciences 270:S96–S99
Huang D, Meier R, Todd PA, Chou LM (2008) Slow Mitochondrial COI Sequence Evolution at the Base of the Metazoan Tree and Its Implications for DNA Barcoding. J Mol Evol 66:167–174
Ingeman K (2016) Lionfish cause increased mortality rates and drive local extirpation of native prey. Mar Ecol Prog Ser 558:235–245
Jasper SA, Thomas JD, Kerstetter D (2018) Diet of the Invasive Lionfish Pterois sp. in Broward County, Florida. 1:
Kimball M, Miller J, Whitfield P, Hare J (2004) Thermal tolerance and potential distribution of invasive lionfish (Pterois volitans/miles complex) on the east coast of the United States. Mar Ecol Prog Ser 283:269–278

Kulbicki M, Beets J, Chabanet P, Cure K, Darling E, Floeter SR, Galzin R, Green A, Harmelin-Vivien M, Hixon M, Letourneur Y, Loma TL de, McClanahan T, McIlwain J, MouTham G, Myers R, O'Leary JK, Planes S, Vigliola L, Wantiez L (2012) Distributions of Indo-Pacific lionfishes Pterois spp. in their native ranges: implications for the Atlantic invasion. Marine Ecology Progress Series 446:189–205

Layman C, Allgeier J (2012) Characterizing trophic ecology of generalist consumers: a case study of the invasive lionfish in The Bahamas. Mar Ecol Prog Ser 448:131–141

de León R, Vane K, Bertuol P, Chamberland V, Simal F, Imms E, Vermeij M (2013) Effectiveness of lionfish removal efforts in the southern Caribbean. Endang Species Res 22:175–182

Leray M, Yang JY, Meyer CP, Mills SC, Agudelo N, Ranwez V, Boehm JT, Machida RJ (2013) A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. Frontiers in Zoology 10:34

Linardich C, Ralph GM, Robertson DR, Harwell H, Polidoro BA, Lindeman KC, Carpenter KE (2019) Extinction risk and conservation of marine bony shorefishes of the Greater Caribbean and Gulf of Mexico. Aquatic Conservation: Marine and Freshwater Ecosystems 29:85–101

Luiz OJ, dos Santos WCR, Marceniuk AP, Rocha LA, Floeter SR, Buck CE, de Klautau AGCM, Ferreira CEL (2021) Multiple lionfish (Pterois spp.) new occurrences along the Brazilian coast confirm the invasion pathway into the Southwestern Atlantic. Biol Invasions 23:3013–3019

Marbuah G, Gren I-M, McKie B (2014) Economics of Harmful Invasive Species: A Review. Diversity 6:500–523

Marques AJD, Mata VA, Velo-Antón G (2022) COI Metabarcoding Provides Insights into the Highly Diverse Diet of a Generalist Salamander, Salamandra salamandra (Caudata: Salamandridae). Diversity 14:89

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet.journal 17:10–12

McCleery C (2011) A comparative study of the feeding ecology of invasive lionfish (Pterois volitans) in the Caribbean | Dutch Caribbean Biodiversity Database. Physis: Journal of Marine Science 9:38–43

Mizrahi M, Chapman J, Gough C, Humber F, Anderson L (2017) Management implications of the influence of biological variability of invasive lionfish diet in Belize. MBI 8:61–70

Mormul RP, Vieira DS, Bailly D, Fidanza K, da Silva VFB, da Graça WJ, Pontara V, Bueno ML, Thomaz SM, Mendes RS (2022) Invasive alien species records are exponentially rising across the Earth. Biol Invasions 24:3249–3261

Morris JA, Akins JL (2009) Feeding ecology of invasive lionfish (Pterois volitans) in the Bahamian archipelago. Environ Biol Fish 86:389–398

Muñoz R, Currin C, Whitfield P (2011) Diet of invasive lionfish on hard bottom reefs of the Southeast USA: insights from stomach contents and stable isotopes. Mar Ecol Prog Ser 432:181–193

NCBI Resource Coordinators (2017) Database Resources of the National Center for Biotechnology Information. Nucleic Acids Research 45:D12–D17

Nuttall M (2014) Lionfish (Pterois volitans [Linnaeus, 1758] and P. miles [Bennett, 1828]) records within mesophotic depth ranges on natural banks in the Northwestern Gulf of Mexico. BIR 3:111–115

O'Farrell S, Bearhop S, McGill RAR, Dahlgren CP, Brumbaugh DR, Mumby PJ (2014) Habitat and body size effects on the isotopic niche space of invasive lionfish and endangered Nassau grouper. Ecosphere 5:art123

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin P, O'Hara B, Simpson G, Solymos P, Stevens H, Wagner H (2024) Vegan: Community Ecology Package.

Peake J, Bogdanoff AK, Layman CA, Castillo B, Reale-Munroe K, Chapman J, Dahl K, Patterson III WF, Eddy C, Ellis RD, Faletti M, Higgs N, Johnston MA, Muñoz RC, Sandel V, Villasenor-Derbez JC, Morris JA (2018) Feeding ecology of invasive lionfish (Pterois volitans and Pterois miles) in the temperate and tropical western Atlantic. Biol Invasions 20:2567–2597

Pereira A, Xavier R, Perera A, Salvi D, Harris DJ (2019) DNA metabarcoding to assess diet partitioning and feeding strategies in generalist vertebrate predators: a case study on three syntopic lacertid lizards from Morocco. Biological Journal of the Linnean Society 127:800–809

Pinheiro HT, Goodbody-Gringley G, Jessup ME, Shepherd B, Chequer AD, Rocha LA (2016) Upper and lower mesophotic coral reef fish communities evaluated by underwater visual censuses in two Caribbean locations. Coral Reefs 35:139–151

Posit team (2024) RStudio: Integrated Development Environment for R.

Pusack TJ, Benkwitt CE, Cure K, Kindinger TL (2016) Invasive Red Lionfish (Pterois volitans) grow faster in the Atlantic Ocean than in their native Pacific range. Environ Biol Fish 99:571–579

Pyle RL (2000) Assessing Undiscovered Fish Biodiversity on Deep Coral Reefs Using Advanced Self-Contained Diving Technology. mar technol soc j 34:82–91

Ratnasingham S, Hebert PDN (2007) bold: The Barcode of Life Data System (http://www.barcodinglife.org). Molecular Ecology Notes 7:355–364

Río L del, Navarro-Martínez ZM, Ruiz-Abierno A, Chevalier-Monteagudo PP, Angulo-Valdes JA, Rodriguez-Viera L (2022) Feeding ecology of invasive lionfish in the Punta Frances MPA, Cuba: insight into morphological features, diet and management. PeerJ 10:e14250

Ritger AL, Fountain CT, Bourne K, Martín-Fernández JA, Pierotti MER (2020) Diet choice in a generalist predator, the invasive lionfish (*Pterois volitans/miles*). Journal of Experimental Marine Biology and Ecology 524:151311

Robertson DR, van Tassell J (2023) Shorefishes of the Greater Caribbean: online information system. Version 3.0 Smithsonian Tropical Research Institute, Balboa, Panamá. https://biogeodb.stri.si.edu/caribbean/en/pages

Robertson DR, Tornabene L, Lardizabal CC, Baldwin CC (2022) Submersibles Greatly Enhance Research on the Diversity of Deep-Reef Fishes in the Greater Caribbean. Frontiers in Marine Science 8:

