UNIVERSITY OF CALIFORNIA SAN DIEGO

Community Ecology of Fishes on Coral Reefs in the South and Central Pacific

A dissertation submitted in partial satisfaction of the requirements for the degree
Doctor of Philosophy

in

Marine Biology

by

Beverly Jeanne French

Committee in charge:

Professor Stuart Sandin, Chair
Professor Eric Allen
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The Dissertation of Beverly Jeanne French is approved, and it is acceptable in quality and form for publication on microfilm and electronically.

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DEDICATION

To those who inducted me into the wonder and mysteries of life, to those who have lit the path along the way with their wisdom or laughter, and to all those “excruciatingly insignificant” beings I love.

EPIGRAPH

My work is loving the world.
Here the sunflowers, there the hummingbird—
equal seekers of sweetness.
Here the quickening yeast; there the blue plums.
Here the clam deep in the speckled sand.

Are my boots old? Is my coat torn?
Am I no longer young, and still half-perfect? Let me
keep my mind on what matters,
which is my work,

which is mostly standing still and learning to be
astonished.

Mary Oliver, *Messenger*

There never was anything else. Only these excruciatingly insignificant creatures we love.

Ellen Bass, *The Big Picture*

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ACKNOWLEDGEMENTS

The work presented here is the product of many years of struggle, culminating at this point of "intersection of increasing quality with decreasing personal expectations." I include here a forewarning that this section will be long, because it is also the product of my intersection with many brilliant and generous minds over the years.

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ABSTRACT OF THE DISSERTATION

Community Ecology of Fishes on Coral Reefs in the South and Central Pacific

by

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Doctor of Philosophy in Marine Biology

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Professor Stuart Sandin, Chair

Coral reefs, and particularly the study of coral reef fishes, have provided fundamental contributions to our understanding of community ecology in part due to their spectacular diversity of life. This dissertation seeks to evaluate facets of the community ecology of fish communities at reefs in the south and central Pacific Ocean via a variety of classical and new approaches, including traditional visual surveys and new techniques in genetics. Collectively, the results of my dissertation address outstanding questions regarding the communities of fishes on coral reefs at islands and atolls in the south and central Pacific, including both well-studied inhabited islands with intimate associations between humans and environments and others from remote environments that have seen only sporadic human visitation and presence. I begin with an investigation of a potential stabilizing mechanism of coexistence through the application of metagenomics to assess dietary niche-partitioning in a guild of hawkfish from remote reefs in the central equatorial Pacific. We observe previously unidentified relationships in this cryptic species complex at its proposed center of distribution and separate clustering of species for both the microbial community of the gut and presumed prey sequences. I next calculate length–weight relationships for abundant coral reef fish species from eight different islands in French Polynesia. These region-specific biological parameters are important for assessing accurate metrics of biomass for coral reef fish communities from underwater visual surveys. I focus on members of the community that are often overlooked and understudied in such contexts, including species of importance for the aquarium trade. I assess temporal patterns in trophic groups of coral reef fishes via remote video surveys on forereefs of Moorea, French Polynesia. I conclude by investigating the "paradox of planktivores"—that is, the substantial number of co-occurring reef fishes that are presumably relying on similar resources. I use metagenomic sequencing and underwater visual surveys to assess the evidence for niche-partitioning and changes in observed

abundance through time that could indicate coexistence in a guild of closely related planktivorous reef fishes.

INTRODUCTION

The diversity of life on Earth is truly spectacular. One recent estimate of global species richness places the figure of total described species at approximately 1.5 million (Costello et al., 2012). Other relatively recent estimates vary by millions, with the highest up to one trillion (Larson et al., 2017). These figures often largely ignore what comprises by far the largest share of diversity on earth: prokaryotes (Whitman et al., 1998) and phages, for which calculations of the latter suggest that there are approximately 4.80×10^{31} (Cobián Güemes et al., 2016) consisting of an estimated 100 million different phage species (Rohwer, 2003). Of the vertebrates, fishes are the most diverse group (Hastings et al., 2015), with 36,294 valid species confirmed in Eschmeyer's Catalog of Fishes as of August 2022 and 591 new species described in the past two years alone (Eschmeyer et al., 2010). It is clear both that the total number of species is much higher than what has been described and that such estimates involve considerable uncertainty. The large ranges in estimates for the species diversity on planet Earth reflect the vast quantity of unknowns that remain in our catalogue of the life of our world's ecosystems.

Regardless of the precise quantification, this diversity poses a challenge to our traditional understanding of natural selection and the principle of competitive exclusion. Theory and early empirical work suggested that two species competing for the same limiting resource cannot be sustained at constant population values, as eventually, the superior competitor would be expected to drive the other to extinction (Gause, 1935; Hardin, 1960). In a presidential address at the annual meeting of the American Society of Naturalists in 1958, British ecologist G. Evelyn Hutchinson famously posed this as the following question: "Why are there not just a few 'super animals' that dominate each habitat (Hutchinson, 1959)?"

Questions relating to the coexistence and diversity of species are not only of interest academically but have also taken on special significance in the Anthropocene. Stemming the tide of biodiversity loss has been considered one of the defining environmental challenges of the 21st century (Hooper et al., 2012). It has been proposed that species are currently going extinct at exceptionally high rates and that these rates are increasing (Ceballos et al., 2015). Some in the scientific community have suggested that this indicates a sixth mass extinction event, unique among the previous five mass extinction events in the fossil record for being driven by human beings (Ceballos et al., 2015; Dirzo et al., 2014). Others conclude that the loss of species is higher than would be expected from the fossil record, but not yet in the category of a mass extinction (Barnosky et al., 2011). Still, the loss of abundance in individuals of many different species—which precedes extinction, as it begins with such loss—including among once-common species (Rosenberg et al., 2019), highlights the need for increased efforts of conservation and understanding of species dynamics in diverse communities.

The study of coral reef fishes in particular has provided fundamental contributions to our understanding of community ecology. Evaluations of presumably coexisting species in the diverse environments of coral reefs became major thrust of research on coral reefs in the 1970s (Sale, 1991) with the increased availability of diving aided by SCUBA, providing substantial natural historical information, while not resolving the question. It did, however, result in a proliferation of hypotheses for these "baubles on the tree of life" (*sensu Bellwood*) that persist together on marine tropical reefs, including the lottery models proposed by Sale (Sale, 1978, 1977) and extended by Chesson and Warner (Warner and Chesson, 1985) that have enriched and challenged our understanding of how ecological communities work. It is worth revisiting these

theories in a changing world, where the "rules of nature" may have changed (Sandin and Sala, 2012; Williams et al., 2015).

Modern coexistence theory is a theoretical framework for why we might not see competitive exclusion even among similar species (Adler et al., 2007; Chesson, 2000; HilleRisLambers et al., 2012). In Chesson's framework (2000), coexistence is a result of stabilizing mechanisms and fitness inequality. Niches—including resource partitioning of limiting resources and differences in environmental tolerance or predators—are considered stabilizing processes in modern coexistence theory because they result in increased intraspecific competition relative to interspecific competition. As such, species limit themselves more than they limit other species, and per capita growth declines as a species' relative abundance within a particular community increases (Adler et al., 2007).

The extent to which the community structure is driven by high levels of resource partitioning or by stochastic processes has been debated for ecological communities more generally (Hubbell, 2001; Velland, 2010), as well as for communities of coral reef fishes specifically. Coral reefs are particularly interesting ecosystems in which to ask these questions, as the high levels of diversity at many different trophic levels would suggest an abundance of opportunities for resource-partitioning. This is supported by studies which have found evidence for fine-scale niche-partitioning among closely-related species (Leray et al., 2019, 2015; McMahon et al., 2016). Still other work has shown remarkable dietary flexibility, high levels of trophic overlap, and the dominance of dietary generalists and omnivores on coral reefs (Bellwood et al., 2006; Frisch et al., 2016; Miller et al., 2019; Zgliczynski et al., 2019). The focus of the aforementioned studies was on dietary niche-partitioning, but species can also partition resources that differ in space (habitat) and time (temporal niche-partitioning). Indeed, in Hutchinson's view, species niches

can be categorized by an "n-dimensional hypervolume" in which the axes correspond to a species' requirements (food, environmental conditions), the boundaries of which delimit the conditions under which growth and reproduction of that species is permitted (Blonder et al., 2014). The question therefore remains: are stochastic processes or high levels of resource partitioning driving coexistence among the diverse communities of fishes on coral reefs?

Furthermore, until recently, many ecological studies of organisms have ignored their intimately associated microbial partners. The advances in "meta-'omics" techniques and decreased costs enable us to look beyond the individual, and more deeply into the "communities" of the organisms themselves, by interrogating their microbial consorts. Whether or not microbial organisms correspond to ecological theories developed via study of macroorganisms remains an open question. An additional challenge lies in considering metazoans as holobionts; that is, a host organism and the many, usually microbial, species that live within or closely associated with it and together form discrete ecological or evolutionary units.

In this thesis, I come back to the question of the mechanisms driving coexistence of the diverse communities of fishes on coral reefs. I conduct natural historical studies in a new century, with new techniques at our disposal, including affordable video monitoring and advances in genetic sequencing. I explore and revisit outstanding questions regarding the communities of fishes on coral reefs to address how closely related species use resources: what are they eating, where are they spending most of their time, what are their patterns of growth and abundance over time, and do patterns of activity for different trophic groups of fishes differ? Effective monitoring and management of wild populations hinges on accurate information. This dissertation seeks to provide information to aid in these efforts, while contributing to our

understanding of the natural history of these fishes and clues into the possible coexistence of species in the diverse ecosystems of the marine tropics.

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CHAPTER 1

DECODING DIVERSITY IN A CORAL REEF FISH SPECIES COMPLEX WITH RESTRICTED RANGE USING METAGENOMIC SEQUENCING OF GUT CONTENTS

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ABSTRACT

AIM: Identification of the processes that generate and maintain species diversity within the same region can provide insight into biogeographic patterns at broader spatiotemporal scales. Hawkfishes in the genus *Paracirrhites* are a unique taxon to explore with respect to niche differentiation, exhibiting diagnostic differences in coloration, and an apparent center of distribution outside of the Indo-Malay-Philippine (IMP) biodiversity hotspot for coral reef fishes. Our aim is to use next-generation sequencing methods to leverage samples of a taxon at their center of maximum diversity to explore phylogenetic relationships and a possible mechanism of coexistence.

LOCATION: Flint Island, Southern Line Islands, Republic of Kiribati.

METHODS: A comprehensive review of museum records, the primary literature, and unpublished field survey records was undertaken to determine ranges for four 'arc-eye' hawkfish species in the *Paracirrhites* species complex, and a potential hybrid. Fish from four *Paracirrhites* species were collected from Flint Island in the Southern Line Islands, Republic of Kiribati. Hindgut contents were sequenced, and subsequent metagenomic analyses were used to assess the phylogenetic relatedness of the host fish, the microbiome community structure, and prey remains for each species.

RESULTS: Phylogenetic analyses conducted with recovered mitochondrial genomes revealed clustering of P. bicolor with *P. arcatus*, and *P. xanthus* with *P. nisus*, which were unexpected on

the basis of previous morphological work in this species complex. Differences in taxonomic composition of gut microbial communities and presumed prey remains indicate likely separation of foraging niches.

MAIN CONCLUSIONS: Our findings point towards previously unidentified relationships in this cryptic species complex at its proposed center of distribution. The three species endemic to the Polynesian province (*P. nisus, P. xanthus, P. bicolor*) cluster separately from the more broadly-distributed *P. arcatus* on the basis of relative abundance of metazoan sequences in the gut (presumed prey remains). Discordance between gut microbial communities and phylogeny of the host fish further reinforce the hypothesis of niche separation.

KEY WORDS: biodiversity, rare species, coexistence, trophic ecology, metagenomics, hawkfish

INTRODUCTION

Although multiple ecological processes for species coexistence have been proposed (Chesson, 2000), a debate remains over what processes maintain morphologically and genetically similar species in a single habitat (Siepielski and McPeek, 2007; Vellend, 2010). By the principle of competitive exclusion, complete competitors should not coexist (Hardin, 1960); yet, closely-related and superficially ecologically-equivalent species do co-occur in nature (Darwin 1859; Cothran, Noyes, and Relyea 2015; Gotelli 1997). One mechanism contributing to initial species' divergence and continued persistence is trophic partitioning. Trophic shifts, especially feeding niche partitioning and associated morphological changes, have frequently been invoked to explain divergence among coral reef fishes on evolutionary timescales (Bellwood et al. 2014; Bellwood et al. 2006), yet current trophic designations of reef fish are coarse and do not reflect likely fine-scale differences in diet (Sandin and Zgliczynski, 2015).

Dietary assessments are often conducted using stable isotope and gut content analysis, but these approaches have limitations. For gut content analysis, an important limitation is linked to difficulties with taxonomic identification of partially digested prey remains. Furthermore, even those identifiable remains may represent limited bouts of feeding (Baker et al., 2014; Dam et al., 1991), especially for carnivorous fish species with rapid digestion processes. The accuracy of the results from stable isotopes is heavily dependent on whether sources have been accurately identified, despite advances in models incorporating uncertainty (Ward et al., 2010).

High-throughput sequencing techniques, including metagenomic sequencing, of coral reef fish intestines and gut homogenates provide an opportunity to close current gaps in knowledge for investigations of high-resolution trophic partitioning amongst coral reef fishes.

Results from metabarcoding studies have already shown promise for fishes (Matthieu Leray et al. 2013; Casey et al. 2019; Leray et al. 2012; Leray, Meyer, and Mills 2015) and other species (Andriollo et al., 2019; Deagle et al., 2013). However, examination of gut contents, both by traditional taxonomy and metabarcoding, provides only a snapshot of short-term feeding behavior, making extrapolation to general ecological behaviors difficult. Metagenomic sequencing, in contrast, provides information on the microbiome in addition to possible prey sequences (Srivathsan et al., 2015). Although prey sequences suffer from same shortcomings of both morphological gut content analysis and metabarcoding in that they are still a snapshot of last meals, and should be interpreted as such, information from the gut microbiome holds more promise. One of the most important environmental factors that influences gut microbiota is host diet (Bolnick et al., 2014a; Muegge et al., 2011; Smriga et al., 2010; Sullam et al., 2012; Wong and Rawls, 2012) and in studies which have parsed the effects of diet and phylogeny, gut microbial communities cluster strongly by diet and only weakly by host species (Hale et al. 2018). In organisms including primates and fish, distinct gut microbiomes are associated with different diets (Hale et al., 2018; Scott et al., 2013; Uchii et al., 2006), providing strong evidence that the gut microbiome can allow us to distinguish feeding habits within closely-related species. For example, fish of the same species with different feeding habits (Lepomis macrochirus) have been shown to have distinct intestinal microbiota associated with different feeding modes, for example specialization on benthic invertebrates, aquatic plants, and zooplankton (Uchii et al., 2006). Thus, metagenomics integrates information on commonly consumed prey species and the intestinal microbiota. Furthermore, metazoan prey remnants may be more readily identifiable via sequencing than taxonomic identification of highly digested remains (Leray et al. 2012; Leray et al. 2013; Leray, Meyer, and Mills 2015).

Exploring the utility and feasibility of this approach, we considered hawkfish in a *Paracirrhites* species complex from the remote island of Flint (Republic of Kiribati), in the likely center of the distribution for this genus in the Polynesian province of the Pacific (Donaldson 1988). Hawkfish, including those in the genus *Paracirrhites*, are harvested for the aquarium trade (Donaldson 2002), and rare species and colorations are highly prized. The arc-eye *Paracirrhites* group has been identified as a particularly interesting species complex for studies of biogeography (Donaldson 1986). Donaldson (1986) noted the existence of two centers of distribution for Cirrhitidae, based on high levels of endemism. The center of maximum diversity for *Paracirrhites* in the arc-eye complex conflicts with a traditional view of Indo-West Pacific reef fish zoogeography in which there is a single center of highest diversity, the Indo-Malay-Philippine (IMP) biodiversity hotspot (Ekman 1953), and instead points towards a hypothesis (Gaither and Rocha, 2013; Woodland, 1983) in which high levels of species richness in the IMP are due to pooling of species numbers from two distinct centers of distribution in confluence (center of overlap). In support of this, of the six Indo-West Pacific species of *Paracirrhites*, all six occur in the Polynesian area; three (*P. nisus, P. xanthus, and P. bicolor*) are endemic (Donaldson 1988).

Collection of species in their center of maximum diversity and endemism in the Polynesia province, along with the use of next-generation sequencing, enabled us to simultaneously re-examine phylogenetic relationships on the basis of new genetic information and assess the potential role for trophic-partitioning in maintaining coexistence among closely-related and morphologically-similar species. For this study, we hypothesized that species of the arc-eye complex would exhibit distinct intestinal microbiota and differences in prey remnants obtained from guts, as a partial explanation for potential coexistence of this enigmatic species complex at

the center of these species' ranges. We further hypothesized that the three species endemic to the Polynesian province (*P. bicolor, P. xanthus, and P. nisus*) would show distinct gut microbiota and prey profiles from the more broadly distributed *P. arcatus*, thus exhibiting a relationship between dietary patterns and biogeographic distribution.