Rocha LA, Rocha CR, Baldwin CC, Weigt LA, McField M (2015) Invasive lionfish preying on critically endangered reef fish. Coral Reefs 34:803–806

Rognes T, Flouri T, Nichols B, Quince C, Mahé F (2016) VSEARCH: a versatile open source tool for metagenomics. PeerJ 4:e2584

Rojas D, Chevalier Monteagudo P, Schmitter-Soto J, Corrada Wong R, Salvat Torres H, Cabrera Sansón E, García Rodríguez A, Fernández Osorio A, Espinosa Pantoja L, Cabrera Guerra

D, Pantoja Echevaria L, Caballero Aragón H, Perera Valderrama S (2016) Density, size, biomass, and diet of lionfish in Guanahacabibes National Park, western Cuba. Aquat Biol 24:219–226
Sancho G, Kingsley-Smith PR, Morris JA, Toline CA, McDonough V, Doty SM (2018) Invasive Lionfish (Pterois volitans/miles) feeding ecology in Biscayne National Park, Florida, USA. Biol Invasions 20:2343–2361
Sandel V, Martínez-Fernández D, Wangpraseurt D, Sierra L (2015) Ecology and management of the invasive lionfish Pterois volitans/miles complex (Perciformes: Scorpaenidae) in Southern Costa Rica. RBT 63:213
Santamaria CA, Locascio J, Greenan TM (2020) First report of lionfish prey from Western Florida waters as identified by DNA barcoding. PeerJ 8:e9922
Savidge JA (1987) Extinction of an Island Forest Avifauna by an Introduced Snake. Ecology 68:660–668
Schizas NV, Dahms H-U, Kangtia P, Corgosinho PHC, Galindo Estronza AM (2015) A new species of Longipedia Claus, 1863 (Copepoda: Harpacticoida: Longipediidae) from Caribbean mesophotic reefs with remarks on the phylogenetic affinities of Polyarthra. Marine Biology Research 11:789–803
Schofield P (2009) Geographic extent and chronology of the invasion of non-native lionfish (Pterois volitans [Linnaeus 1758] and P. miles [Bennett 1828]) in the Western North Atlantic and Caribbean Sea. AI 4:473–479
Shearer TL, van Oppen MJH, Romano SL, Wörheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Molecular Ecology 11:2475–2487
Shepherd B, Pinheiro HT, Phelps TAY, Easton EE, Pérez-Matus A, Rocha LA (2020) A New Species of Chromis (Teleostei: Pomacentridae) from Mesophotic Coral Ecosystems of Rapa Nui (Easter Island) and Salas y Gómez, Chile. Copeia 108:326
Smith-Vaniz WF, Tornabene L, Macieira RM (2018) Review of Brazilian jawfishes of the genus Opistognathus with descriptions of two new species (Teleostei, Opistognathidae). Zookeys 95–133
Soares MO, Pereira PHC, Feitosa CV, Maggioni R, Rocha RS, Bezerra LEA, Duarte OS, Paiva SV, Noleto-Filho E, Silva MQM, Csapo-Thomaz M, Garcia TM, Arruda Júnior JPV, Cottens KF, Vinicius B, Araújo R, Eirado CB do, Santos LPS, Guimarães TCS, Targino CH, Amorim-Reis Filho J, Santos WCR dos, Klautau AGC de M, Gurjão LM de, Machado DAN, Maia RC, Santos ES, Sabry R, Asp N, Carneiro PBM, Rabelo EF, Tavares TCL, Lima GV de, Sampaio CLS, Rocha LA, Ferreira CEL, Giarrizzo T (2023) Lessons from the invasion front: Integration of research and management of the lionfish invasion in Brazil. Journal of Environmental Management 340:117954
de Sousa LL, Silva SM, Xavier R (2019) DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. Environmental DNA 1:199–214
South J, Dick JTA, McCard M, Barrios-O'Neill D, Anton A (2017) Predicting predatory impact of juvenile invasive lionfish (Pterois volitans) on a crustacean prey using functional response analysis: effects of temperature, habitat complexity and light regimes. Environ Biol Fish 100:1155–1165
Steell SC, Van Leeuwen TE, Brownscombe JW, Cooke SJ, Eliason EJ (2019) An appetite for invasion: digestive physiology, thermal performance and food intake in lionfish (Pterois spp.). Journal of Experimental Biology 222:jeb209437
Tennekes M (2023) treemap: Treemap Visualization.

Tornabene L, Baldwin C, Weigt L, Pezold F (2010) Exploring the diversity of western Atlantic Bathygobius (Teleostei: Gobiidae) with cytochrome c oxidase-I, with descriptions of two new species. Aqua 16:141–170

Tornabene L, Baldwin CC (2017) A new mesophotic goby, Palatogobius incendius (Teleostei: Gobiidae), and the first record of invasive lionfish preying on undescribed biodiversity. PLOS ONE 12:e0177179

Tornabene L, Van Tassell JL, Robertson DR, Baldwin CC (2016) Repeated invasions into the twilight zone: evolutionary origins of a novel assemblage of fishes from deep Caribbean reefs. Molecular Ecology 25:3662–3682

Villaseñor-Derbez JC, Herrera-Perez R (2014) Brief description of prey selectivity and ontogenetic changes in the diet of the invasive lionfish Pterois volitans (Actinopterygii, Scorpaenidae) in the Mexican Caribbean. Pan-American Journal of Aquatic Sciences 9:131–135

Vinson MR, Angradi TR (2011) Stomach Emptiness in Fishes: Sources of Variation and Study Design Implications. Reviews in Fisheries Science 19:63–73

Weigt LA, Baldwin CC, Driskell A, Smith DG, Ormos A, Reyier EA (2012) Using DNA Barcoding to Assess Caribbean Reef Fish Biodiversity: Expanding Taxonomic and Geographic Coverage. PLOS ONE 7:e41059

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis.

Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H (2019) Welcome to the {tidyverse}. Journal of Open Source Software 4(43):1686

Wickham H, François R, Henry L, Müller K, Vaughan D (2023) dplyr: A Grammar of Data Manipulation.

Wood SN (2011) Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models. Journal of the Royal Statistical Society Series B: Statistical Methodology 73:3–36

Supplementary Material

Supplementary Material 1. Summary of previously published lionfish diet and stable isotopes studies from the western Atlantic in chronological order. Sometimes the exact number of species detected or depth of collection was not reported in text and was inferred based on figures. Studies that collected fish from below 40 meters are in bold. *Maximum depth not reported by authors but inferred based on method of collection or figures.

# of Samples	# of Prey Teleost Species Detected	Method of Analysis	Maximum Sampled Depth	Study Region	Reference
52	14	Visual ID	10 m	Bahamas	(Albins & Hixon, 2008)
1069	41	Visual ID	30 m	Bahamas	(Morris & Akins, 2009)
183	**24**	**Visual ID / Stable Isotope**	**45 m**	**North Carolina, USA**	**(Munoz et al. 2011)**
70	5 Families	Visual ID	Unknown	Bonaire	(McCleery 2011)
567	42	Visual ID	20 m*	Bahamas	(Green et al. 2012a)
122	10	Stable Isotopes / Visual ID	2.4 m	Abaco Island, Bahamas	(Layman and Allgeier 2012)
157	34	Barcoding	Not Reported	Eastern Yucatan Peninsula	(Valdez-Moreno et al. 2012)
130	37	Barcoding	20 m	Bahamas	(Cote et al. 2013)
934	30	Visual ID	35 m	Northern Gulf of Mexico	(Dahl and Peterson III 2014)
54	N/A	Stable Isotopes	12 m	Bahamas	(O'Farrell et al. 2014)
637	33	Visual ID / Barcoding	20 m	Bahamas	(Green and Côté 2014)
44	16	Visual ID / Barcoding	40 m*	Belize	(Rocha et al. 2015)
63	39	Metabarcoding	30 m	Puerto Rico	(Harms-Tuohy et al. 2016)
962	47	Visual ID	40 m*	Eastern Yucatan Peninsula	(Arredondo-Chávez et al. 2016)
411	35	Visual ID	25 m	Cuba	(Rojas et al. 2016)
1023	22 Order /Families	Visual ID	30 m	Belize	(Mizrahi et al. 2016)
1352	**44**	**Visual ID**	**60 m**	**Bermuda**	**(Eddy et al. 2016)**
392	**14 Families**	**Visual ID**	**72 m**	**Utila**	**(Andradi-Brown et al. 2017)**
899	30 Families	Visual ID	Not Reported	Cuba	(Echevarría et al. 2017)
934	41	Barcoding	35 m	Northern Gulf of Mexico	(Dahl et al. 2017)
311	Presence / Absence of teleost/ inverts	Stable Isotopes / Visual ID	25 m	Florida, USA	(Curtis et al. 2017)
513	**46**	**Visual ID/ Barcoding**	**50 m**	**Florida, USA**	**(Sancho et al. 2018)**
343	23 Genera	Visual ID	Not reported	Cozumel	(Bogdanoff et al. 2018)
229	23	Visual ID	40 m*	Florida, USA	(Jasper et al. 2018)
50	21	Barcoding	30 m	Florida, USA	(Santamaria et al. 2020)
13	N/A	Stable Isotopes	25 m	Florida, USA	(Curtis et al. 2020)
218	**N/A**	**Stable Isotopes**	**60 m***	**Bermuda**	**(Eddy et al. 2020)**
17	**2 genera, 1 species**	**Visual ID**	**71 m**	**Veracruz, Mexico**	**(Aguilar-Medrano and Vega-Cendejas 2020)**
523	23	Visual ID	30 m	Cuba	(Río et al. 2022)
76	**N/A**	**Stable Isotopes**	**179 m**	**Curacao**	**(Ewing et al. In Press)**
136	**51**	**Metabarcoding**	**189 m**	**Curacao**	**This study**

Supplementary Material 2. Summary of items visually identified from gut contents including the number of individuals found, the number of stomachs in which the species was found, and depth or range of depths from which the stomachs were collected.

Family	Species or Item	Number of Individuals	Number of stomachs (depth range)
Apogonidae	Apogonidae sp.	3	3 (22 – 89 m)
Apogonidae	Phaeoptyx sp.	2	2 (19 – 31 m)
Chaetodonidae	*Prognathodes guyanensis*	1	1 (109 m)
Chaetodonitdae	Chaetodontidae sp.	1	1 (115 m)
Epidonidae	*Sphyraenops bairdianus*	3	1 (157 m)
Gobiidae	*Coryphopterus personatus/ hyalinus/ sp.*	13	10 (19 – 159 m)
Gobiidae	Gobiidae sp.	1	1 (106 m)
Grammatidae	*Gramma loreto*	3	3 (21 – 37 m)
Holocentridae	Holocentridae sp.	4	4 (9 – 23 m)
Holocentridae	*Sargocentron*	1	1 (178 m)
Pomacentridae	*Azurina multilineata*	1	1 (22 m)
Pomacentridae	Pomacentridae sp.	4	4 (15 – 106 m)
Pomacentridae	*Stegastes partitus*	3	2 (18 – 18 m)
Pomacentridae	Stegastes sp.	1	1 (16 m)
Priacanthidae	Priacanthidae sp.	1	1 (20 m)
Scorpeanidae	Scorpaenidae sp.	1	1 (178)
Serranidae	Serranidae sp.	2	2 (109 – 122 m)
	Crab	1	1 (109 m)
	Fish	94	54 (7 – 189 m)
	Invertebrate	1	1 (106 m)
	Octopus	2	1 (109 m)
	Shrimp	110	39 (7 – 189 m)

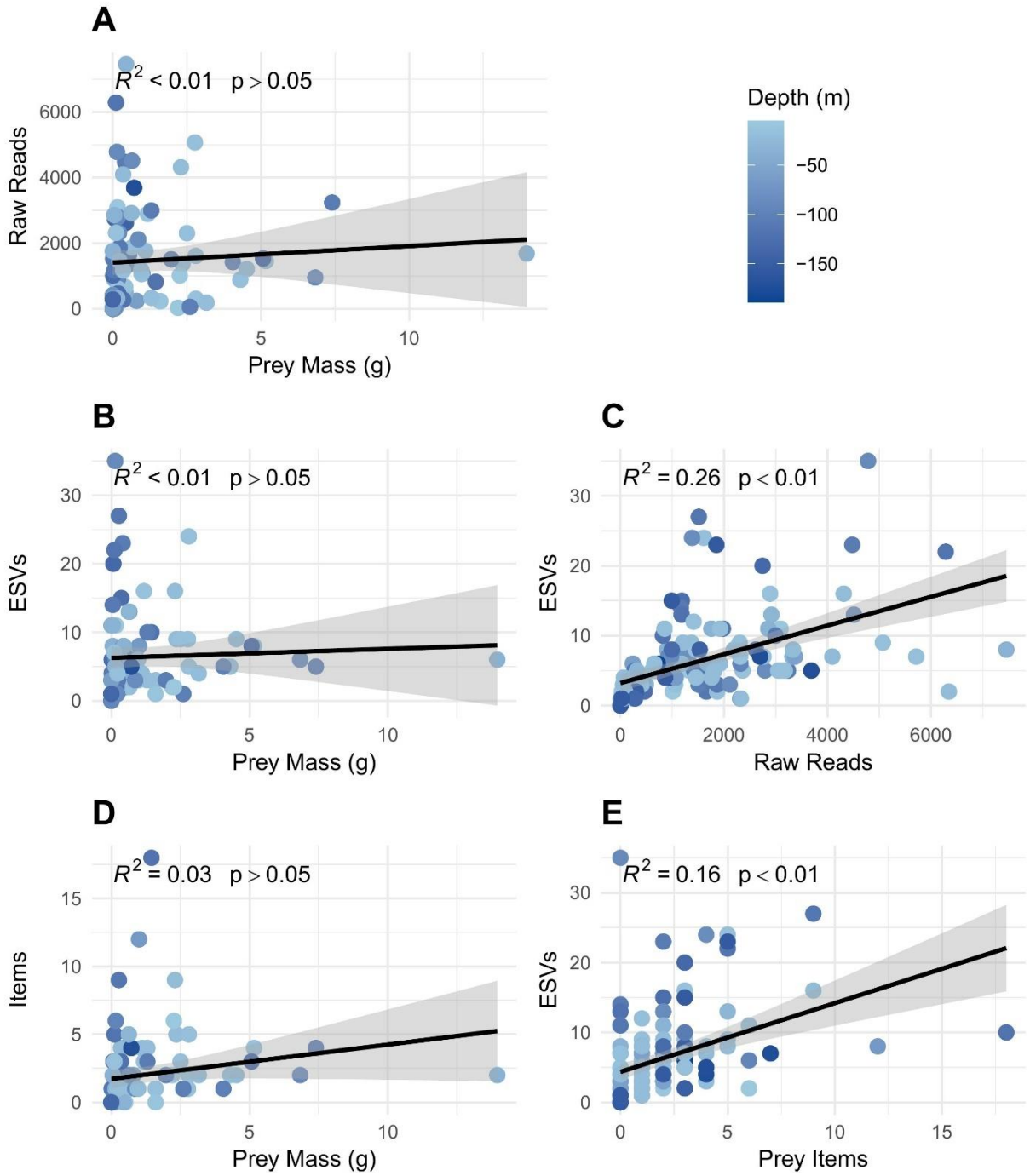
Supplementary Material 3. Teleost families identified using gut content metabarcoding and frequency of occurrence in all lionfish, lionfish collected 40 m and above, and lionfish collected below 40 m. A visual representation of taxa frequency of occurrence can be seen in figs 6 - 9. Families that are either recorded for the first time or contain species that are recorded for the first time from lionfish stomach contents are indicated with an asterisk (*). See novel species in supplementary material 6.