METHODS

Study system

Paracirrhites arcatus species complex: Hawkfish in the genus *Paracirrhites* are small (maximum published length 29 cm) coral reef fish in the family Cirrhitidae found across the Indo-Pacific (Randall 1963; Randall, Allen, & Steene 1990). These fish are sit-and-wait mesopredators lacking a swim bladder, generally found perched on branching coral species, such as *Pocillopora* (DeMartini and Donaldson, 1996). Morphological analysis of prey remains in gut contents from *P. arcatus* indicate a general diet of small fishes, shrimps, crabs, and other crustaceans (Randall, Allen & Steene 1990), and a recent DNA metabarcoding approach revealed unexpected prey items such as *Paracalanus parvus*, a pelagic copepod species, in addition to coral crabs (genus Trapezia), species in the genus *Galathea* (squat lobster), and traces of DNA of *Pocillopora* coral (Leray et al., 2015, 2013). Although there are six recognized species in the genus *Paracirrhites*, there are only four recognized species of *Paracirrhites* with a characteristic and colorful oblique U-shaped mark behind the eye; throughout the rest of this manuscript, we will refer to this group as the "arc-eye complex." Of the four species in the arc-eye complex, one (*P. bicolor*) is listed as data deficient by the IUCN, and remaining three as least concern (*P. arcatus, P. nisus, P. xanthus*). *P. arcatus* is one of the more abundant and widespread of the cirrhitids, known from east Africa to Polynesia. This species occurs in two

different color morphs: a light grayish brown to reddish body with a pale pink to white band over the region of the lateral line, and a variety that is olive to dark brown and lacks the pale band on the body. These color morphs appear to be behaviorally unmodifiable, and are unrelated to ontogeny, sex, body size, or maturation (Randall 1963; DeMartini and Donaldson 1996; Sadovy and Donaldson 1995; Donaldson 1988), but differences in microhabitat have been described (DeMartini and Donaldson, 1996; Jonathan L Whitney et al., 2018), and they have been considered a potential instance of incipient speciation (Jonathan L. Whitney et al., 2018). Across the Pacific where the species spans a depth range of 1-27m, the light color morph was found to be more abundant at depths greater than 10m (DeMartini and Donaldson, 1996). A more recent examination of microhabitat associations in Hawaii found that the light color morph was observed more frequently in deeper, sub-surge zones, the dark morph was found more frequently in shallow, steep surge zones, and phenotypic intermediates were restricted to intermediate habitats (Jonathan L Whitney et al., 2018). The distribution and ecology of the endemic species (*P. xanthus, P. nisus, and P. bicolor*) are understudied and less understood than for *P. arcatus*. Representative photographs of each species and color morph, as well as distributions from occurrence records, are shown in Figure 1.

Range determination and implications for biogeography

Occurrences of the four species were tabulated based on review of the literature, museum records, and observations from belt-transect surveys (see Table S3 and S4 in Supporting Information). Most surveys did not distinguish between color morphs of *P. arcatus*, so we have shown the range as one group in Figure 1. Some reports included relatively broad ranges (island groups; eg., Gambier, Society Islands, Tuamotus) and so we also included shaded ranges

encompassing the entire region (e.g., Siu et al. 2017), as detailed species lists were not always available by individual island in these areas. Observations of a presumed hybrid (eg., *P. xanthus by P. nisus*) in transect surveys are shown on a map in Fig. S2, in the context of observations of *P. xanthus, P. nisus, and P. bicolor*. Observations of *P. nisus* via visual census have also been recorded in Maratua Island, Indonesia (Madduppa et al., 2012). It is possible that these observations of *P. nisus* are misidentifications of *P. arcatus*, as *P. nisus* is considered endemic to the eastern central Pacific, and there are no voucher specimens or photographs to provide a definitive identification.

Data collection.

Samples were collected from Flint Island (11.43˚S, 149.82˚W) in the Southern Line Islands (central Pacific) in the Republic of Kiribati. Sampling included five adult individuals of each of the five groups: *P. arcatus* (light morph), *P. arcatus* (dark morph), *P. bicolor, P. nisus, and P. xanthus*. Sampling for species was conducted from October 17th-21st and restricted to forereef habitat at an average depth of 10m to minimize variation due to sampling. (We will refer to these five groups operationally as 'species' for brevity. Although the two color morphs of *P. arcatus* are not presumed to be taxonomically distinct, Whitney, Bowen, and Karl 2018 found evidence for partial reproductive isolation and potential incipient speciation. Individual fish were collected with 3-prong spears (Hawaiian slings) and fish anesthetic (clove oil). Upon collection, fish were stored on ice and brought back to the research ship for initial processing. Each fish was assigned a unique identification tag and basic morphometric information was collected. Intestines were dissected, and hindgut homogenates from the five individuals per species were combined for DNA extraction using the MO-BIO Powersoil® DNA Isolation Kit, following the manufacturer's instructions. Sequence libraries were prepared using the Ion XpressTM Plus

Fragment Library Kit (Life Technologies, NY, USA) with slight protocol modification and each library was barcoded using the Ion XpressTM Barcode Adaptors 1-16 Kit. SPRI beads-based size selection was performed according to the published New England Bioscience (NEB) E6270 protocol (https://www.neb.com/protocols/1/01/01/size-selection-e6270) for 200-300 bp fragment size-selection after adaptor ligation. Emulsion PCR was performed on an 8-cycle amplified library using the OneTouch supplemented with Ion Torrent PGM Template OT2 200 Kit. Template libraries were sequenced on the Ion Torrent PGM using the Ion Torrent PGM Sequencing 200 Kit v2 and Ion 318TM Chip Kit v2 on the research vessel M/Y Hanse Explorer (Lim et al., 2014).

Bioinformatic Analyses.

DNA isolated from gut homogenates was pooled for five individuals per species and sequenced to yield 5 metagenomic libraries (*P. arcatus*: light morph, *P. arcatus*: dark morph, *P. xanthus*, *P. nisus*, and *P. bicolor*). Metagenomic sequence reads were filtered for quality using the Preprocessing and Information of Sequences tool (PRINSEQ; Schmieder and Edwards 2011). The sequenced reads were translated in silico into predicted protein sequences and compared with the SEED database to provide taxonomic identifications and information on organism abundance using Blastx (Altschul et al., 1990) with a maximum e-value cut-off of 1x10-5, a minimum alignment cut-off of 15, and a minimum percent identity of 80%. Metagenomes were deposited in the MG-RAST metagenomics analysis server (Meyer et al., 2008) and made publicly available using the unique identifiers listed in Table S1. Taxonomic assignment of bacterial species was confirmed using a blastn search against whole bacteria genomes deposited in GenBank (ftp://ftp.ncbi.nlm.nih.gov/genomes/Bacteria/). Bacterial taxa were categorized at the phylum level, with the exception of Proteobacteria and Firmicutes, which were categorized

by class. Unclassified bacteria were categorized as “other bacteria.” While conducting analyses at this taxonomic level aids in comparability to results from other studies conducted on fish gut microbiome, there may be hidden diversity in the identity and function of the gut microbial community that warrants a deeper look. For this reason, the 16s rRNA, rpoB, and recA gene sequences were extracted from the unassembled reads of each genome and clustered into OTUs using the program GenomePeek (McNair and Edwards, 2015). Further analyses focused on the results for 16S rRNA exclusively, as the database for this marker has the most coverage, thereby making comparisons of relative sequence abundance more accurate. For the analysis of putative prey items, the metagenomes were subset into 20,000 sequences to speed processing time. We used Midori-UNIQUE, a curated reference dataset of metazoan mitochondrial DNA sequences containing all unique haplotypes for each species for 16,697 unique species (Leray et al. 2018) as our reference dataset. The Midori reference datasets are divided into multiple markers, including two ribosomal RNA and thirteen mitochondrial protein-coding genes found in most phyla of metazoans. The cytochrome oxidase subunit I (COI) gene had the largest number of sequences, so we conducted our analyses with this dataset. We performed an initial blastn search for each metagenome in CLC Genomics Workbench (Qiagen Bioinformatics). Retaining only those sequences with a maximum e-value cutoff of 1x10-4, we then removed sequences with matches to *Paracirrhites*, as we assumed these were likely the result of host tissue contamination, rather than cannibalism, as customary for these analyses (Casey et al., 2019). We took all remaining annotations and calculated relative abundance within each metagenome at the phylum level. At lower taxonomic levels (e.g. species), the best sequence hit sometimes matched to an organism that either 1) does not occur in the study location, according to existing censuses, or 2) does not occur in the marine environment (e.g., species in the orders Lepidoptera and Hemiptera within

the phylum Arthropoda). To avoid any erroneous assumptions produced by using false species IDs or excluding important prey that could not be confidently assigned to lower taxonomic levels, we therefore conducted all analyses with metazoan sequences at the phylum level.

Phylogenetic Analyses.

Sequence reads from the metagenome for each species and color morph were aligned against the reference genome for the *P. arcatus* mitochondrion (17336 bp, NCBI RefSeq AP006012.1) using alignment tools in CLC Genomics Workbench 7 (Qiagen, CA, USA). Using *Cheilodactylus quadricornis* (NCBI RefSeq: KT357695.1), a species in the sister group to Cirrhitidae, and *Cirrhitichthys aprinus* (NCBI RefSeq: AP006011.1) as outgroups, the resultant FASTA mitochondrial DNA (mtDNA) sequences were aligned using MAFFT, a multiple alignment program for amino acid or nucleotide sequences. Maximum likelihood (ML) maximum parsimony (MP), and Bayesian inference (BI) analyses were conducted in order to assess congruence between the methods. An ML tree was constructed with RaxML-HPC2 (Randomized Axelerated Maximum Likelihood) using the GTRGAMMA model with 100 bootstrap replications (Stamatakis, 2014) run on the CIPRES portal (Miller, Pfeiffer & Schwartz). MP was carried out using PAUP 4.0a166 (Swofford 2003). Node support was assessed using jackknifing of sites with 100 replicates to produce a 50% majority-rule consensus tree (Fig S3). BI was conducted in MrBayes v.3.2.7a (Ronquist and Huelsenbeck, 2003) using two runs of four chains with ten million generations and a sampling frequency of 1000 generations for the Markov chain Monte Carlo analysis. The first 25% of trees were discarded as burn-in and the posterior probabilities were estimated by combining the remaining trees from each run into a majority-rule consensus tree.

Statistical Analyses.

In order to assess differences in bacterial community composition and metazoan prey composition between the four species and two color morphs, separate similarity profile routine (SIMPROF) tests were conducted on relative abundance of bacterial taxonomic groups and the relative abundance of metazoan sequences using the *clustsig* package (Whitaker & Christman 2010) implemented in R (R Development Core Team, www.r-project.org). SIMPROF is a permutation procedure which tests for the presence of sample groups in a priori unstructured sets of samples (K. R. Clarke et al., 2008). Analyses were based on 10,000 random permutations of the annotated metagenomic data. Following procedure which has been used in other analyses of bacterial structure in metagenomic samples, the two most abundant phyla (Firmicutes and Proteobacteria) were further divided into the lower taxonomic grouping of class to provide clearer resolution of the groups driving differences in microbiome composition between species(Kelly et al., 2014). Shannon-Weiner (H′) diversity indices were calculated using the R package *vegan* (Okansen et al. 2012). All statistics were conducted using R 3.1.2.

RESULTS

Phylogenetic analyses of hawkfish.

The ML and MP phylogenetic analyses of the whole mitochondrial genome (mtDNA) for all four species, including the two color morphs analyzed separately, provided almost identical reconstructions with strong node support for the separation of two clades within the arcatus complex, with one clade composed of *P. arcatus* (both color morphs) and *P. bicolor*, and one clade composed of *P. xanthus* and *P. nisus*. The maximum likelihood tree showed clustering of *P. xanthus* with *P. nisus*, in addition to clustering of the two color morphs of *P. arcatus* with *P. bicolor*. Under maximum parsimony and Bayesian inference, the phylogenetic tree showed

additional structure among species, with *P. arcatus* (dark morph) and *P. bicolor* forming a sister clade to *P. arcatus* (light morph) (Figure 3, right).

Fish gut microbiomes and metabolic functions

Investigation of the bacterial community structure shows divergence between species, but not color morphs (SIMPROF, $\alpha<0.05$, see Figure 2). Firmicutes and Proteobacteria together made up 94-96% (Firmicutes=81-91%; Proteobacteria=5-13%) of the bacterial community, and thus were divided into class. SIMPROF analysis of bacterial community structure in the five composite metagenomes for each of the five members of the arc-eye complex revealed three significant ($\alpha=0.05$) groupings: (1) *P. arcatus* (light), *P. arcatus* (dark), and *P. xanthus*; (2) *P. nisus*, and (3) *P. bicolor* (Figure 2c and Fig. 3). From the analyses of 16S rRNA OTUs, *Clostridium perfringens* was found in highest abundance of all bacterial taxa for all species, but comprises only 42.6% and 37.4% of the relative abundance of all 16S contigs for *P. nisus* and *P. bicolor*, respectively, in comparison to 78-94% of the bacterial community for *P. xanthus* and *P. arcatus* (both morphs). Accordingly, Shannon-Weiner diversity indices of the gut microbial communities using 16S data are highest for *P. bicolor* ($H'=2.27$) and *P. nisus* ($H'=1.51$), and consistent with the clustering identified at higher taxonomic levels (Fig. 2c).

Putative prey

Chordate sequences were found in higher relative abundance for the species with a more restricted range (*P. nisus, P. xanthus, P. bicolor*); ie. 24-36% compared to 0.9-2% for *P. arcatus* (dark) and *P. arcatus* (light), respectively (Fig 2b). SIMPROF analysis of the metazoan data ($\alpha=0.15$) show two groupings; grouping of the two color morphs of *P. arcatus*, and a separate grouping with the three endemic species (*P. bicolor*, *P. xanthus*, and *P. nisus*), see Fig. 2d.

One limitation of this approach is the difficulty in distinguishing secondary predation (that is, prey remains in a prey item's gut) from targeted prey, or incidental consumption in the course of feeding. For instance, sequences with a best match to Cnidara were found in the metagenome for the dark morph of *P. arcatus*. *P. arcatus* are known to feed on mutualistic coral crab species of the genus Trapezia (Leray et al., 2015), which themselves feed on coral mucus (Knudsen, 1967). Given the low relative abundance of sequences matching to Cnidaria (>1%), it is certainly possible these sequences are from secondary prey.

DISCUSSION

The importance of the expansion of feeding forms over evolutionary time for the diversification of reef fishes has been well-supported, with niche-partitioning and morphological innovation identified as playing key roles in the development of herbivory and detritivory (Bellwood et al. 2006). Behavioral innovations may be equally as important as morphological innovations but have not received the same attention likely due to difficulties in inference from the fossil record. Diversification by this mode, however, may have left traces in the current biogeography of reef fishes, and may be more readily studied by examining potential ecological differentiation in present-day genera. Local adaptation may reduce the likelihood of successful colonization of novel habitats after dispersal, and dietary specialization has been linked to reduced species durations (Balisi et al., 2018), as many of the traits that increase the chance of speciation also increase the chances of extinction (Jablonski, 2008). Compressed ranges may therefore be the result of either (1) limitations in dispersal or successful colonization, or (2) the result of past extinction events. Simultaneous examination of phylogenetic relationships, potential ecological differentiation, and current ranges within a genus—especially one for which incipient speciation has been proposed (Jonathan L. Whitney et al., 2018; Whitney, 2016)—may provide clues to the

mechanisms involved in creating and maintaining the observed species' distributions. To that end, our findings point towards previously unidentified relationships in a cryptic species complex at its proposed center of distribution, and discordance between gut microbial communities and phylogeny of the host fish which support the hypothesis of niche separation.

The findings here further demonstrate that metagenomic techniques can provide data comprising three important lines of information for a variety of uses: (1) host genetics, (2) microbial communities in the gut, as well as (3) the identity of putative prey items. When these tools were applied to understudied and closely related fish from a remote island, the value of the multi-dimensional data are revealed well. Species-level designations for hawkfish included in this study were originally determined on the basis of morphometric differences. In the initial description by Randall (Randall, 1963), it was suggested that, of the five species with postocular marks in *Paracirrhites*, *P. bicolor* might be most closely related to *P. nisus*. Our phylogenetic analysis of mtDNA, in contrast, suggests that *P. nisus* and *P. xanthus* are more closely related than *P. bicolor* and *P. nisus*. This finding further matches observations of *P. nisus* by *P. xanthus* hybrids observed in the wild (see Fig. S1, Table S3, and Table S4), as species that are more closely related are more likely to hybridize. The phylogenetic analyses conducted here further raise the possibility that P. bicolor is a color morph variation of *P. arcatus*, given that mtDNA sequences for the *P. arcatus* dark morph and *P. bicolor* were nearly identical (>99% similarity). As mtDNA is inherited solely from the female parent, it is also possible that P. bicolor is a hybrid arising from a spawning event in which *P. arcatus* was the female parent with an unknown male species. In support of this possibility is the extreme rarity of *P. bicolor*. Rarity of at least one parental species has been shown to play an important role in hybrid formation for some reef fishes, with hybridization resulting from mating of closely related species due to

scarcity of conspecific partners (Hobbs et al., 2009). Incorporating nuclear markers with the mtDNA results, as well as microsatellite markers, would further clarify phylogenetic relationships between species in this study. These possibilities are being investigated with additional molecular research.

A unique gut microbiome and differential relative abundance of likely dietary items suggests that there may be some ecological differences among species, despite high genetic similarity, that warrant further investigation. Previous studies have demonstrated an unexpected negative relationship between diet diversity and diversity of gut microbiota for two fish species; that is, dietary generalists had a less diverse microbiome (Bolnick et al., 2014b). Indeed, we found that *P. bicolor* and *P. nisus* have the most diverse microbiota, perhaps indicating dietary specialization. Additionally, although the two color morphs of *P. arcatus* cluster in analyses of gut microbial communities and metazoan sequences, differences in relative abundance of sequences for prey items found in the guts warrant further study of potential fine-scale differences. As noted previously, it has been suggested that *P. arcatus* may be an example of incipient speciation in a sympatric species (Jonathan L. Whitney et al., 2018). Differences in composition of diet between color morphs could be driven by these differences in microhabitat associations.

Analyses of putative prey remains revealed broad convergence with what was generally known regarding *Paracirrhites* diets, including a high relative percentage of sequences belonging to the phyla Arthropoda and Chordata. This is consistent with a reported diet consisting predominantly of crustaceans and other fishes, but also revealed some interesting differences. In particular, the three species with the most restricted ranges had a higher relative abundance of sequences matching Chordata. An analysis of gut contents by morphologic

identification of prey remains for 199 individuals of *Paracirrhites arcatus* (both color morphs) show a range of 0.62-9.4% relative abundance for other fish species (Cordner 2013, see Table S2), suggesting that other fish species may make up a higher proportion of the diet for those species with more restricted ranges (*P. bicolor*, *P. nisus*, and *P. xanthus*). One potential caveat is that all sequences with a match to the host organism were removed to avoid conflation of host tissue with estimates of prey consumption. Any instances of intraspecific predation would therefore be impossible to detect. As this processing was applied equally across all species, if intraspecific predation is more frequent among *P. arcatus*, for instance, the estimates of relative abundance of prey fish would be underestimated in comparison.

There are some considerations that must be noted for the interpretation of our results, and in particular what the relative abundance of metazoan sequences indicates for hawkfish diets over long timescales. The number of High-scoring Segment Pairs (HSPs) is not a direct correlate to the number of organisms in the gut; a single large organism could result in many HSPs, and gene copy number differs among eukaryotes (Prokopowich et al., 2003). Furthermore, carnivorous fish have relatively short gut passage times (~8 hours, Clements et al. 2014), so the identification of prey remains, even with combined samples to homogenize variability, only reflects a limited selection of what these fish are eating over time. Gut microbiomes may therefore be a more stable predictor of integrated food habits over time. Dietary interventions in well-studied human gut microbiomes have shown community changes on timescales of months (Ley et al., 2006) to years (Faith et al., 2013), and others showing return to original structure of gut microbiota only 2 days after the end of a dietary intervention (David et al., 2014). Studies that have examined this for fish have had similarly varying results. A study on captive clownfish found that certain bacterial groups such as *Clostridia perfringens* did not vary significantly with

time post-feeding, whereas other groups such as *Bacilli* (Firmicutes) and *Photobacterium sp*. increased after feeding (Parris, Morgan & Stewart 2019). Other studies found no or minimal changes in diversity, but observed changes in relative abundance of bacterial phyla 3-24 hours after feeding (Zhang et al., 2018) and 3-12 days post-fasting (Xia et al., 2014). Taken together, there does appear to be a core microbiome for fish that does not change with feeding status but is sensitive to diet on longer timescales. We further posit that the general concordance between the metazoan and microbial data that we show here provides strong evidence for real ecological differences between species.

Some of the limitations associated with this method can help guide additional research in this field and contribute to the discussion on best practices in using next-generation sequencing of gut contents as a technique for assessing dietary habits within animals. As guts were pooled to produce composite metagenomes, information on individual variability between microbiomes and diet within species was impossible to obtain for this study, although we had a priori evidence that intraspecific variability in this genus would be low. In analyses excluding all empty stomachs (~10%), stomach contents analysis of 199 *P. arcatus* individuals were indistinguishable across islands (Zgliczynski et al., 2019). In instances where there are constraints to the number of samples that can be analyzed, whether financial or logistical, specimen pooling is often promoted as an option with very little loss of statistical power (Weinberg and Umbach, 1999). (Schisterman and Vexler, 2008) show that estimations based on pooled data increase efficiency over the use of individual measurements when an assay has a detection threshold, as pooling samples minimizes the amount of information lost below the detection threshold. This technique is used extensively in the literature for population-based epidemiological studies (Schisterman and Vexler, 2008), and other situations with high-

dimensional biological data, such as that generated via high-throughput sequencing techniques (Clarke et al. 2008).

Sample sizes for this study were also quite limited. *P. bicolor* in particular is so rare that a photograph of one was only published recently (Bacchet, Zysman, & Lefevre 2017), and this species is known from only four specimens from two localities (Randall, 1963). Future studies that incorporate analyses of intraspecific variability with higher sample sizes for species where additional collection is both logistically possible and ethically defensible are warranted to verify and extend the findings here.

Notwithstanding the very real limitations, a major strength of this approach includes the ability to look at the host genetics, microbiome and the presumed prey fragments simultaneously. This approach further allows the investigation of the results using multiple genes, rather than one pre-selected marker that must be chosen a priori (as in the use of metabarcoding). In most situations, and particularly for highly complex ecosystems, reference barcodes are not available for all potential prey species. Combined with the knowledge that no single marker is ideal for resolving all taxonomic groups (Deagle et al., 2014; Hebert et al., 2003), the use of this technique represents a significant advancement in the available tools for investigating realistic patterns of prey consumption in wild animals. As databases further improve through international efforts, our ability to address complex ecological questions with genetic tools will continue to grow. It is our hope that this study will encourage more community ecologists and conservation scientists to consider metagenomics as a powerful tool that can be used to leverage multidimensional and ecologically informative data from rare and understudied species.

DATA ACCESSIBILITY STATEMENT

Metagenomes were deposited in the MG-RAST metagenomics analysis server (Meyer et al. 2008) and made publicly available using the following unique identifiers: *P. arcatus*; light morph: 4632779.3, *P. arcatus*; dark morph: 4632778.3, *P. xanthus*: 4632786.3, *P. nisus*: 4631833.3, and *P. bicolor*: 4632780.3.

ACKNOWLEDGMENTS

This work was carried out under research permits from the Environment and Conservation Division of the Republic of Kiribati. For logistical support, we thank the Captain Martin Graser, officers and crew of the M/Y Hanse Explorer. Dive support was provided by Christian McDonald (SIO). We thank Life Technologies for providing library preparation and sequencing reagents for the study. The SDSU Vice President of Research, Director's Office of Scripps Institution of Oceanography, Moore Family Foundation, and several private donors provided cruise support. Computing resources and support were provided by NSF grant CNS-1305112 to Robert A. Edwards. We further thank Adi Khen and Kiefer Forsch for the artwork, Gregory Rouse, Emily Mclaughlin, and Josefine Stiller for conversations and recommendations regarding phylogenetic analyses, Katelyn McNair and Genivaldo Gueiros Z. Silva for bioinformatics assistance and advice, and Gilles Siu for providing detailed information on hawkfish observations in the Tuamotus. The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript. This manuscript is dedicated to Rachel A. Morrison, whose work laid the foundations for the investigation conducted here.

Chapter One, in full, is a reprint of the material as it appears in *Ecology and Evolution*, 2020. B. J. French, Y. W. Lim, B. J. Zgliczynski, R. A. Edwards, F. Rohwer, S. A. Sandin.

“Decoding diversity in a coral reef fish species complex with restricted range using metagenomic sequencing of gut contents.” The dissertation author was the primary investigator and author of this paper.

FIGURES

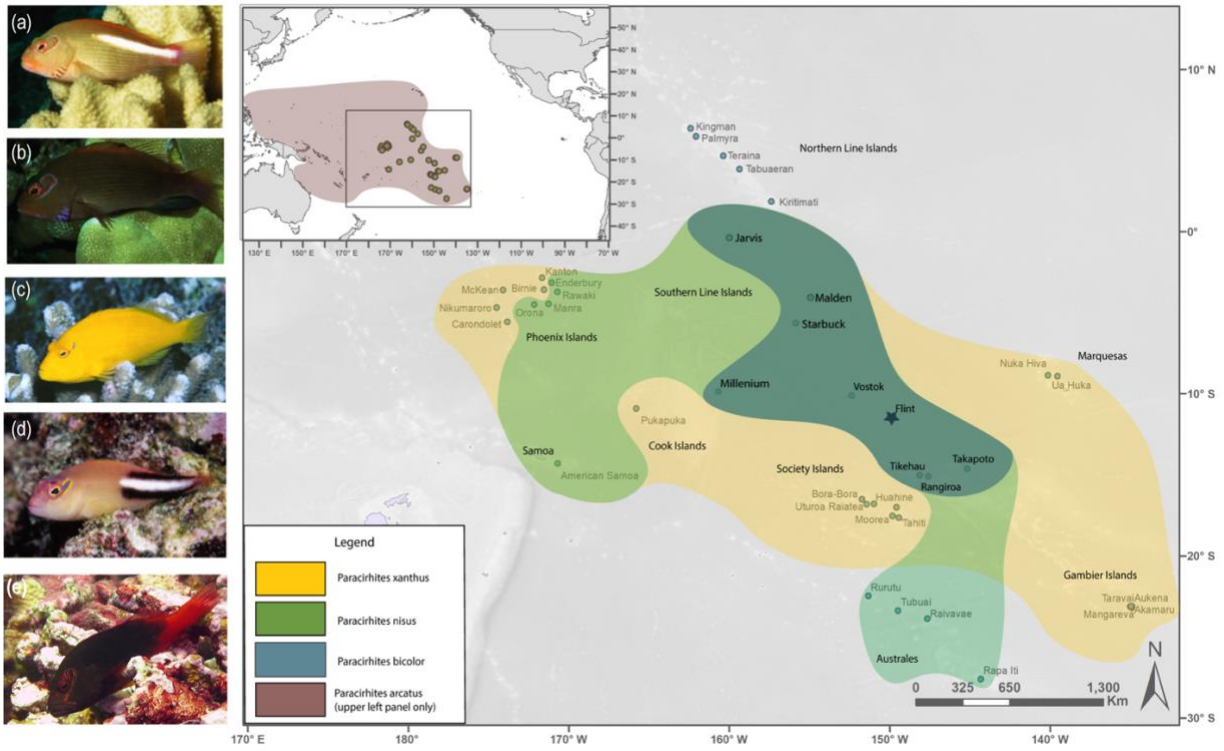

Figure 1.1 (*Left panel*) Illustration of color and morphological differentiation for species in the *arc-eye* species complex. Panel (a) corresponds to *Paracirrhites arcatus*; light morph; panel (b) corresponds to *Paracirrhites arcatus*, dark morph; panel (c) is *Paracirrhites xanthus*; panel (d) is *Paracirrhites nisus*; and panel (e) is *Paracirrhites bicolor*. (*Right panel*) Biogeographical range for species in the *arc-eye* complex. The range of *Paracirrhites arcatus* is shown in the upper left (smaller figure) only, as this range encompasses the ranges of the other species. The square box denotes the enlarged portion. The sampling site for collection of specimens for metagenomic analysis is labeled with a star (Flint Island, Republic of Kiribati). (Photos: B.J. Zgliczynski and M.J. Adams).

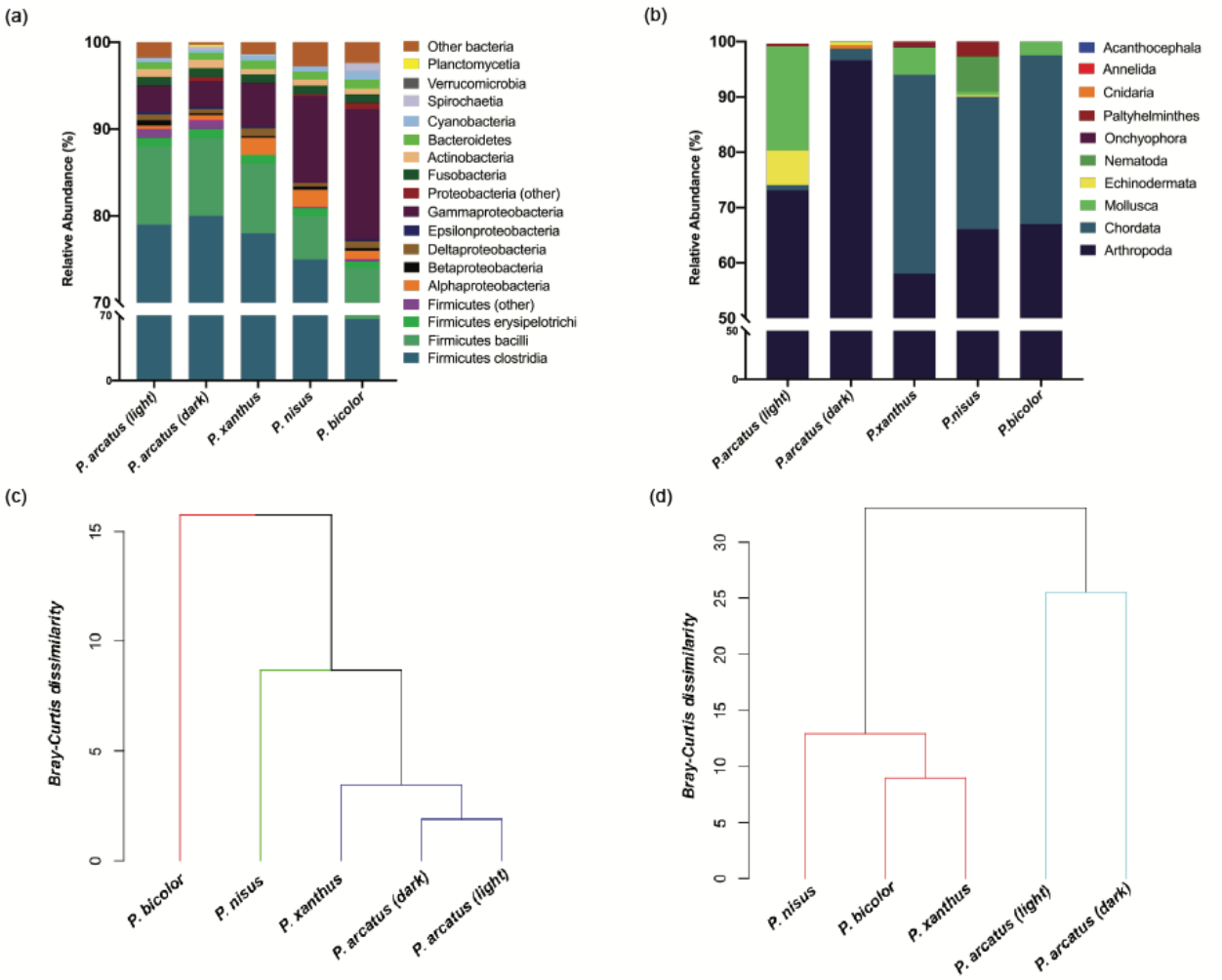

Figure 1.2. (a) *Gut microbial community structure and metazoan composition of metagenomes*. Stacked barplot illustrating relative abundance of bacterial phyla within total bacterial reads. The two most abundant phyla (Proteobacteria and Firmicutes) are broken down into class. (b) *Putative prey sequences in hawkfish gut contents*. Stacked barplot illustrating relative abundance of all High-scoring Segment Pairs (HSPs) with e<$1x10^{-4}$ from BLASTn search of the Midori-UNIQUE reference dataset for the cytochrome oxidase subunit I (COI) gene, after removal of HSPs where the accession was a match to *Paracirrhites*. (c) Dendrogram from Similarity Profile Routine (SIMPROF) test on hawkfish gut microbial community structure. Colors indicate significantly different clusters at α=0.05. (d) Dendrogram from Similarity Profile Routine (SIMPROF) test on metazoan prey sequence composition in hawkfish guts. Colors indicate significantly different clusters at α=0.15.

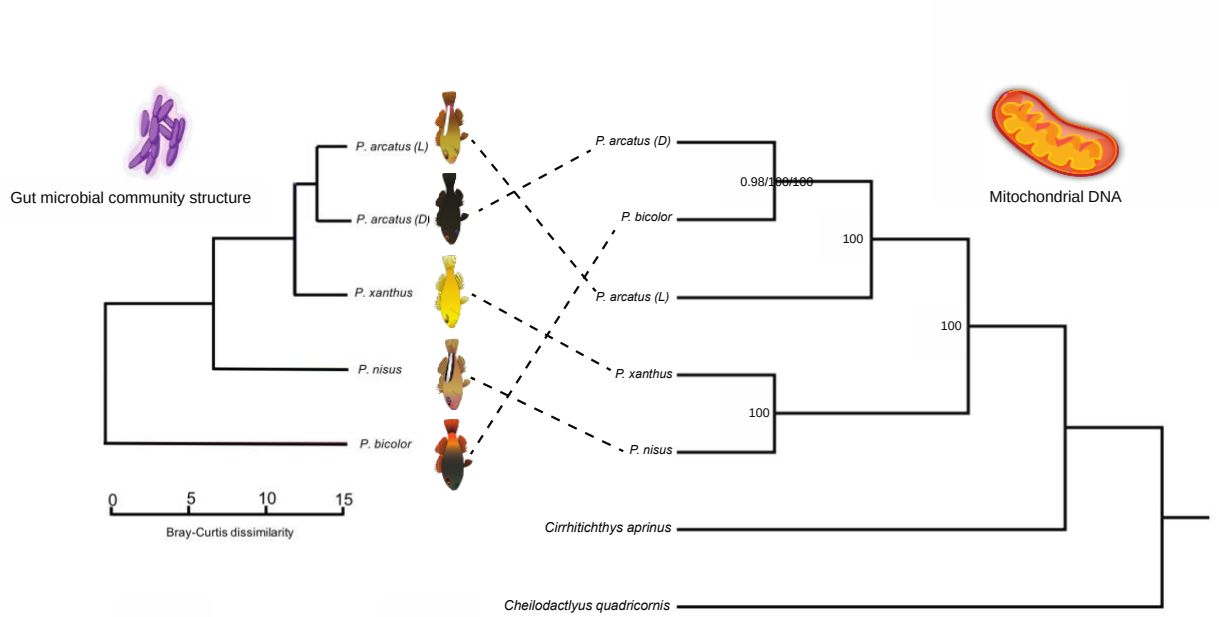

Figure 1.3. Dendrogram from SIMPROF analysis on hawkfish gut microbial communities (left). *Paracirrhites bicolor* and *Paracirrhites nisus* show significant ($p<0.05$) separation from *Paracirrhites arcatus* (both color morphs) and *Paracirrhites xanthus*. The microbiomes of the two color morphs and *Paracirrhites xanthus* are not significantly different from one another. Phylogenetic analysis of hawkfish species using genome sequences for the mitochondrion (right). Depicted is a phylogenetic tree for *Paracirrhites* species with *Cheilodactlyus quadricornis* (KT357695.1) and *Cirrhitichthys aprinus (*AP006011.1) as outgroups. Support values are indicated at the nodes in the order Bayesian inference/maximum likelihood/maximum parsimony. Maximum support values (1/100/100) are indicated at the nodes by black circles.