Family	**All**	**> 40 m**	**≤ 40 m**
Pomacentridae	27.21	12.68	43.08
Apogonidae	19.85	14.08	26.15
Gobiidae *	18.38	14.08	23.08
Scorpaenidae	17.65	18.31	16.92
Serranidae *	11.76	18.31	4.62
Labridae	7.35	2.82	12.31
Grammatidae	5.15	2.82	7.69
Holocentridae *	5.15	4.23	6.15
Priacanthidae	1.47	0.00	3.08
Acropomatidae	0.74	1.41	0.00
Blennidae	0.74	0.00	1.54
Chaenopsidae	0.74	1.41	0.00
Chaetodontidae	0.74	1.41	0.00
Epigonidae *	0.74	1.41	0.00
Paralichthyidae	0.74	1.41	0.00
Symphysanodontidae *	0.74	1.41	0.00
Synodontidae	0.74	1.41	0.00
Tripterygiidae	0.74	0.00	1.54

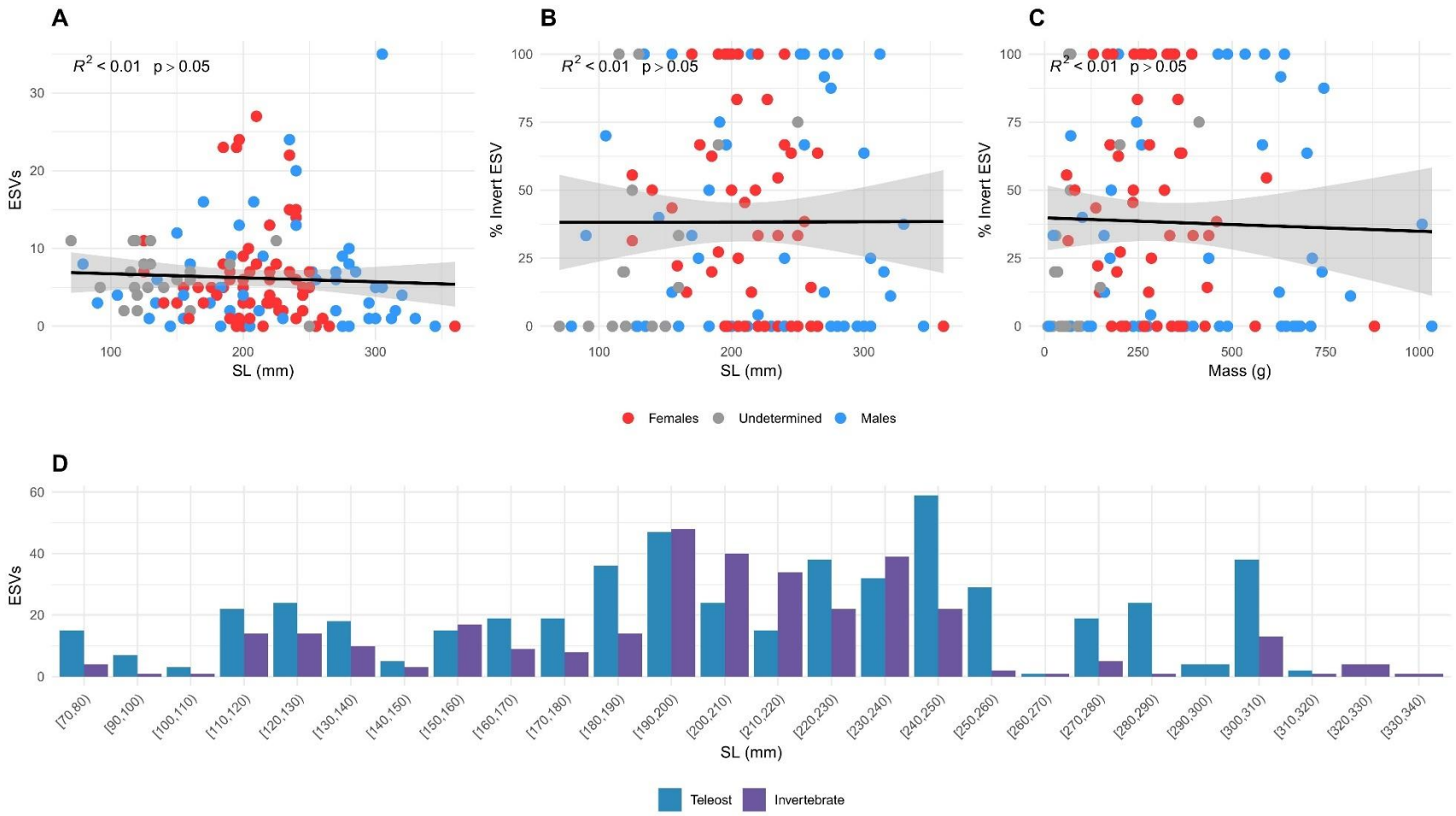
Supplementary Material 4. Summary of taxa identified using gut content metabarcoding with > 98% sequence similarity to a vouchered specimen and frequency of occurrence in all lionfish, lionfish collected 40 m and above, and lionfish collected below 40 m. Taxa recorded for the first time in lionfish guts are indicated with an asterisk (*).