Ecology and Evolution

Supplementary Material for:

Decoding diversity in a coral reef fish species complex with restricted range using metagenomic sequencing of gut contents

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Supplementary Methods

Methods for novel and unpublished fish survey records

For the unpublished survey records, quantitative underwater surveys (belt transect surveys) of reef fish assemblages were conducted, following methodological details described elsewhere (DeMartini et al. 2008, Friedlander et al. 2010, Sandin et al. 2008). We analyzed quantitative survey data collected from islands, atolls, and reefs spanning the equatorial Pacific between 2009-2017. Surveys were restricted to the forereef slope at depths between 8 and 15 m. The unit of replication within islands was the station. Although belt transects can underestimate the presence of cryptic fishes and cryptic species (Willis 2001), it is a non-extractive method that can be used to capture high diversity and species with patchy distributions (Caldwell et al. 2015), and was able to capture all of the species in surveys in the arc-eye complex in their region of maximum diversity in the central Pacific (Line Islands and Tuamotus). Records are provided in Table S4.

Museum Records

We used FishNet2, a collaborative effort of global fish collections designed to share and distribute data on specimen holdings from around the world, to search for all records of species in the arc-eye *Paracirrhites* species complex. A total of 723 individual specimen records, and their metadata, were downloaded and are provided in Table S4.

Parameters for CLC Genomics Workbench BLAST analyses (metazoan sequences):

Match = 2, mismatch = 3, existence = 5, extension = 2; expectation value = 10; word size = 11; mask lower case = no; filter low complexity = yes; maximum number of hits =20000; number of threads =10. Retaining only those sequences with a maximum e-value cutoff of $1x10^{-4}$ resulted in 31 annotations for *P. nisus*, 57 annotations for *P. bicolor*, 68 annotations for *P. arcatus* (dark), 61 annotations for *P. arcatus* (light), and 42 annotations for *P. xanthus*.

Supplementary Figures

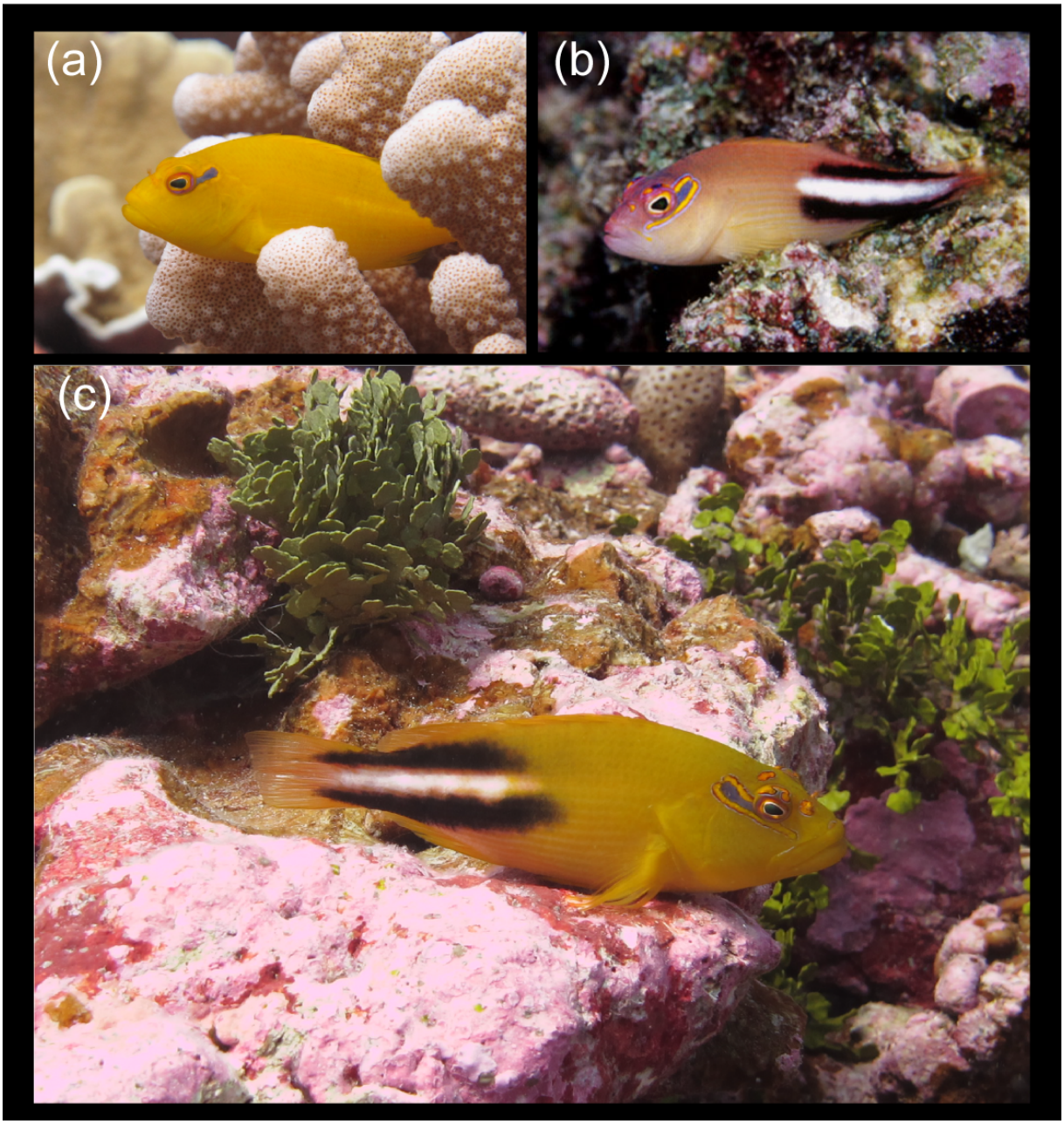

Figure 1.S1. *Comparison of P. xanthus, P. nisus and apparent P. xanthus x nisus hybrid observed in the Southern Line Islands.* (a) Image of *Paracirrhites xanthus*, photograph taken by Brian Zglicznski (b) Image of *Paracirrhites nisus*, photograph taken by Mary Jane Adams at Nikumaroro Island (c) Apparent *Paracirrhites xanthus* x *nisus* hybrid observed in the Southern Line Islands, photograph taken by Brian Zgliczynski. *P. xanthus x P. nisus* "hybrids" have been observed in Tikei (Tuamotu, French Polynesia), Millenium Atoll, Vostok, Starbuck, and Flint (Southern Line Islands, Republic of Kiribati).

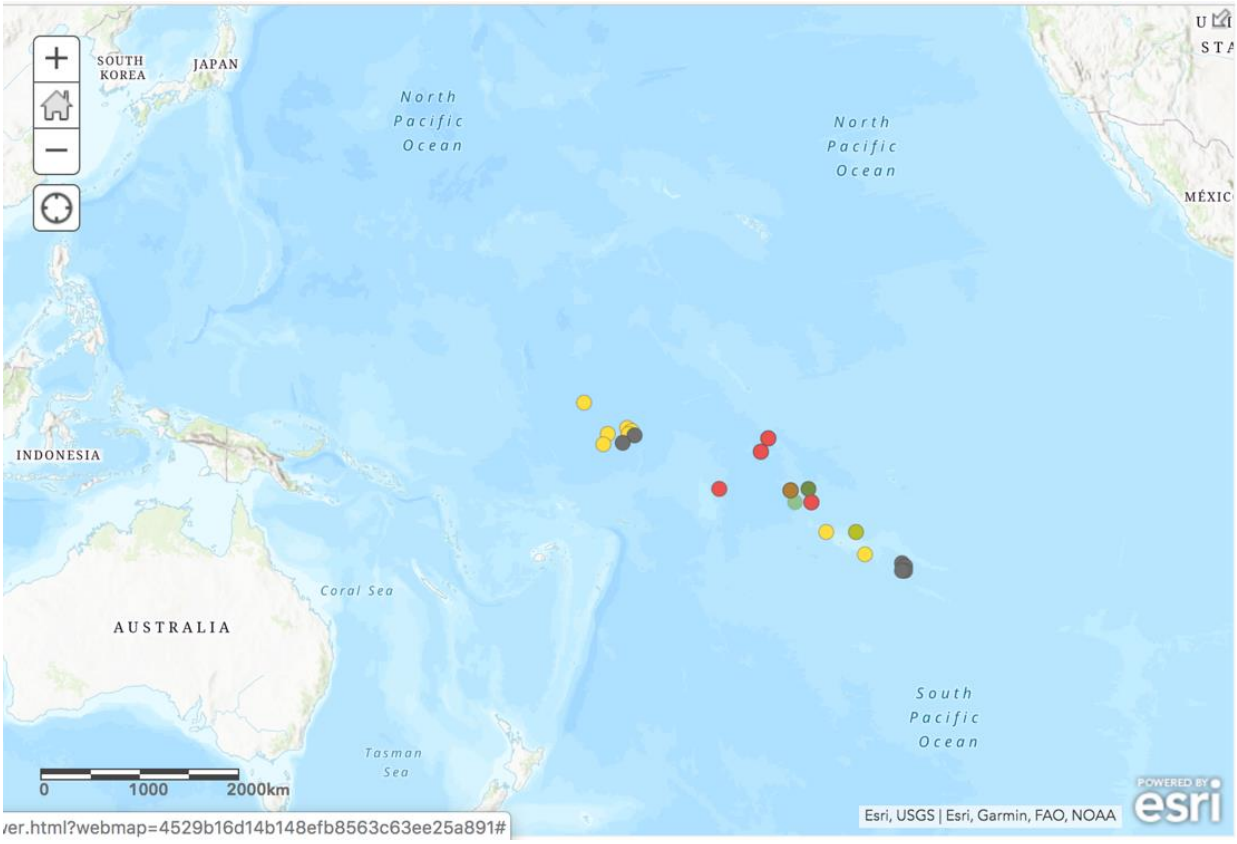

Figure 1.S2. *Map displaying occurrence records from visual census surveys of the species considered endemic to the Polynesian province, plus a putative hybrid of P. nisus x xanthus.* Yellow circles correspond to *P. xanthus*, black circles to *P. nisus*, red circles to *P. bicolor*, and green circles to putative *P. nisus x P. xanthus hybrids*. All occurrence records correspond to the survey records included in Table S4.

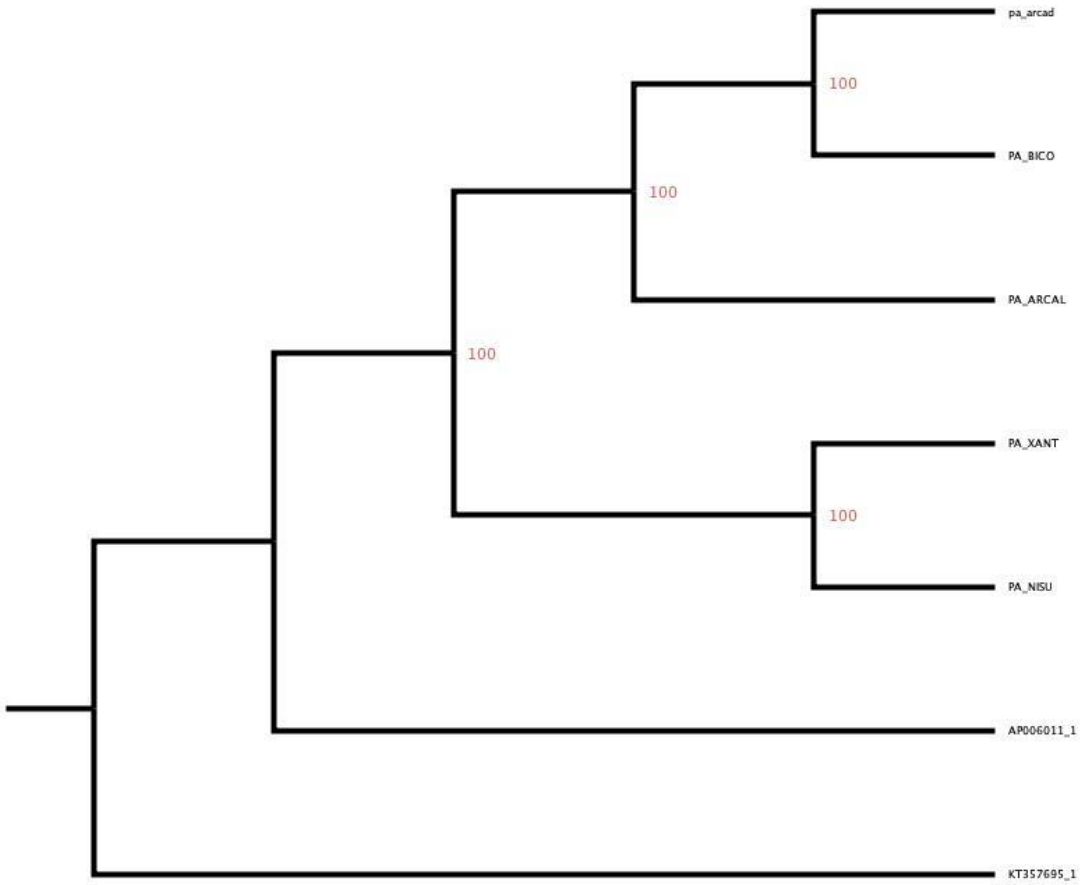

Figure 1.S3. *Jackknife 50% majority-rule consensus tree from maximum parsimony phylogenetic analysis.* KT357695.1 is the NCBI GenBank reference for the complete mitochondrial genome for *Cheilodactlyus quadricornis*. AP006011.1 is the NCBI GenBank reference for the complete mitochondrial genome for *Cirrhitichthys aprinus.*

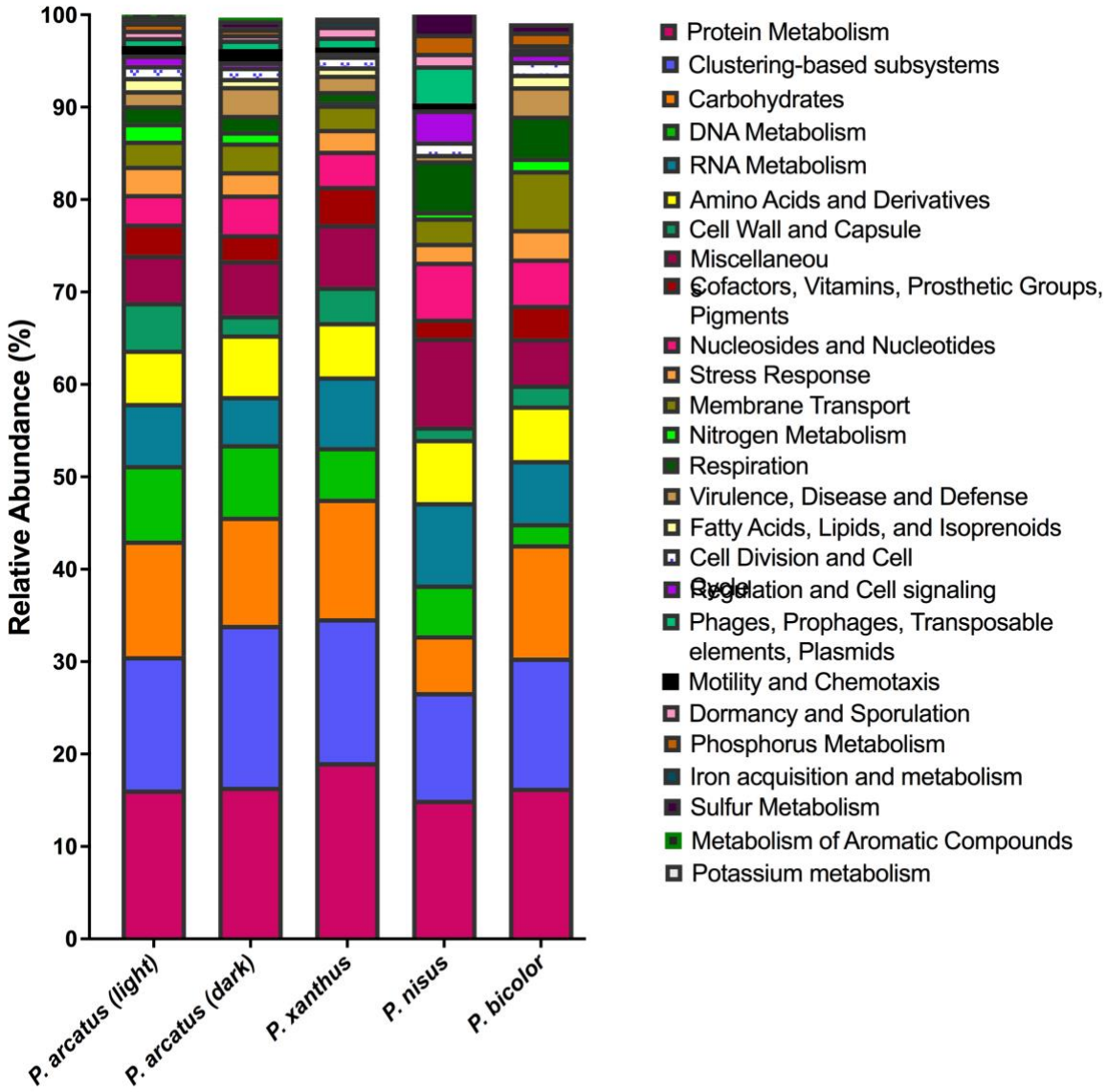

Figure 1.S4. *Metabolic functions in metagenomes.* Stacked barplot indicating the relative abundance of genes in functional categories, using level-one SEED subsystem-based functional annotations.

Supplementary Tables

Table 1.S1. Details of metagenomic libraries deposited in the MG-RAST server under the study name "SLI_FishGutMetagenomes."

Sample Name	MG-RAST ID	Total no. of reads	Mean read length (bp)	% GC content
Paracirrhites arcatus (light morph)	4632779.3	5,709,803	153 ± 56	38 ± 10
Paracirrhites arcatus (dark morph)	4632778.3	1,218,165	158 ± 33	39 ± 9
Paracirrhites xanthus	4632786.3	1,277,645	151 ± 27	41 ± 9
Paracirrhites nisus	4631833.3	6,223,957	152 ± 52	42 ± 9
Paracirrhites bicolor	4632780.3	1,594,441	137 ± 46	42 ± 9

Table 1.S2. Mean percentages of prey groups identified in 199 *Paracirrhites arcatus* guts from the Northern Line Islands. Islands listed from North to South (*reproduced from* Cordner 2013). **indicates an inhabited island

Prey Group	Kingman	Palmyra	Teraina**	Tabuaeran**	Jarvis
Crustaceans	91.44 (5.9)	81.34 (6.37)	80.15 (5.72)	80.34 (5.68)	88.7 (4.17)
Decapoda	37.59 (9.15)	42.56 (9.39)	34.91 (7)	28.12 (6.15)	43.82 (7.8)
Amphipoda	0	0.24 (0.24)	0.03 (0.02)	3 (2.32)	1.47 (0.96)
Copepoda	7.1 (4.39)	0.01 (0.01)	0.37 (0.32)	0.45 (0.45)	0.1 (0.1)
Stomatapoda	6.97 (4.95)	5.33 (4.16)	2.47 (2.47)	4.53 (2.58)	4.4 (3.13)
Megalopes	5.82 (4.29)	12.21 (5.97)	1.48 (1.48)	10.2 (4.03)	0
Unidentifiable	33.97 (8.23)	21 (7.18)	40.89 (7.25)	34.03 (6.53)	38.91 (7.27)
Fish	4.35 (4.35)	9.4 (5.48)	8.7 (4.09)	8.64 (4.19)	0.62 (0.62)
Gobiidae	0	1.75 (1.75)	0	1.83 (1.83)	0
Pseudanthias spp.	0	0	2.47 (2.47)	0	0
Unidentifiable	4.35 (4.35)	7.65 (5.3)	6.23 (3.38)	6.82 (3.84)	0.62 (0.62)
Other	4.21 (4.19)	9.25 (3.67)	11.15 (4.57)	11.01 (4.37)	10.68 (4.16)
Ophiuroidea	0	1.24 (1.23)	2.49 (2.49)	0	2.52 (1.88)
Gastropod	0	0	0.56 (0.56)	0.07 (0.07)	0.17 (0.15)
Halimeda	0	1.27 (0.97)	0.07 (0.06)	0.13 (0.13)	0.19 (0.19)
Algae	0.02 (0.02)	4.42 (3.13)	0.28 (0.2)	3.8 (2.45)	2.92 (1.57)
Sand	0	0	7.68 (3.69)	3.75 (2.62)	4.81 (2.91)
Worm	0.07 (0.07)	0	0	1.79 (1.71)	0
Unknown	4.12 (4.12)	2.33 (1.92)	0.08 (0.08)	1.11 (1.11)	0.07 (0.07)

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CHAPTER 2

LENGTH–WEIGHT RELATIONSHIPS FOR ABUNDANT CORAL REEF FISH SPECIES FROM EIGHT ISLANDS IN FRENCH POLYNESIA

Beverly J. French, Brian J. Zgliczynski, Chelsea L. Wood, Alison J. Haupt, Serge Planes and Stuart A. Sandin

ABSTRACT

English:

Here we present length-weight relationships (LWR) for 11 reef fish species from eight islands and atolls in French Polynesia. A total of 1,930 fish were collected from five islands in the Society Archipelago (Moorea, Tahiti, Raiatea, Huahine, Tetiaroa) and in three atolls of the Tuamotu Archipelago (Takapoto, Tikehau, and Rangiroa). These fishes span trophic levels, including planktivores, herbivores, and carnivores, and are among the most abundant species for the region. Estimates include LWRs for species never previously published or available in the literature or accessible databases. Measurements of total length (TL: 0.1 cm precision) and total weight (W: 0.01 g precision) were taken. These estimates increase the number of available and robust LWRs for coral reef fishes, including those without length-weight parameters previously available in the literature, providing a better understanding of life and growth history traits of these species. With a particular focus on small-bodied species, among the most abundant observed in underwater visual censuses, these estimates will allow marine resource managers and local scientists to better characterize fish biomass communities in French Polynesia with greater precision.

French:

Nous présentons ici les relations longueur-poids (LWR) pour 11 espèces de poissons de récif coralliens de huit îles de Polynésie française. Au total, 1 930 poissons ont été collectés dans 5 l'île de l'Archipel de la Société (Moorea, Tahiti, Raiatea, Huahine, Tetiaroa) et dans 3 atolls de l'archipel des Tuamotu (Takapoto, Tikehau et Rangiroa). Ces poissons couvrent plusieurs

niveaux trophiques, avec des planctonivores, des herbivores et des carnivores, et comprennent parmi les espèces les plus abondantes de la région. Les estimations incluent les LWR pour des espèces jamais précédemment publiés ou disponibles dans la littérature ou dans les bases de données accessibles. Les mesures de longueur totale (TL : précision de 0,1 cm) et de poids total (P : précision de 0,01 g) ont été mesurées. Ces estimations augmentent le nombre de LWR disponibles et robustes pour les poissons des récifs coralliens, y compris celles sans paramètres longueur-poids précédemment disponibles dans la littérature, apportant une meilleure compréhension des traits d'histoires de vie et de croissance de ces espèces. Avec un focus plus particulier sur des espèces de petite taille, parmi les plus abondantes observées dans les comptages visuels sous-marin de poissons, ces estimations permettront aux gestionnaires des ressources marines et aux scientifiques locaux de mieux caractériser les biomasses de poissons en Polynésie française, avec une plus grande précision.

Introduction

Coral reef fish assemblages and their associated fisheries experience distinct challenges for management. In particular, the combination of high species diversity among coral reef fishes and limited availability of critical life history information makes accurate assessments of reef fish assemblage structure difficult. Resource managers and scientists frequently conduct underwater visual census surveys to assess the structure of fish assemblages, with the goal of tracking patterns of ecological change and providing insight into the fisheries potential of an area (Caldwell et al., 2016; DeMartini et al., 2008; Friedlander and DeMartini, 2002; Sandin et al., 2008; Williams et al., 2011). A common goal is to calculate biomass based on the known relationship between fish length and weight, which allows estimation of overall biomass, as well as biomass in specific categories of interest (fisheries targets, trophic category), given the importance of size to fisheries value and ecological function (e.g., predation, fecundity). Size-based assessments are conducted to examine patterns of exploitation and to estimate the health of populations of interest. However, length-weight relationships are known only for a restricted set of species, and robust data are available for even fewer (Froese, 2006; Kulbicki et al., 2005). In this study, we report length-weight relationships (LWR) for 11 reef fish species from eight islands in French Polynesia. While only a few of these species are of interest as food fish, they are some of the most abundant members of the community – in one case, a single species accounts for 37% of the total fish density at a given site (Moussa, 2009). In addition, several species are of high potential commercial interest to the aquarium industry as ornamental fishes, whose populations are often understudied and life histories poorly understood (Green, 2003; Wabnitz et al., 2003). These estimates should therefore be of substantial value for local science

and management, not only as they reflect the area-specific LWR, but because they provide information for an industry with critical knowledge gaps.

Materials and methods:

Fishes were collected from five islands in the Society Islands archipelago of French Polynesia in November 2018, February–March 2019, and May–June 2021, and from three islands in the Tuamotu archipelago in September–October 2021. All fishes were collected from 5–15 m depth using three-prong spears or fish anesthetic with hand nets (1-mm mesh) and barrier nets (6-mm mesh). Morphometric data, including standard length, fork length, total length (all length measurements: 0.1 cm precision) and weight (W: 0.01 g precision) were collected shortly after collection wherever possible. When field conditions prevented immediate collection of accurate length and weight data due to the difficulty of obtaining such measurements on small boats, fishes were photographed immediately in the field with rulers and frozen for later analysis in the laboratory. In the laboratory, frozen and thawed weights were obtained for all fishes collected from the Society Islands in 2019, regardless of whether in-field weight data was obtained. For fishes collected from the Society Islands and Tuamotus in 2021, weights were only taken in the laboratory. Measurements of standard length, fork length, total length, and body depth were taken using ImageJ (Schneider et al. 2012) for all fishes collected from the Society Islands in 2019 and 2021 and from the Tuamotus in 2021. Calipers were used to collect measurements of standard length, fork length, total length, and body depth for all fishes collected from Moorea in 2018. To account for the potential effects of freezing on fish body weight, we used conversion factors to convert frozen weights to presumed ‘field weights’ using established conversion factors (Akiona et al. 2021) to account for the loss of mass that occurs during the freezing

process. Smaller fishes lose a higher proportion of weight during the freezing process due to greater surface-to-volume ratios. However, no appreciable differences in parameter estimates for the conversion function were observed from species spanning different ranges of body sizes, and we therefore calculated 'field' or 'fresh' weights using the following equation:

$$W_{FIELD} = W_{FROZEN} + (0.0946)W_{FROZEN}^{(0.5620)}$$

All subsequent analysis used the mean weight from three values (frozen weight, thawed weight, converted field weight) to determine LWR.

To calculate LWR, we fit a standard length-weight model to all species using:

$$W = aL^b$$

where W is the weight of the fish, L is the length of the fish, parameter *a* is the scaling coefficient for the weight at length of the fish species, and parameter *b* is the shape parameter for the body form of the fish species. In all cases we used total length in cm for our measure of L. Parameters *a* and *b* were calculated using the linear regression of the log-transformed equation to account for heteroscedasticity in the untransformed relationship:

$$\log(W) = \log(a) + b\log(\mathrm{L})$$

where log(*a*) is the intercept and *b* is the slope. Statistical analysis was conducted in the statistical language R Version 3.6.1 (R Core Team, 2020). As measurement errors can cause outliers that bias the estimates of length-weight parameters, we removed any outliers that were likely to be the result of errors in measurement. Outliers were defined as any datum that was four or more standard deviations away from the mean model fit in log-log space, as in Kamikawa et al. (2015). We then re-fit the regression equations after omitting the outliers. Species were included only if the coefficient of determination (r^2) of the model fit was 0.6 or greater. All

parameter estimates were compared to the Bayesian prediction of parameters for each species using FishBase (Froese & Pauly, 2022).

Results

A total of 1,930 individual fish were collected and analyzed for length-weight relationships as reported in Table 2.1. LWR for *Pycnochromis xanthura* (n=7) and *Pycnochromis acares* (n=26) are not reported, on the basis of poor model fits (r^2 = 0.538 and r^2 =0.237, respectively). We report increases in reported maximum lengths for two species (*Pseudanthias mooreanus* and *Pycnochromis vanderbilti*) when compared with values available in the published literature and FishBase version 04/2022 (Froese & Pauly, 2022). Nine of the estimated parameters fall outside the range of previous predictions on accessible databases based on Bayesian predictions generated for each species based on body shapes (Froese, Thorson, & Reyes, 2014), with *a* values higher than the 95% confidence interval of Bayesian prediction and *b* values lower than the 95% confidence interval of Bayesian prediction.

Discussion

Parameter estimates for length-weight relationships in fishes are known to vary by region and with environmental conditions, making region-specific parameters especially important, although challenging to collect for high-diversity fisheries with little available data. The aim of our study was therefore two-fold: 1) to increase the set of robust data on these fishes by providing parameter estimates for species that do not have published LWR in the literature or databases and for those with parameter estimates based on small sample sizes, and 2) to provide the area-specific LWR for French Polynesia for the target reef fish species. Our estimates include length-weight parameters for multiple species that are not represented in the literature or databases

(*Pycnochromis vanderbilti*, *Pseudanthias mooreanus*), for those with reported relationships that are based on low sample sizes (*Pseudanthias pascalus, Pycnochromis iomelas*), and for those with parameters that are under doubt due to low sample size (*Stegastes fasciolatus*) in databases (FishBase version 04/2022, Froese & Pauly, 2022). Many of our estimated parameters fall outside the range of the Bayesian predictions for each species based on body shapes (Froese, Thorson, & Reyes, 2014).

French Polynesia hosts one of the longest-running long-term datasets on coral and fish assemblages on the island of Moorea (Galzin et al., 2016; Galzin & Legendre, 1987). Monitoring data on the fish assemblages at multiple permanent sites at this island began in 1983 and continues to be conducted by researchers at CRIOBE. Our parameter estimates cover some of the most abundant species observed in the underwater visual surveys at these long-term monitoring sites and several of the species are of commercial interest as ornamental fishes in the aquarium industry (Lecchini et al., 2006). It has been estimated that between 90–99% of exploited marine ornamental fishes are directly collected from the environment (Sadovy and Vincent, 2002; Wabnitz et al., 2003), in part due to difficulties of maintenance and captive breeding in aquaria. As such, more accurate information on the growth and condition of these fishes in their natural environments is essential. The provision of these LWR therefore allows for improved accuracy of biomass calculations and growth of coral reef fishes from the region, with a particular focus on some of the most abundant, small-bodied species encountered in underwater visual surveys in the region.

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Table 2.1. *Length-weight relationships ($W=aL^b$) for 11 species from French Polynesia (Society and Tuamotu Archipelagos).* Abbreviations: a, intercept; b, slope; CI, confidence interval; Lmax, maximum total length; Lmin, minimum total length; n, number of individuals; r^2, coefficient of determination; Wmax, maximum total weight; Wmin, minimum total weight. [a] Expanded size range. * Higher than 95% CI of Bayesian prediction. ** Lower than 95% CI of Bayesian prediction. Gray shading: one or less prior reported a-b values in the literature.

Family	Scientific Name	n	Lmin (cm)	Lmax (cm)	Wmin (g)	Wmax (g)	a	95% CI of a	b	95% CI of b	r^2	Length type
Acanthuridae	*Acanthurus nigricans*	218	5.9	20.4	10.36	207.43	0.02400	0.0565-0.0822	2.988	2.8952-3.0809	0.949	TL
Acanthuridae	*Ctenochaetus striatus*	224	8.8	22.3	14.76	199.14	0.08471*	0.0509-0.1411	2.424**	2.2491-2.5995	0.778	TL
Cirhhitidae	*Paracirrhites arcatus*	215	3.4	11.8	0.59	44.5	0.01810	0.0140-0.0233	3.002	2.8867-3.1180	0.927	TL
Pomacentridae	*Pycnochromis iomelas*	266	2.1	6.7	0.18	4.23	0.12548*	0.0370-0.0568	2.409**	2.2757-2.5429	0.829	TL
Pomacentridae	*Pycnochromis margaritifer*	203	2.8	8.2	0.34	8.06	0.03503*	0.0271-0.0452	2.607**	2.4619-2.7518	0.883	TL
Pomacentridae	[a]*Pcynochromis vanderbilti*	25	3.0	6.5	0.50	2.80	0.03424*	0.0201-0.0582	2.446**	2.0846-2.8079	0.923	TL
Pomacentridae	*Stegastes aureus*	37	6.3	11.3	4.75	29.44	0.03243*	0.0095-0.1103	2.854**	2.3122-3.3396	0.794	TL
Pomacentridae	*Stegastes fasciolatus*	238	5.9	10.4	7.43	28.90	0.08345*	0.0451-0.1546	2.428**	2.1425-2.7136	0.600	TL
Serranidae	*Cephalopholis urodeta*	217	11.0	21.0	18.24	148.46	0.03894*	0.0242-0.0625	2.707**	2.5371-2.8759	0.823	TL
Serranidae	*Pseudanthias pascalus*	187	2.1	18.2	0.08	28.28	0.02925*	0.0231-0.0371	2.530**	2.3950-2.6651	0.907	TL
Serranidae	[a]*Pseudanthias mooreanus*	67	3.5	10.6	1.61	8.30	0.12891*	0.1183-0.2318	1.813**	1.6346-1.9921	0.871	TL

Electronic Supplementary Material for:

Length-weight relationships for abundant coral reef fish species from eight islands in French Polynesia

Beverly J. French*, Brian J. Zgliczynski, Chelsea L. Wood, Alison J. Haupt, Serge Planes and Stuart A. Sandin

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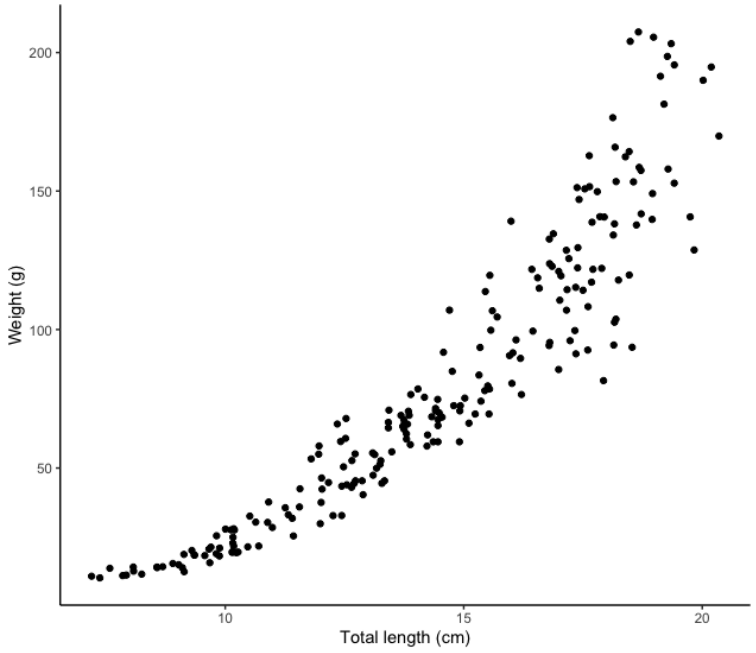

Figure S1. Plot of total length (cm) by weight (g) for *Acanthurus nigricans* (n=218).