				Frequency of Occurrence		
Phylum	**Order**	**Family**	**Species**	**All**	**> 40 m**	**≤ 40 m**
Annelida	Eunicida	Eunicidae	*Marphysa madrasi*	1.47	0.00	3.08
Annelida	Spionidae	Spionidae	*Spionidae sp.*	0.74	0.00	1.54
Arthropoda	Calanoida	Candaciidae	*Candacia sp.*	2.21	1.41	3.08
Arthropoda	Calanoida	Candaciidae	*Candacia curta*	0.74	0.00	1.54
Arthropoda	Decapoda	Calappidae	*Cryptosoma sp.*	0.74	1.41	0.00
Arthropoda	Decapoda	Hippolytidae	*Lysmata grabhami*	0.74	1.41	0.00
Arthropoda	Decapoda	Munididae	*Munida sp.*	1.47	2.82	0.00
Arthropoda	Decapoda	Palaemonidae	*Palaemonidae*	0.74	0.00	1.54
Arthropoda	Decapoda	Penaeidae	*Metapenaeopsis sp.*	12.50	5.63	20.00
Arthropoda	Decapoda	Penaeidae	*Metapenaeopsis gerardoi*	1.47	1.41	1.54
Arthropoda	Decapoda	Penaeidae	*Penaeus brasiliensis*	0.74	1.41	0.00
Arthropoda	Decapoda	Portunidae	*Achelous depressifrons*	0.74	0.00	1.54
Arthropoda	Decapoda	Portunidae	*Achelous sebae*	0.74	0.00	1.54
Arthropoda	Decapoda	Portunidae	*Portunidae sp.*	0.74	1.41	0.00
Arthropoda	Decapoda	Rhynchocinetidae	*Cinetorhynchus sp.*	4.41	0.00	9.23
Arthropoda	Stomatopoda	Gonodactyloidea	*Gonodactyloidea sp.*	0.74	0.00	1.54
Chordata	Aulopiformes	Synodontidae	*Synodus synodus*	0.74	1.41	0.00
Chordata	Blenniiformes	Blennidae	*Parablennius marmoreus*	0.74	0.00	1.54
Chordata	Blenniiformes	Tripterygiidae	*Enneanectes sp.*	0.74	0.00	1.54
Chordata	Gobiiformes	Gobiidae	*Antilligobius nikkiae* *	0.74	1.41	0.00
Chordata	Gobiiformes	Gobiidae	*Bollmannia sp.**	0.74	1.41	0.00
Chordata	Gobiiformes	Gobiidae	*Coryphopterus eidolon*	3.68	0.00	7.69
Chordata	Gobiiformes	Gobiidae	*Coryphopterus hyalinus / personatus*	10.29	2.82	18.46
Chordata	Gobiiformes	Gobiidae	*Coryphopterus lipernes*	0.74	0.00	1.54
Chordata	Gobiiformes	Gobiidae	*Coryphopterus thrix*	2.21	1.41	3.08
Chordata	Gobiiformes	Gobiidae	*Coryphopterus venezuelae*	2.94	0.00	6.15
Chordata	Gobiiformes	Gobiidae	*Gnatholepis thompsoni*	0.74	0.00	1.54
Chordata	Gobiiformes	Gobiidae	*Lythrypnus sp.*	0.74	0.00	1.54
Chordata	Gobiiformes	Gobiidae	*Palatogobius incendius* *	2.21	4.23	0.00
Chordata	Holocentriformes	Holocentridae	*Holocentrus adscensionis*	0.74	0.00	1.54
Chordata	Holocentriformes	Holocentridae	*Neoniphon marianus*	0.74	1.41	0.00
Chordata	Holocentriformes	Holocentridae	*Ostichthys trachypoma* *	1.47	2.82	0.00
Chordata	Kurtiformes	Apogonidae	*Apogon aurolineatus*	1.47	2.82	0.00
Chordata	Kurtiformes	Apogonidae	*Apogon binotatus*	0.74	1.41	0.00
Chordata	Kurtiformes	Apogonidae	*Apogon lachneri*	3.68	2.82	4.62
Chordata	Kurtiformes	Apogonidae	*Apogon maculatus*	0.74	0.00	1.54
Chordata	Kurtiformes	Apogonidae	*Apogon phenax*	2.21	0.00	4.62
Chordata	Kurtiformes	Apogonidae	*Apogon pillionatus*	2.94	2.82	3.08

Chordata	Kurtiformes	Apogonidae	*Apogon pseudomaculatus*	0.74	1.41	0.00
Chordata	Kurtiformes	Apogonidae	*Apogon townsendi*	2.21	0.00	4.62
Chordata	Kurtiformes	Apogonidae	*Paroncheilus affinis*	1.47	2.82	0.00
Chordata	Kurtiformes	Apogonidae	*Phaeoptyx conklini*	8.09	0.00	16.92
Chordata	Kurtiformes	Apogonidae	*Phaeoptyx pigmentaria*	1.47	0.00	3.08
Chordata	Labriformes	Labridae	*Clepticus parrae*	2.94	1.41	4.62
Chordata	Labriformes	Labridae	*Halichoeres garnoti*	0.74	0.00	1.54
Chordata	Labriformes	Labridae	*Scarus taeniopterus*	0.74	0.00	1.54
Chordata	Labriformes	Labridae	*Sparisoma viride*	2.21	0.00	4.62
Chordata	Labriformes	Labridae	*Thalassoma bifasciatum*	0.74	0.00	1.54
Chordata	Ovalentaria	Grammatidae	*Gramma loreto*	3.68	0.00	7.69
Chordata	Ovalentaria	Grammatidae	*Liprogramma klayi* *	1.47	2.82	0.00
Chordata	Ovalentaria	Pomacentridae	*Azurina cyanea*	0.74	0.00	1.54
Chordata	Ovalentaria	Pomacentridae	*Azurina multilineata*	14.71	2.82	27.69
Chordata	Ovalentaria	Pomacentridae	*Chromis insolata*	2.21	4.23	0.00
Chordata	Ovalentaria	Pomacentridae	*Chromis scotti*	0.74	1.41	0.00
Chordata	Ovalentaria	Pomacentridae	*Stegastes partitus*	11.03	5.63	16.92
Chordata	Ovalentaria	Pomacentridae	*Stegastes planifrons*	0.74	0.00	1.54
Chordata	Pempheriformes	Epigonidae	*Sphyraenops bairdianus* *	0.74	1.41	0.00
Chordata	Pempheriformes	Symphysanodontidae	*Symphysanodon berryi* *	0.74	1.41	0.00
Chordata	Perciformes	Chaetodontidae	*Prognathodes aculeatus*	0.74	1.41	0.00
Chordata	Perciformes	Serranidae	*Baldwinella cf. vivanus* *	1.47	2.82	0.00
Chordata	Perciformes	Serranidae	*Bullisichthys caribbaeus* *	2.21	4.23	0.00
Chordata	Perciformes	Serranidae	*Cephalopholis cruentata*	1.47	0.00	3.08
Chordata	Perciformes	Serranidae	*Cephalopholis fulva* *	1.47	1.41	1.54
Chordata	Perciformes	Serranidae	*Liopropoma rubre*	0.74	0.00	1.54
Chordata	Perciformes	Serranidae	*Pronotogrammus martinicensis*	5.15	9.86	0.00
Chordata	Perciformes	Serranidae	*Serranus tigrinus*	0.74	0.00	1.54
Chordata	Perciformes	Serranidae	*Serranus tortugarum*	0.74	1.41	0.00
Chordata	Pleuronectiformes	Paralichthyidae	*Citharichthyes cornutus* *	0.74	1.41	0.00
Chordata	Pleuronectiformes	Paralichthyidae	*Paralichthyidae sp.*	0.74	1.41	0.00
Chordata	Priacanthiformes	Priacanthidae	*Heteropriacanthus cruentatus*	1.47	0.00	3.08
Mollusca	Octopoda	Octopoda	*Octopoda sp.*	0.74	14.10	0.00



Supplementary Material 5. Number of raw reads (A), ESVs (B) and identifiable prey items (D) in stomachs plotted against prey mass (g) where prey mass is the mass of all stomach contents regardless of state of digestion excluding the mass of the stomach. (C) ESVs plotted against raw reads. (E) ESVs plotted against the number of identifiable prey items in a stomach. An outlier point on plot E represents a lionfish with a stomach full of heavily digested material from which no single prey item could be identified but over 30 ESVs were recovered from the digested material.



Supplementary Material 6. Total unique ESVs plotted against SL of the lionfish (A) and percent invertebrate ESVs in the stomach plotted against SL of the lionfish (B) and the mass of the lionfish (C). (D) Number of unique teleost and invertebrate ESVs in lionfish stomachs grouped into 10 mm SL bins.