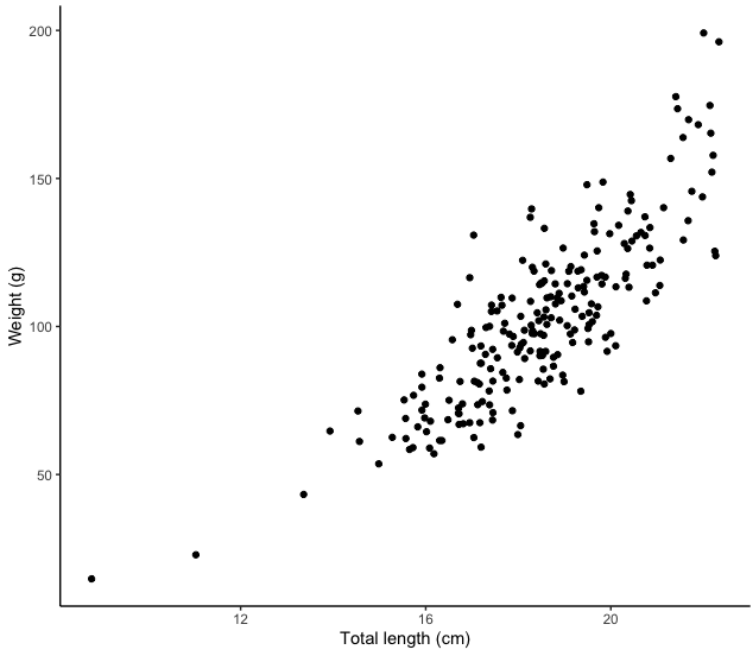

Figure S2. Plot of total length (cm) by weight (g) for *Ctenochaetus striatus* (n=224).

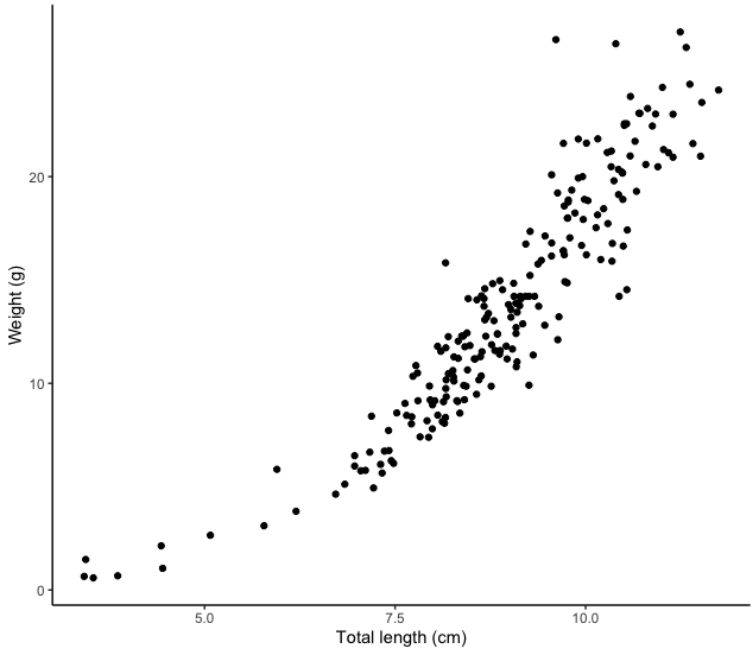

Figure S3. Plot of total length (cm) by weight (g) for *Paracirrhites arcatus* (n=215).

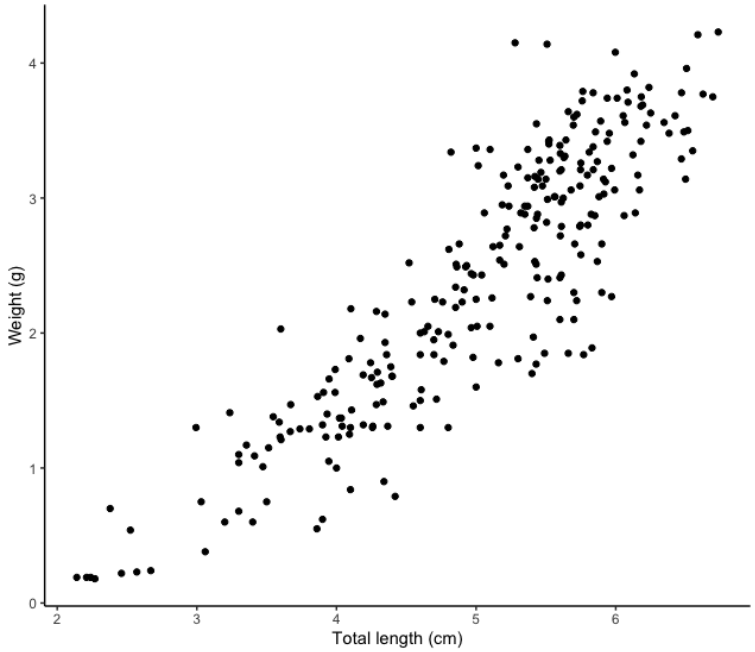

Figure S4. Plot of total length (cm) by weight (g) of *Pycnochromis iomelas* (n=265).

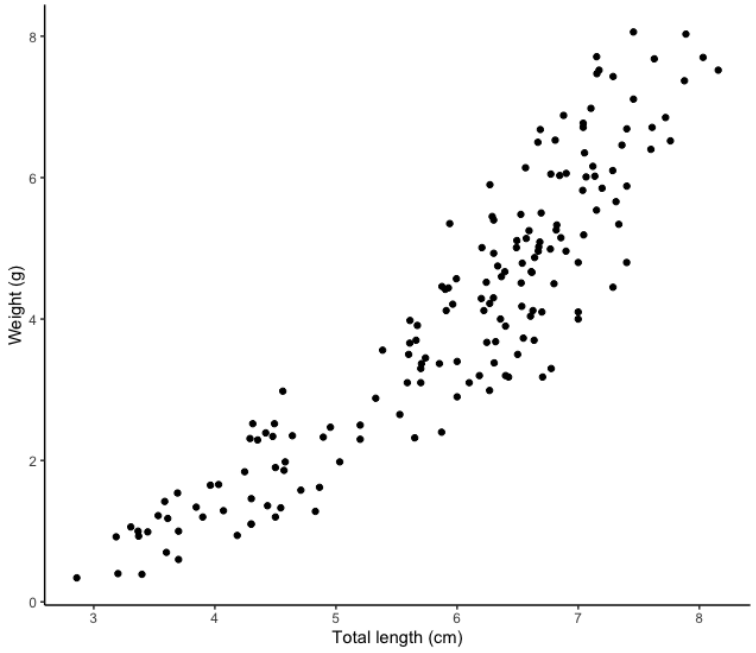

Figure S5. Plot of total length (cm) by weight (g) for *Pycnochromis margaritifer* (n=203).

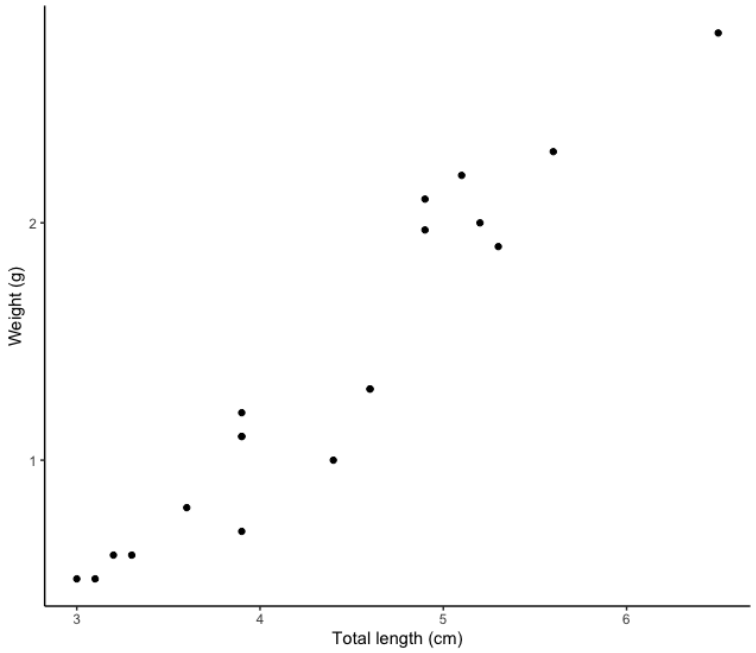

Figure S6. Plot of total length (cm) by weight (g) of *Pycnochromis vanderbilti* (n=17).

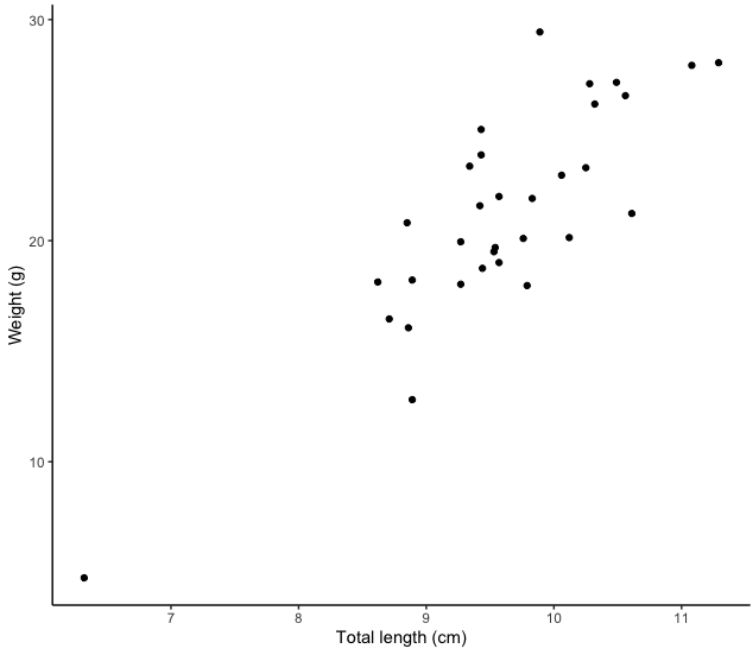

Figure S7. Plot of total length (cm) by weight (g) for *Stegastes aureus* (n=37).

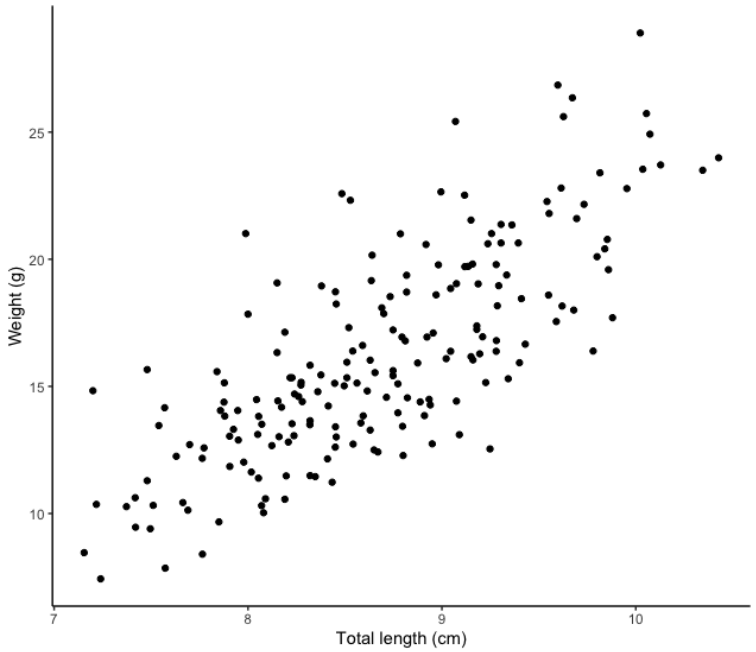

Figure S8. Plot of total length (cm) by weight (g) for *Stegastes fasciolatus* (n=238)

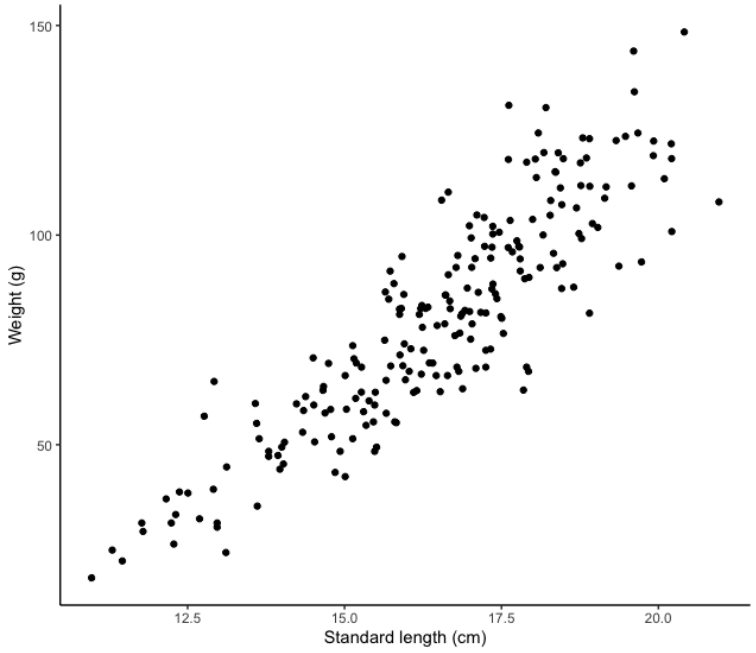

Figure S9. Plot of total length (cm) by weight (g) for *Cephalopholis urodeta* (n=217).

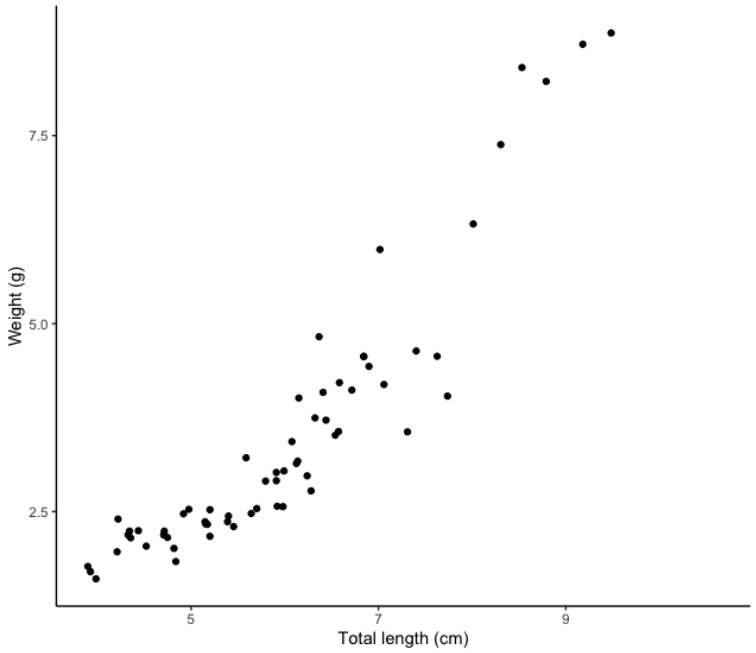

Figure S10. Plot of total length (cm) by weight (g) for *Pseudanthias mooreanus* (n=67).

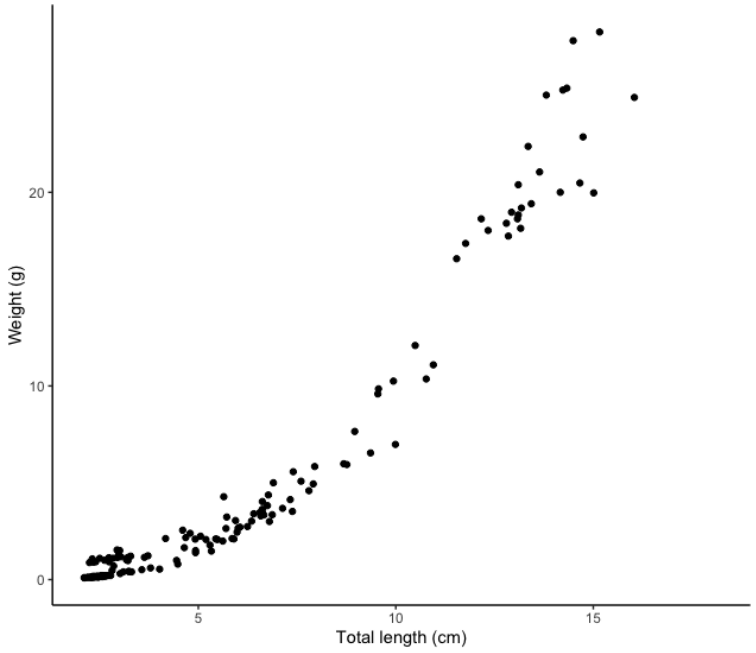

Figure S11. Plot of total length (cm) by weight (g) for *Pseudanthias pascalus* (n=187).