Supplementary Material 7. A list of 229 taxa reported from 50 families identified in lionfish stomachs from the western Atlantic. Prey items identified using visual identification methods, DNA barcoding, and DNA metabarcoding. Families or genera without species indicates that the prey item was only identified to that taxonomic level. Taxa marked by an asterisk (*) were also reported in this study. Taxa marked by two asterisks (**) are novel prey species reported by this study.

Family	Species	Source(s)
Acanthuridae	*Acanthurus bahianus*	(Morris and Akins 2009) (Sandel et al. 2015) (Eddy et al. 2016) (Peake et al. 2018)
	Acanthurus chirurgus	(Layman and Allgeier 2012) (Eddy et al. 2016) (Peake et al. 2018)
	Acanthurus coeruleus	(Sandel et al. 2015) (Peake et al. 2018)
	Acanthurus tractus	(Harms-Tuohy et al. 2016)
Acropomatidae	*Verilus* **	This study
Antennariidae	*Fowlerichthys radiosus*	(Dahl et al. 2017)
Apogonidae	*Apogon aurolineatus* *	(Albins and Hixon 2008) (Santamaria et al. 2020)
	Apogon binotatus *	(Morris and Akins 2009) (Cote et al. 2013) (Rocha et al. 2015) (Eddy et al. 2016) (Dahl et al. 2017) (Peake et al. 2018) (Río et al. 2022)
	Apogon lachneri *	(Valdez-Moreno et al. 2012)
	Apogon maculatus *	(Valdez-Moreno et al. 2012) (Sandel et al. 2015) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
	Apogon mosavi	(Valdez-Moreno et al. 2012)
	Apogon phenax *	(Cote et al. 2013)
	Apogon pillionatus *	(Harms-Tuohy et al. 2016)
	Apogon planifrons	(Green et al. 2012a) (Arredondo-Chávez et al. 2016)
	Apogon pseudomaculatus *	(Munoz et al. 2011) (Dahl and Peterson III 2014) (Eddy et al. 2016) (Dahl et al. 2017) (Peake et al. 2018)
	Apogon townsendi *	(Morris and Akins 2009) (Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Eddy et al. 2016) (Peake et al. 2018)
	Astrapogon puncticulatus	(Valdez-Moreno et al. 2012)
	Paroncheilus affinis *	(Peake et al. 2018)[a] [a] Reported as *Apogon affinis*
	Phaeoptyx conklini *	(Harms-Tuohy et al. 2016)
	Phaeoptyx pigmentaria *	(Green et al. 2012a) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Dahl et al. 2017) (Peake et al. 2018)
	Zapogon evermanni	(Río et al. 2022)
Atherinidae	*Atherinomorus stipes*	(Peake et al. 2018)
Aulostomidae	*Aulostomus maculatus*	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Arredondo-Chávez et al. 2016)

		(Eddy et al. 2016) (Rojas et al. 2016) (Jasper et al. 2018) (Peake et al. 2018) (Río et al. 2022)
Balistidae	*Balistes vetula*	(Rojas et al. 2016)
	Canthidermis sufflamen	(Peake et al. 2018)
Blennidae	*Ophioblennius macclurei*	(Peake et al. 2018)
	Entomacrodus nigricans	(Eddy et al. 2016) (Peake et al. 2018)
	Hypleurochilus geminatus	(Munoz et al. 2011) (Peake et al. 2018)
	Ophioblennius atlanticus	(Villaseñor-Derbez and Herrera-Perez 2014) (Sandel et al. 2015) (Peake et al. 2018)
	Parablennius marmoreus *	(Rocha et al. 2015) (Eddy et al. 2016) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
Bothidae	*Bothus lunatus*	(Valdez-Moreno et al. 2012) (Arredondo-Chávez et al. 2016)
	Bothus ocellatus	(Cote et al. 2013)
	Bothus robinsi	(Dahl et al. 2017)
Callionymidae	*Diplogrammus pauciradiatus*	(Santamaria et al. 2020)
Carangidae	*Alectis ciliaris*	(Arredondo-Chávez et al. 2016)
	Decapterus punctatus	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
	Selar crumenophthalmus	(Munoz et al. 2011) (Peake et al. 2018)
	Trachurus lathami	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
Chaenopsidae	*Acanthemblemaria aspera*	(Green et al. 2012a) (Cote et al. 2013)
	Acanthemblemaria maria	(Arredondo-Chávez et al. 2016)
	Emblemaria pandionis	(Peake et al. 2018)
	Emblemariopsis arawak	(Harms-Tuohy et al. 2016)
	Lucayablennius zingaro	(Morris and Akins 2009) (Green et al. 2012a) (Rojas et al. 2016) (Río et al. 2022)
Chaetodontidae	*Chaetodon capistratus*	(Harms-Tuohy et al. 2016) (Eddy et al. 2016) (Peake et al. 2018)
	Chaetodon ocellatus	(Eddy et al. 2016) (Peake et al. 2018)
	Prognathodes aculeatus *	(Peake et al. 2018)[b] [b] Reported as *Chaetodon aculeatus*
	Syacium gunteri	(Aguilar-Medrano and Vega-Cendejas 2020)
	Syacium papillosum	(Dahl et al. 2017)
Chlorophthalmidae		(Dahl et al. 2017)
Cirrhitidae	*Amblycirrhitus pinos*	(Morris and Akins 2009) (Rojas et al. 2016) (Peake et al. 2018)
Clupeidae	*Jenkinsia lamprotaenia*	(Eddy et al. 2016)
Cyclopsettidae	*Citharichthys cornutus* **	This study
	Cyclopsetta fimbriata	(Dahl et al. 2017)
	Syacium papillosum	(Dahl et al. 2017)
Dactylosopidae	*Gillellus greyae*	(Arredondo-Chávez et al. 2016)
Dussumieriidae	*Etrumeus teres*	(Dahl et al. 2017)
Engralidae	*Anchoviella*	(Arredondo-Chávez et al. 2016)
Epigonidae	*Sphyraenops bairdianus* **	This study
Gerreidae		(Peake et al. 2018)
Gobiidae	*Antilligobius nikkiae* **	This study

	Bathygobius soporator	(Arredondo-Chávez et al. 2016)
	Bollmannia **	This study
	Coryphopterus dicrus	(Morris and Akins 2009) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Coryphopterus eidolon *	(Morris and Akins 2009) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Peake et al. 2018)
	Coryphopterus glaucofraenum	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Rocha et al. 2015) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Coryphopterus lipernes	(Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016)
	Coryphopterus personatus / hyalinus *	(Morris and Akins 2009) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Rocha et al. 2015) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Coryphopterus thrix *	(Valdez-Moreno et al. 2012) (Peake et al. 2018)
	Coryphopterus tortugae	(Valdez-Moreno et al. 2012) (Harms-Tuohy et al. 2016)
	Coryphopterus venezuelae *	(Valdez-Moreno et al. 2012) (Green et al. 2012a)[c] (Cote et al. 2013)[c] [c] Reported as *C. bol*
	Elacatinus oceanops	(Peake et al. 2018)
	Elacatinus prochilos	(Villaseñor-Derbez and Herrera-Perez 2014)[d] (Peake et al. 2018)[d] [d] Reported as *Gobiosoma prochilos*
	Elacatinus xanthiprora	(Jasper et al. 2018)[e] [e] Reported as *Gobiosoma xanthiprora*
	Gnatholepis thompsoni *	(Green et al. 2012a) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Eddy et al. 2016) (Jasper et al. 2018)
	Gobiosoma grosvenori	(Sandel et al. 2015) (Peake et al. 2018)
	Gobiosoma robustum	(Jasper et al. 2018)
	Lythrypnus minimus	(Valdez-Moreno et al. 2012)
	Lythrypnus spilus	(Green et al. 2012a) (Cote et al. 2013)
	Microgobius carri	(Dahl et al. 2017)
	New Genus **	This study (genus reported by Tornabene et al. 2016)
	Oxyurichthys stigmalophius	(Jasper et al. 2018)
	Palatogobius incendius **	This study
	Priolepis hipoliti	(Morris and Akins 2009) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Rojas et al. 2016) (Peake et al. 2018)
Grammatidae	*Gramma loreto* *	(Albins and Hixon 2008) (Morris and Akins 2009) (Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) Villaseñor-Derbez and Herrera-Perez 2014) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018) (Río et al. 2022)