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CHAPTER 3

TEMPORAL PATTERNS OF CLUSTERING IN PLANKTIVOROUS CORAL REEF FISHES: A COMMUNITY PERSPECTIVE

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Abstract

Understanding and quantifying variability and predictability in community assemblages is necessary for ecologists attempting to derive accurate interpretations from observations of community structure. However, documenting accurate changes in community composition has proven challenging, particularly for species-rich ecosystems, in part because all methods of observation have limitations. Here we investigate temporal patterns in the coral reef fish communities on the island of Moorea in French Polynesia. Divers deployed remote video on the forereef of reefs on the northern shore of the island at the 10-14 m depth range from morning to evening to encompass the daily activities of the entire diurnally-active, non-cryptic assemblage of coral reef fishes. Using a metric of temporal dispersion, we tested for departures from randomness which indicate clustering or regularity of fishes in time, to address whether there are temporal patterns of aggregation in coral reef fishes and if these patterns differ across trophic guilds. Our results demonstrate that the coral reef fish community in the shallow (10-14 m depth) forereef habitats of Moorea revealed patterns of temporal regularity and clustering, with most groups demonstrating either regularity or randomness in time, and planktivores exhibiting high levels of clustering across all time categories. Importantly, these findings suggest that planktivores are likely to represent a significant source of variability for underwater visual surveys, and they appear to be responding to episodic environmental events throughout the day as a coordinated trophic guild.

Introduction

Understanding and quantifying variability and predictability in community assemblages is necessary for ecologists attempting to derive accurate interpretations from observations of community structure (Blonder et al., 2017; Giron-Nava et al., 2017; McGill, 2019). These observations are of great general interest, as they have the potential to yield insights into long-standing questions in community ecology (Paine, Deasey, & Duthie, 2018) and enable the tracking of populations and community change through time for improved monitoring and management.

It is axiomatic that the accuracy of these interpretations and any subsequent predictions are only as good as the underlying data. However, documenting accurate changes in community composition has proven challenging, particularly for species-rich ecosystems, in part because all methods of observation have limitations (Lawton, 1999; Preston, 1948). These limitations remain despite robust efforts to minimize bias and maximize accuracy of approaches.

The status of coral reef fish populations is of urgent interest, given reports of dramatic changes in populations and broad concerns about the status of coral reefs globally (Moritz et al., 2018). The dramatic declines documented in the Caribbean (Cramer et al., 2021; Jackson, Donovan, Cramer, & Lam, 2012) raised concerns about the future of coral reefs in the Pacific, given the immediate to near-term effects of increasing coastal development and fishing pressure, as well as the longer-term impacts of climate change (Knowlton & Jackson, 2008; Malhi et al., 2020; Sandin et al., 2008). Strona and colleagues recently conducted a thought experiment in which they carried out a statistical extrapolation of the response of coral reef fish communities to complete coral loss (Strona et al., 2021). They found that, were this to occur, current global tropical reef fish richness would decline by approximately 50 percent. While clearly an extreme

example, it is undeniable that anthropogenic effects are causing changes to natural communities (Graham et al., 2017; Williams, Gove, Eynaud, Zgliczynski, & Sandin, 2015; Wood, Sandin, Zgliczynski, Guerra, & Micheli, 2014; Wood et al., 2018), with some of the most striking changes to loss of abundance occurring across large species assemblages, including still-common species (Rosenberg et al., 2019). Furthermore, coral reefs are highly variable and dynamic ecosystems, complicating the underlying causes of these changes. These characteristics highlight the importance of accurate monitoring and assessment of natural communities through periods of change.

One of the primary ways that fish community structure is assessed for coral reefs in a non-extractive way is through underwater visual censuses (UVCs), which aim to provide snapshots of the entire diurnally-active, non-cryptic reef fish community (Brock, 1954; Edgar, Moverley, Barrett, Peters, & Reed, 1997). A report which assessed the use of underwater survey methods found that the majority of diver-based methods used for community-wide assessments of reef fish assemblages use a variation on one of three main methods: belt transect surveys, sometimes called strip or line transects; stationary point count surveys; and timed swim surveys, sometimes called roving diver surveys (Caldwell, Zgliczynski, Williams, & Sandin, 2016). These methods have differences in relative performance, with tradeoffs and biases that users must consider when determining their choice of assessment methodology (McClanahan et al., 2007; Usseglio, 2015).

Compared with community surveys such as UVCs, extended observational behavioral studies are used to provide detailed insights into focal animal behavior (Altmann, 1974). Although such studies provide high-resolution data and rich insights into our understanding of animal behavior, including coral reef fishes, due to the time-intensive nature of detailed

behavioral studies, these are usually focused on individual species or functional groups (Gil & Hein, 2017).

The reef fish communities of Moorea are the site of long-term monitoring by researchers at CRIOBE, and more recently, researchers at the University of California Gump Research Station. At CRIOBE, the reef fish community has been monitored annually since 1987 using underwater transect surveys (Rene Galzin, 1987; René Galzin et al., 2016; Rene Galzin & Legendre, 1988).

Here we investigate temporal patterns in the coral reef fish communities on the forereef on the northern shore of the island of Moorea in French Polynesia. Divers deployed remote video at the 10-14 m depth range from morning to evening to encompass the daily activities of the diurnally-active, non-cryptic assemblage of coral reef fishes. All fishes within the camera field-of-view were then enumerated to the lowest possible taxonomic level at 10-second intervals for these recordings. Using a metric of temporal dispersion, we tested for departures from randomness which indicate clustering or regularity of fishes in time. These community-level data were used to address two fundamental questions of temporal patterns in animal activity that have the potential to affect our interpretations from synoptic observations of community structure: (i) are there temporal patterns of dispersion in coral reef fishes, and (ii) do these patterns differ across coral reef fish trophic guilds?

Materials and Methods

Study location and sites

All work was conducted on Moorea (17°30’S, 149°50’W), an island in the Society Islands archipelago of French Polynesia that has been the subject of intensive biological and oceanographic study (Adam et al., 2014; Adjeroud, 1997; Adjeroud et al., 2018; Edmunds,

Leichter, & Adjeroud, 2010; Leichter et al., 2013). Moorea is a high-relief volcanic island with an offshore fringing/barrier reef that separates a protected inshore lagoon from the open ocean (Leichter, Stokes, Hench, Witting, & Washburn, 2012). The north coast hosts two large bays, Opunohu Bay and Cook Bay, of ~5 km in length and ~1km in width. The coral reef ecosystem can be divided into three major reef types: a fringing reef, a barrier reef separated by a narrow sandy channel, and an outer reef slope, or forereef, which is separated from the barrier reef by the reef crest. The outer reef slopes on the north shore are characterized by spur-and-groove formations that run approximately perpendicular to the reef slope isobaths. The forereef of Moorea has been strongly impacted in recent decades by cyclones and crown-of-thorns starfish (COTS) outbreaks, with benthic communities showing high resilience and recovery (Adjeroud et al., 2018), despite a shift to dominance of corals in the genus *Pocillopora*. Live coral cover declined from an island-wide average of ~40 percent in 2007 to less than 1 percent, due to the combined effects of a COTS outbreak and the tropical cyclone Oli in 2010. Total coral cover in the reefs in the shallow lagoons remained relatively constant. Fish and benthic communities on the forereef and lagoon of Moorea at Tiahura have been monitored annually since 1987. The fish community is monitored by visual census using a 2 x 50 m benthic transect, in which fishes are systematically counted and identified to species level between the months of September and October.

In this study, videos were recorded at 10-14 m depth on the outer reef slope at three sites on the north shore of Moorea (*see Figure 1*) in 2018. These include 1) the Tiahura long-term monitoring site (17° 29’ 00.6" S, 149° 54’ 20.9" W), which is contained within the Tiahura marine protected area, a fully protected, no-take zone as established by the Plan de Gestion de l’Espace Maritime (PGEM) of Moorea in 2004, 2) a forereef site frequented by recreational

divers near to the mouth of Opunohu bay, which we designate as ‘Opunohu’ (17° 28' 54.552" S 149° 51' 21.24" W) for the remainder of this work, and 3) the site of Vaipahu (17° 28′ 39.4″ S 149° 50′ 56.2″ W) considered a control area of comparison with the marine protected area of Tiahura, where moderate research activities and monitoring takes place.

Video Recordings

Video recordings were conducted within discrete time periods (5:00-7:00, 8:00-10:00, 11:00-13:00, 14:00-16:00, and 17:00-18:00) designed to encompass the entire diurnal period of activity for reef fishes (daylight hours). Each recording lasted for ~1-1.5 hours depending upon the duration of the camera batteries. A pair of divers deployed the underwater stereo-video systems on the benthos in order to record the fish community without the presence of divers. Brightly colored zip ties were attached to the benthos so that the video systems could be placed in the same orientation for the different recordings and removed at the conclusion of the observation season. Two video systems consisted of two small action cameras (GoPro Hero5 Black), mounted at a distance of 90 cm from each other at an angle of 6° on steel frames. This set-up enabled three-dimensional (3D) measurements of objects within the frame of view. Two additional video systems without stereo-video capabilities consisted of a single small action camera (GoPro Hero4) mounted on flexible tripods. In total, 39 video recordings were taken between June 1st, 2018 and December 12th, 2018, of which 20 videos were recorded using the stereo-video systems.

Video annotation

Video from each dive was segmented into still frames for every 10-seconds of recording. These still frames were annotated by hand by trained observers, with all fishes within the field-of-view counted and identified to the finest possible taxonomic resolution. Accurate

identifications often required review of the video footage preceding and following the still shot in order to determine fish identities from variable approach angles with confidence. All taxonomic annotations were reviewed and any challenging identifications were discussed and identified to the next highest taxonomic resolution by experts. Although divers departed the recording area as rapidly as possible after camera system placement, the first two minutes of video after camera placement were discarded from analysis in order to avoid effects of the divers on the behavior of the reef fish community.

Statistical Analyses

The variance-to-mean ratio (VMR), or coefficient of dispersion, is a metric which allows for identification of departures from spatial and temporal randomness (Banerjee, 1976; Dale et al., 2002; Whitlock & Schluter 2009). In this work, we use VMR to look at departures from temporal randomness in patterns of activity in coral reef fishes from video surveys at different times of day.

First, using a bootstrapping approach, we estimated the VMR across the three time periods for each trophic group at each site. For each group i and each time period j at each site k, we calculated the mean $\mu_{i,j,k}$ and the variance ${\sigma^2}_{i,j,k}$ to find the VMR ($v_{i,j,k}$) where $\left(v_{i,j,k}\right) = \frac{{\sigma^2}_{i,j,k}}{\mu_{i,j,k}}$. We then repeated this process 10,000 times for each plot to obtain a distribution of VMR values for each trophic group. From this distribution, we estimated the bootstrapped mean VMR for each group. We then estimated the 95% confidence interval of this distribution $v_{i,0.025}$ and $v_{i,0.975}$ for each group i where $v_{i,\alpha}$ is the α^{th} quartile of the vector of bootstrapped mean VMR for each i. For those groups with values of $v_{i,0.025} > 1$, the group is clustered or aggregated in time, groups with $v_{i,0.975} < 1$ are even or regular in time, and groups where $v_{i,0.025} < 1$ and $v_{i,0.975} > 1$ are randomly distributed in time. This approach has been used before to test for

departures from spatial randomness in simulated quadrats from large area imaging approaches (Edwards et al., 2017).

All statistical analyses were completed using R version 4.1.3 and RStudio (R Development Core Team; www.r-project.org).

Results

In total, we counted 29,963 individual fishes from a total of 90 unique species (6,302 still frames). At Opunohu, we counted a total of 16,981 individual fishes from a total of 56 unique species (3,113 still frames). At Tiahura, we counted a total of 11,271 individual fishes from a total of 65 unique species (2,777 still frames). At Vaipahu, we counted a total of 1,711 individual fishes from a total of 36 unique species (414 still frames).

The most abundant fishes observed were planktivores in the genus *Pycnochromis*, especially *Pycnochromis iomelas* and *Pycnochromis acares*. Together, these two species made up 82.2 percent of the total abundance of all observed fishes combined across the three sites.

Of the three sites, only Opunohu had a sufficient number of sampling intervals with replication across trophic groups and species to estimate VMR at different times of day, with segments of the day divided broadly into morning (5:00-11:59), afternoon (12:00-15:59), and evening (16:00-sunset) categories. Tiahura had sufficient replication across sampling intervals to look at VMR for different trophic groups in the morning and evening categories. Due to the relatively consistency of the patterns across the different times of day, we grouped all of the trophic categories across timepoints for the site-specific comparisons. Top predators were not sufficiently abundant at Vaipahu for an across-site comparison (n=15), so we do not present results from this group for this analysis.

Depicted in Figure 3 are the temporal dispersion patterns of the trophic groups in this study at Opunohu. As shown here, only the planktivore trophic group is significantly clustered in time (VMR>1). Most trophic groups, including top predators, carnivores, and herbivores/detritivores, showed evidence for regularity (VMR <1). The coarse trophic categories of top predators, lower carnivores, and herbivores are all more regular in time than the expectation from a Poisson distribution for randomness, with the exception of the herbivore/detritivore group in the morning (v_i =0.410, $v_{i,0.025}$ = 0.19, $v_{i,0.975}$ = 1.15), and the lower carnivore group in the evening (v_i = 1.085, $v_{i,0.025}$ = 0.61, $v_{i,0.975}$ = 1.75), which are not significantly different from random. Planktivores exhibit higher levels of clustering in the morning (v_i = 5.745, $v_{i,0.025}$= 5.30, $v_{i,0.975}$ = 6.25) and afternoon (v_i = 7.954, $v_{i,0.025}$= 7.49, $v_{i,0.975}$ = 8.46) when compared to the evening (v_i = 3.338, $v_{i,0.025}$= 7.49, $v_{i,0.975}$ = 8.46).

At Tiahura, the patterns were also consistent between different times of day (*Figure 4*). Planktivores had high VMRs in both the morning (v_i =17.912, $v_{i,0.025}$= 15.83, $v_{i,0.975}$ = 20.79) and in the evening (v_i =11.438, $v_{i,0.025}$ = 7.96, $v_{i,0.975}$ = 15.97), once again exhibiting clustered distributions in time. Herbivores/detritivores and top predators were not significantly different from random in the evening, and lower carnivores were not significantly different from random in the morning, but otherwise exhibited evenness in time.

The site comparison, averaging across all time points for each site, also shows consistent patterns, with planktivores exhibiting clustering (VMR>1) for Tiahura, Opunohu and Viapahu (*Figure 5*). Planktivores had higher site-specific VMRs at Tiahura (v_i = 11.453, $v_{i,0.025}$= 9.93, $v_{i,0.975}$ = 13.48) and Vaipahu (v_i = 12.509, $v_{i,0.025}$= 10.63, $v_{i,0.975}$ = 15.07) when compared to Opunohu (v_i = 6.895, $v_{i,0.025}$= 6.57, $v_{i,0.975}$ = 7.24), but clustering was also more variable at Tiahura and Vaipahu.

Discussion

Our results demonstrate that the coral reef fish community in the shallow (10-14 m depth) forereef habitats of Moorea revealed consistent patterns of temporal regularity and clustering, with herbivores/detritivores, lower carnivores, and top predators demonstrating either regularity or randomness in time and planktivores exhibiting high levels of clustering. These patterns of clustering for this trophic group were likely strongly driven by the numerically dominant species *Pycnochromis iomelas* and *Pycnochromis acares.*

It is perhaps unsurprising that we found little evidence for random distributions in time for most of the trophic groups assessed in this study. Mutual attraction in natural populations should lead to aggregation, whereas mutual repulsion should lead to regularity, both of which should occur with some frequency due to competition and schooling behavior, such that truly random patterns should be rare. It is, however, surprising that herbivores/detritivores were not found to cluster, as some species within this category are known to form both intraspecific and mixed-species aggregations. In several instances, this group was not significantly different from random. We suggest that this pattern may be due to groups with opposing patterns of aggregation and competition causing repulsion such that it is impossible to distinguish when all species are grouped into one trophic category. As one example, large aggregations of *Ctenochaetus striatus* are known to occur in the Pacific between October-February and have been observed in Moorea (Weideli, Mourier, & Planes, 2015). *Ctenochaetus striatus* is the fourth most abundant species observed in this study but was not observed to cluster at any of the study locations. It is possible that such aggregations occurred at the time of this study, but that our recordings missed this occurrence, either because of depth differences in schooling behavior, as large spawning

aggregations have been observed in the shallow waters close to the pass of the barrier reef, or by chance.

The observations that predators and lower carnivores are more evenly distributed suggest avoidance behavior of competitors. In support of this, observations of species in the genera *Cephalopholis* and *Paracirrhites* in which individuals conduct circuits of repeated patterns in the area throughout the recording which suggest territoriality and perhaps patrolling of specific territories. It would be informative to repeat these studies in areas of higher predator density and at times of predator aggregation, as one might expect to see corresponding changes in the other trophic guilds in reponse to heightened predation risk.

Importantly, these findings suggest that planktivores are likely to represent a significant source of variability for underwater visual surveys, as they appear to be responding to episodic environmental events throughout the day as a coordinated trophic guild. Notably, this pattern was consistent throughout the entire diurnal period of monitoring, regardless of time of day. Recently, an extensive survey at 122 sites along a single island found that even models with site-specific anthropogenic, physical and biological predictors had only limited predictive power for the spatial variability in fish biomass (Sandin et al., 2022). We suggest that the patterns of clustering and evenness of different trophic groups found in this study may partly explain these results, as such behavioral patterns will contribute to statistical variability in sampling conducted with underwater visual surveys.

Our analysis identified divergence from a random temporal distribution and compared aggregation of different trophic guilds but did not directly test the processes underlying the observed patterns. We suggest that there are likely several processes involved, including both abiotic and biotic factors. It has been suggested, for example, that planktivorous reef fishes form

a “wall of mouths” that is capable of removing nearly all zooplankton from the seaward face of reefs (Hamner, Colin, & Hamner, 2007; Hamner, Jones, Carleton, Hauri, & Williams, 1988).