	Gramma melacara	(Morris and Akins 2009) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Lipogramma klayi **	This study
Haemulidae	*Anisotremus surinamensis*	(Sandel et al. 2015) (Peake et al. 2018)
	Haemulon aurolineatum	(Munoz et al. 2011) (Dahl and Peterson III 2014) (Eddy et al. 2016) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
	Haemulon chrysargyreum	(Peake et al. 2018)
	Haemulon flavolineatum	(Valdez-Moreno et al. 2012) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Peake et al. 2018)
	Haemulon plumierii	(Albins and Hixon 2008) (Peake et al. 2018)
	Haemulon striatum	(Jasper et al. 2018)
	Haemulon vittata	(Green et al. 2012a)[f] (Cote et al. 2013) [f] Reported as *Inermia vittata*
Holocentridae	*Holocentrus adscensionis* *	(Eddy et al. 2016) (Peake et al. 2018)
	Holocentrus rufus	(Cote et al. 2013) (Sandel et al. 2015) (Peake et al. 2018)
	Myripristis jacobus	(Rojas et al. 2016)
	Neoniphon marianus *	(Rojas et al. 2016) (Río et al. 2022)
	Ostichthys trachypoma **	This study
	Sargocentron coruscum	(Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) (Eddy et al. 2016) (Peake et al. 2018)
	Sargocentron vexillarium	(Morris and Akins 2009) (Eddy et al. 2016) (Jasper et al. 2018) (Peake et al. 2018)
Labridae	*Bodianus rufus*	(Morris and Akins 2009) (Green et al. 2012a) (Layman and Allgeier 2012) (Harms-Tuohy et al. 2016) (Peake et al. 2018)
	Clepticus parrae *	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Cryptotomus roseus	(Villaseñor-Derbez and Herrera-Perez 2014) (Peake et al. 2018)
	Decodon **	This study
	Halichoeres bathyphilus	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
	Halichoeres bivittatus	(Albins and Hixon 2008) (Morris and Akins 2009) (Green et al. 2012a) (Layman and Allgeier 2012) (Cote et al. 2013) (Dahl and Peterson III 2014) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Dahl et al. 2017) (Jasper et al. 2018) (Peake et al. 2018) (Santamaria et al. 2020) (Río et al. 2022)
	Halichoeres garnoti *	(Albins and Hixon 2008) (Morris and Akins 2009) (Layman and Allgeier 2012) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Jasper et al. 2018) (Peake et al. 2018) (Río et al. 2022)

	Halichoeres maculipinna	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Eddy et al. 2016) (Jasper et al. 2018) (Peake et al. 2018)
	Halichoeres pictus	(Morris and Akins 2009) (Peake et al. 2018)
	Halichoeres radiatus	(Peake et al. 2018)
	Halichoeres socialis	(Rocha et al. 2015)
	Nicholsina usta	(Arredondo-Chávez et al. 2016) (Peake et al. 2018) (Río et al. 2022)
	Scarus iseri	(Albins and Hixon 2008) (Morris and Akins 2009) (Valdez-Moreno et al. 2012) (Rocha et al. 2015) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Scarus taeniopterus *	(Valdez-Moreno et al. 2012) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Peake et al. 2018) (Río et al. 2022)
	Scarus vetula	(Harms-Tuohy et al. 2016) (Peake et al. 2018)
	Sparisoma atomarium	(Rocha et al. 2015) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Sparisoma aurofrenatum	(Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) (Rocha et al. 2015) (Eddy et al. 2016) (Peake et al. 2018) (Río et al. 2022)
	Sparisoma radians	(Munoz et al. 2011) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Sparisoma viride *	(Morris and Akins 2009) (Rocha et al. 2015) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Thalassoma bifasciatum *	(Albins and Hixon 2008) (Morris and Akins 2009) (Layman and Allgeier 2012) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Villaseñor-Derbez and Herrera-Perez 2014) (Sandel et al. 2015) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Jasper et al. 2018) (Peake et al. 2018) (Río et al. 2022)
	Xyrichtys martinicensis	(Eddy et al. 2016) (Peake et al. 2018)
	Xyrichtys novacula	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
	Xyrichtys novacula	(Peake et al. 2018)
Labrisomidae	*Gobioclinus gobio*	(Arredondo-Chávez et al. 2016)
	Gobioclinus haitiensis	(Green et al. 2012a)[g] (Cote et al. 2013)[g] [g] Reported as *Labrisomus haitiensis*
	Malacoctenus boehlkei	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013)
	Malacoctenus gilli	(Albins and Hixon 2008)
	Malacoctenus macropus	(Layman and Allgeier 2012) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Malacoctenus triangulatus	(Morris and Akins, 2009) (Valdez-Moreno et al. 2012) (Villaseñor-Derbez and Herrera-Perez 2014) (Peake et al. 2018) (Arredondo-Chávez et al. 2016) (Río et al. 2022)
	Paraclinus fasciatus	(Peake et al. 2018)

	Starksia langi	(Valdez-Moreno et al. 2012)
	Starksia nanodea	(Arredondo-Chávez et al. 2016)
	Starksia occidentalis	(Rocha et al. 2015)
	Starksia ocellata	(Valdez-Moreno et al. 2012)
	Starksia williamsi	(Harms-Tuohy et al. 2016)
Lutjanidae	*Lutjanus campechanus*	(Dahl et al. 2017)
	Lutjanus synagris	(Peake et al. 2018)
	Ocyurus chrysurus	(Morris and Akins 2009) (Peake et al. 2018)
	Pristipomoides aquilonaris	(Dahl et al. 2017)
	Rhomboplites aurorubens	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
Microdesmidae	*Ptereleotris calliura*	(Dahl et al. 2017) (Santamaria et al. 2020)
Monacanthidae	*Alusterus heudelotii*	(Jasper et al. 2018)
	Aluterus schoepfii	(Eddy et al. 2016) (Peake et al. 2018)
	Aluterus scriptus	(Eddy et al. 2016)
	Cantherhines pullus	(Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Monacanthus ciliatus	(Munoz et al. 2011) (Rocha et al. 2015) (Eddy et al. 2016) (Peake et al. 2018) (Santamaria et al. 2020)
	Monacanthus tuckeri	(Morris and Akins 2009) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Stephanolepis hispidus	(Peake et al. 2018)
Mullidae	*Mulloidichthys martinicus*	(Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018) (Río et al. 2022)
	Pseudupeneus maculatus	(Morris and Akins 2009) (Green et al. 2012a) (Eddy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018) (Río et al. 2022)
Opistognathidae	*Opistognathus aurifrons*	(Peake et al. 2018)
	Opistognathus macrognathus	(Peake et al. 2018)
	Opistognathus robinsi	(Dahl et al. 2017)
Paralichthyidae	*Paralichthys albigutta*	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
Pempheridae	*Pempheris schomburgkii*	(Eddy et al. 2016) (Peake et al. 2018)
Pleuronectidae		(Dahl and Peterson III 2014)
Pomacathidae	*Holacanthus tricolor*	(Rojas et al. 2016) (Peake et al. 2018)
Pomacentridae	*Abudefduf saxatilis*	(Layman and Allgeier 2012) (Valdez-Moreno et al. 2012) (Sandel et al. 2015) (Peake et al. 2018)
	Azurina cyanea *	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018) (Río et al. 2022)
	Azurina multilineata *	(Morris and Akins 2009) (Green et al. 2012a) (Cote et al. 2013) (Villaseñor-Derbez and Herrera-Perez 2014)(Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)

	Chromis enchrysurus	(Munoz et al. 2011) (Dahl et al. 2017) (Dahl and Peterson III 2014) (Peake et al. 2018)
	Chromis flavicauda	(Eddy et al. 2016) (Peake et al. 2018)
	Chromis insolata *	(Morris and Akins 2009) (Eddy et al. 2016) (Peake et al. 2018)
	Chromis scotti *	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
	Dascyllus	(Jasper et al. 2018)
	Microspathodon chrysurus	(Sandel et al. 2015) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Stegastes adustus	(Sandel et al. 2015) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Stegastes fuscus	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
	Stegastes leucostictus	(Morris and Akins 2009) (Layman and Allgeier 2012) (Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Stegastes partitus *	(Albins and Hixon 2008) (Morris and Akins 2009) (Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) (Villaseñor-Derbez and Herrera-Perez 2014) (Harms-Tuohy et al. 2016) (Rojas et al. 2016) (Jasper et al. 2018) (Peake et al. 2018) (Río et al. 2022)
	Stegastes planifrons *	(Arredondo-Chávez et al. 2016) (Peake et al. 2018)
	Stegastes xanthurus	(Morris and Akins 2009)[h] (Green et al. 2012a)[h] (Cote et al. 2013)[h] (Rocha et al. 2015)[h] (Harms-Tuohy et al. 2016)[h] (Eddy et al. 2016)[h] (Dahl et al. 2017)[h] (Peake et al. 2018)[h] (Santamaria et al. 2020)[h] [h] Reported as *Stegastes variabilis*
Priacanthidae	*Heteropriacanthus cruentatus* *	(Harms-Tuohy et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
Sciaenidae	*Pareques acuminatus*	(Peake et al. 2018)
Scorpaenidae	*Pontinus* **	This study
	Scorpaena brasiliensis	(Dahl et al. 2017)
	Scorpaenodes caribbaeus	(Peake et al. 2018)
Serranidae	*Baldwinella c.f. vivanus* **	This study
	Baldwinella vivanus	(Dahl and Peterson III 2014)
	Bullisichthys caribbaeus **	This study
	Centropristis ocyurus	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
	Cephalopholis cruentata *	(Valdez-Moreno et al. 2012) (Green et al. 2012a) (Cote et al. 2013) (Arredondo-Chávez et al. 2016) (Rojas et al. 2016) (Peake et al. 2018)
	Cephalopholis fulva **	This study
	*Choranthias c.f. tenuis***	This study
	Diplectrum bivittatum	(Jasper et al. 2018) (Peake et al. 2018)
	Diplectrum formosum	(Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018) (Santamaria et al. 2020)
	Epinephelus adscensionis	(Peake et al. 2018)

	Epinephelus striatus	(Morris and Akins 2009) (Green et al. 2012a) (Peake et al. 2018)
	Hypoplectrus aberrans	(Harms-Tuohy et al. 2016)
	Hypoplectrus floridae	(Santamaria et al. 2020)
	Hypoplectrus nigricans	(Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016)
	Hypoplectrus puella	(Rocha et al. 2015) (Harms-Tuohy et al. 2016) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Peake et al. 2018)
	Hypoplectrus unicolor	(Jasper et al. 2018)
	Liopropoma carmabi	(Villaseñor-Derbez and Herrera-Perez 2014) (Peake et al. 2018)
	Liopropoma rubre *	(Morris and Akins 2009) (Green et al. 2012a) (Valdez-Moreno et al. 2012) (Cote et al. 2013) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Peake et al. 2018)
	Mycteroperca venenosa	(Villaseñor-Derbez and Herrera-Perez 2014) (Peake et al. 2018)
	Paralabrax dewegeri	(Arredondo-Chávez et al. 2016)
	Paranthias furcifer	(Eddy et al. 2016) (Peake et al. 2018)
	Parasphyraenops **	This study
	Pronotogrammus martinicensis *	(Dahl et al. 2017)
	Schultzea beta	(Munoz et al. 2011) (Peake et al. 2018)
	Serraniculus sp.	(Dahl et al. 2017)
	Serranus baldwini	(Peake et al. 2018)
	Serranus flaviventris	(Rocha et al. 2015)
	Serranus phoebe	(Munoz et al. 2011) (Peake et al. 2018)
	Serranus subligarius	(Munoz et al. 2011) (Dahl and Peterson III 2014) (Dahl et al. 2017) (Peake et al. 2018)
	Serranus tabacarius	(Green et al. 2012a)
	Serranus tigrinus *	(Morris and Akins 2009) (Green et al. 2012a) (Munoz et al. 2011) (Cote et al. 2013) (Arredondo-Chávez et al. 2016) (Jasper et al. 2018) (Peake et al. 2018)
	Serranus tortugarum **	This study
Sparidae	*Diplodus bermudensis* *Pagrus pagrus*	(Eddy et al. 2016) (Dahl et al. 2017)
Symphysanodontidae	*Symphysanodon berryi* **	This study
Syngnathidae	*Anarchopterus criniger*	(Peake et al. 2018)
Synodontidae	*Saurida brasiliensis*	(Dahl et al. 2017)
	Saurida normani	(Munoz et al. 2011)
	Synodus intermedius	(Green et al. 2012a) (Harms-Tuohy et al. 2016) (Peake et al. 2018) (Santamaria et al. 2020)
	Synodus macrostigmus	(Dahl et al. 2017)
	Synodus poeyi	(Dahl et al. 2017)
	Synodus saurus	(Green et al. 2012a) (Santamaria et al. 2020)
	Synodus synodus *	(Green et al. 2012a) (Cote et al. 2013) (Dahl and Peterson III 2014) (Arredondo-Chávez et al. 2016) (Eddy et al. 2016) (Rojas et al. 2016) (Dahl et al. 2017) (Peake et al. 2018)

Tetraodontidae	*Canthigaster rostrata*	(Morris and Akins 2009) (Villaseñor-Derbez and Herrera-Perez 2014) (Peake et al. 2018)
	Sphoeroidea spengleri	(Eddy et al. 2016)
Triglidae	*Bellator brachychir*	(Dahl and Peterson III 2014) (Peake et al. 2018)
	Bellator militaris	(Dahl et al. 2017)
	Prionotus carolinus	(Dahl and Peterson III 2014)
Tripterygiidae	*Enneanectes altivelis*	(Valdez-Moreno et al. 2012)
	Enneanectes boehlkei	(Valdez-Moreno et al. 2012)
	Enneanectes jordani	(Arredondo-Chávez et al. 2016)[i] [i] Reported as *Enneanectes pectoralis*
Uranoscopidae		(Dahl et al. 2017)