Additionally, planktivorous fishes should face a trade-off between foraging high in the water column, which receives rich material influxes of allochthonous plankton, but require them to forage further from the benthos, where they are more exposed to predators. This trade-off should result in aggregation and promote schooling behavior, as individuals seek to optimize their foraging opportunities while avoiding predation.

Furthermore, internal wave activity in Moorea, which result in surges of upward and onshore flow, peaks in October-May (Leichter et al., 2012), which partly overlaps with the recordings conducted in this study. Video observations showed periods of intense foraging activity and movement by planktivorous reef fishes, potentially coinciding with these fluxes of water flow and allochthonous plankton and nutrient input. These observations also suggest that there should be a relationship between current speed and enhanced foraging activity by planktivores. Further study with estimation of current speeds would allow for the testing of the hypothesis that planktivorous fishes are more active at times of higher current speed.

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Chapter Three, in part, is currently being prepared for submission for publication of the material: B. J. French, N. M. D. Schiettekatte, G. Siu, J. Pena, B. J. Zgliczynski, S. Planes, and S. A. Sandin. "Temporal patterns of clustering in planktivorous coral reef fishes: a community perspective." The dissertation author was the primary investigator and author of this material.

FIGURES

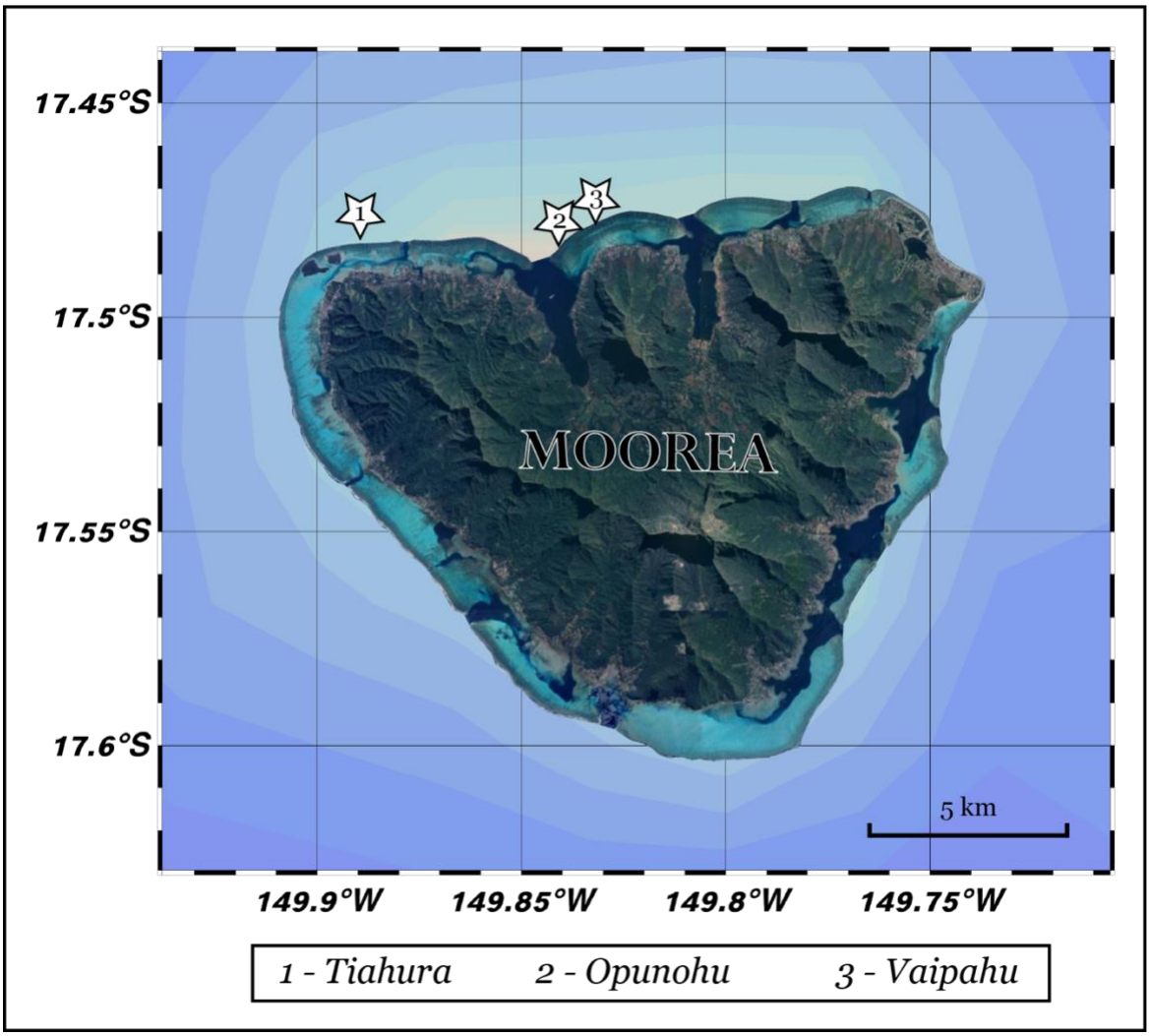

Figure 3.1. Map of study locations in Moorea, French Polynesia. All video recordings were conducted between 10-14m of depth on the forereef. Latitude and longitude are as follows, from the west, Site 1 = Tiahura (17° 29’ 00.6" S, 149° 54’ 20.9" W), Site 2 = ‘Opunohu’ (17° 28' 54.552" S 149° 51' 21.24" W), and Site 3 = Vaipahu (17° 28′ 39.4″ S 149° 50′ 56.2″ W).

Figure 3.2. Example still frames for analysis from the video recordings at the site of Vaipahu, showing variability in the fish community assemblages through time at a single site.

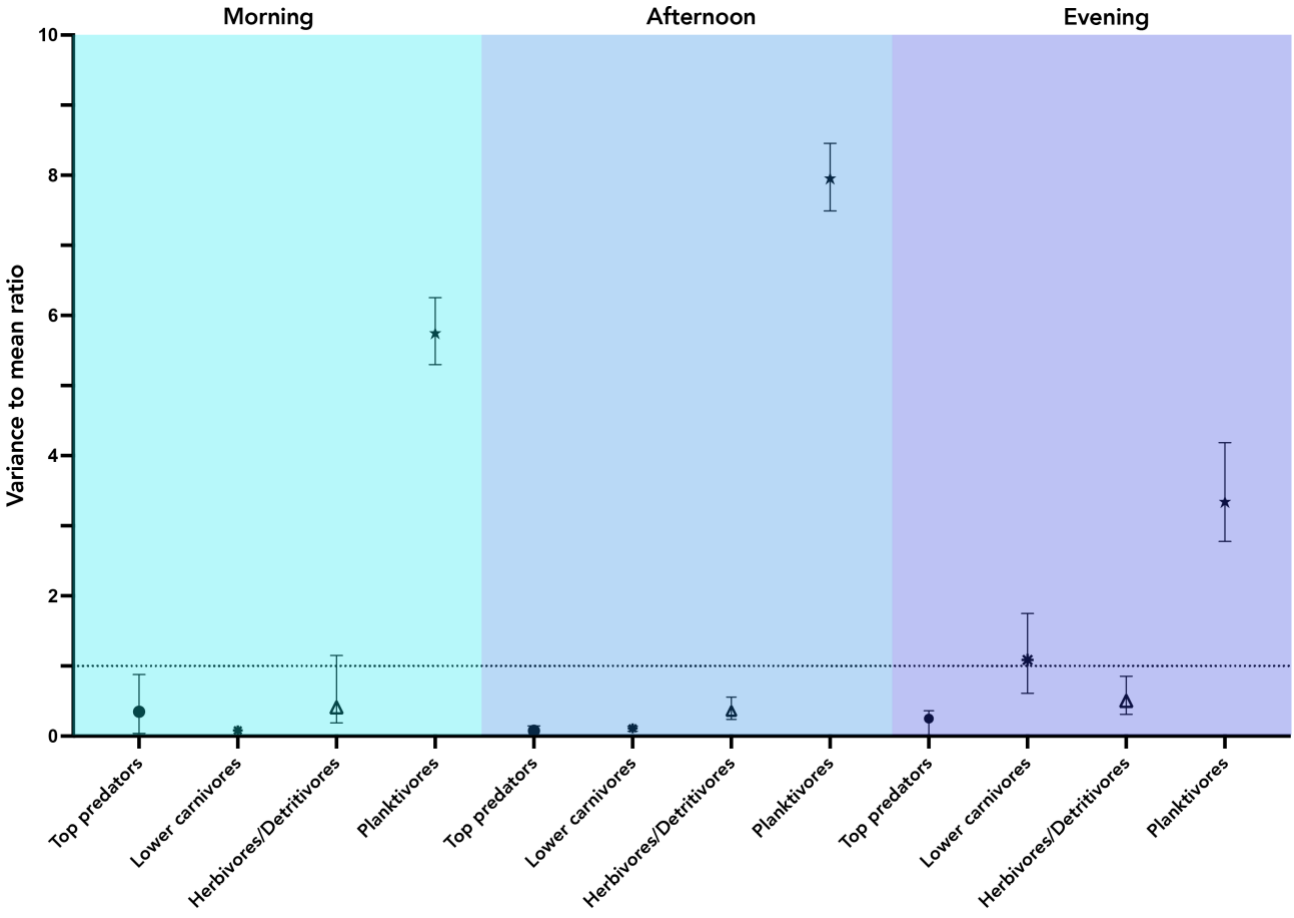

Figure 3.3. Diel dispersion patterns of trophic groups at Opunohu. Symbols represent the mean and 95% confidence intervals of the VMR distributions for each discrete time category. The dotted line indicates a VMR of 1 (random pattern in time as modeled by a Poisson distribution). 95% confidence intervals that do not overlap 1 indicate significant departures from randomness, with lines above representing clustering in time and lines below one demonstrating regularity or evenness in time.

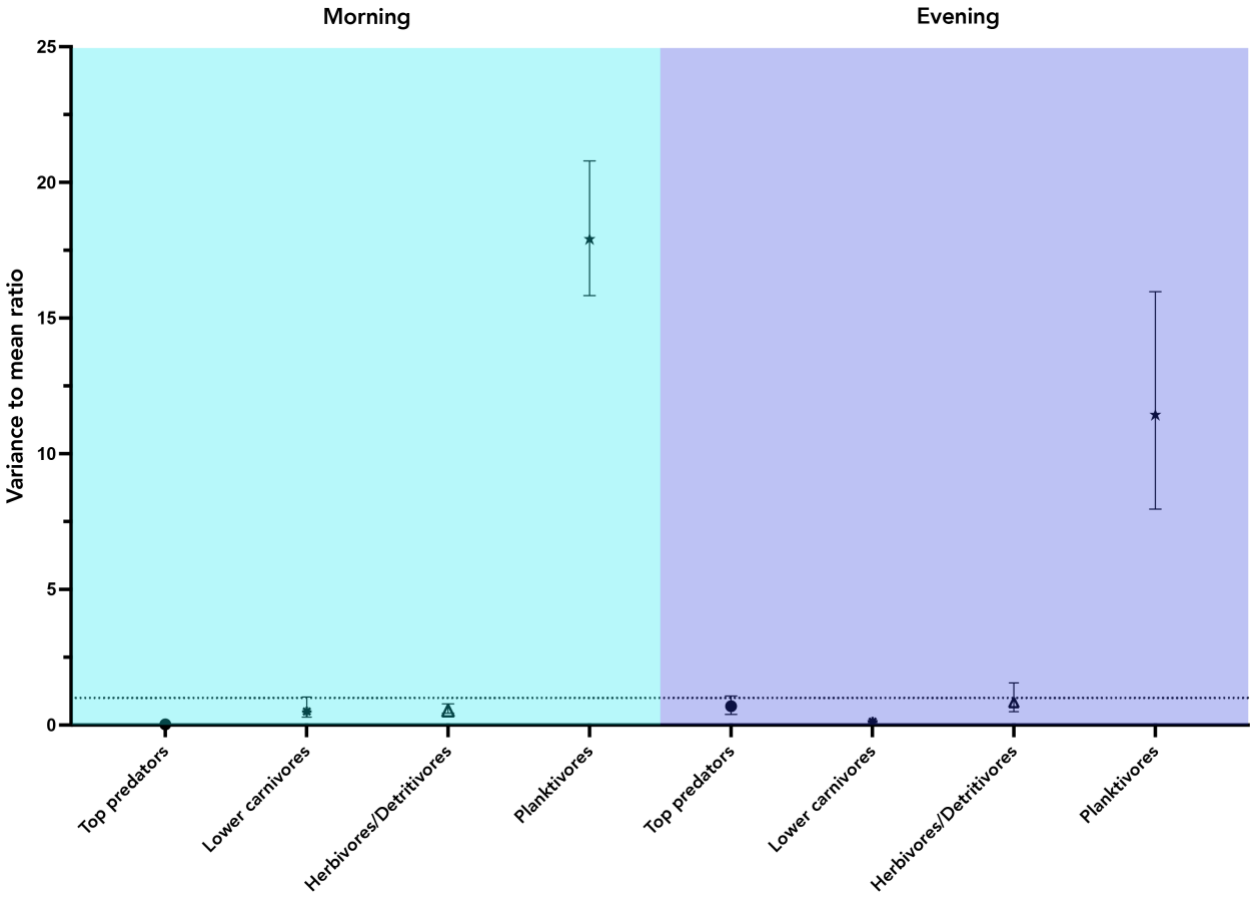

Figure 3.4. Dispersion patterns of trophic groups at Tiahura in the morning and evening. Symbols represent the mean and 95% confidence intervals of the VMR distributions for each discrete time category. The dotted line indicates a VMR of 1 (random pattern in time as modeled by a Poisson distribution). 95% confidence intervals that do not overlap 1 indicate significant departures from randomness, with non-overlapping groups above one representing clustering in time and non-overlapping groups below one demonstrating regularity or evenness in time.

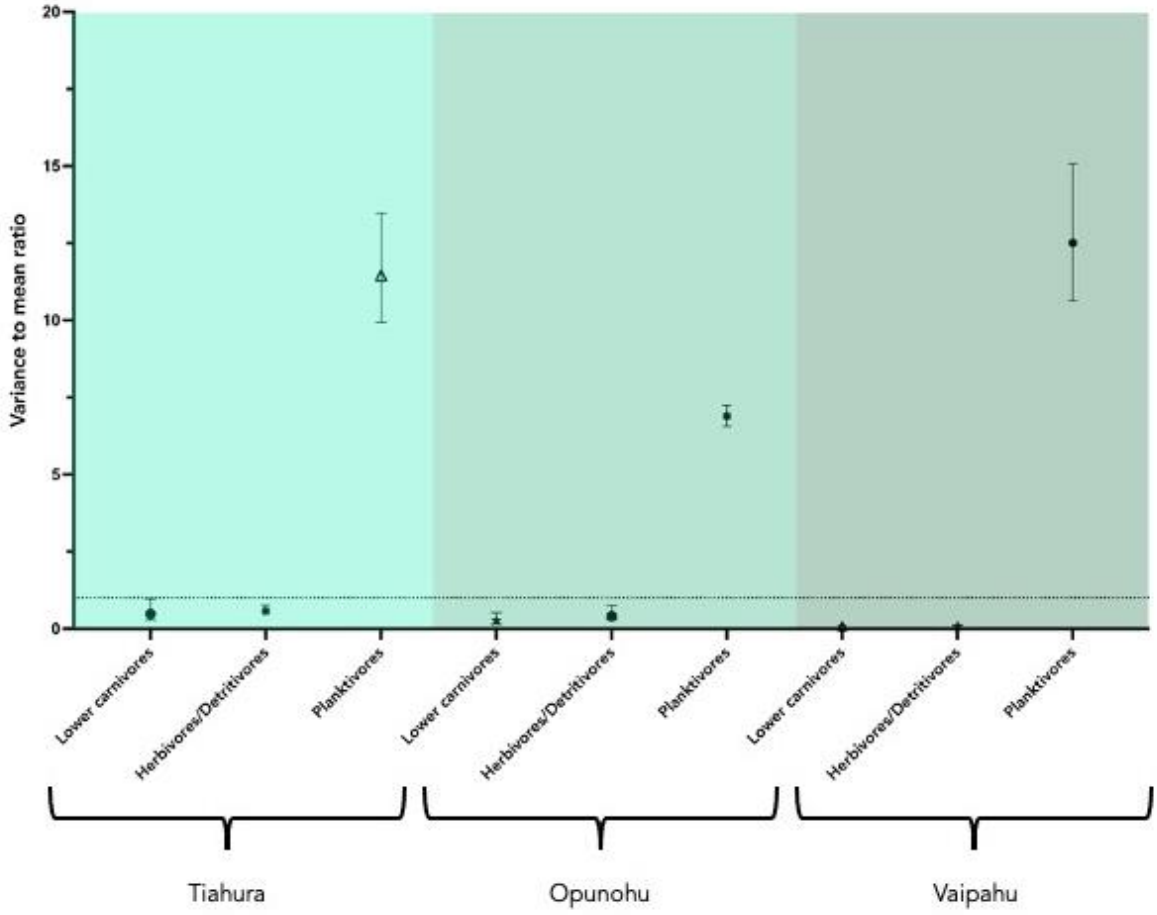

Figure 3.5. Dispersion patterns of trophic groups across all three sites. Symbols represent the mean and 95% confidence intervals of the VMR distributions for each discrete time category. The dotted line indicates a VMR of 1 (random pattern in time as modeled by a Poisson distribution). 95% confidence intervals that do not overlap 1 indicate significant departures from randomness, with non-overlapping groups above one representing clustering in time and non-overlapping groups below one demonstrating regularity or evenness in time. There was insufficient replication for the top predator group at Vaipahu so this group was removed from the comparisons.

TABLES

Table 3.1. List of fish species observed across the three sites on the forereef of Moorea, with trophic group designations, sorted by number of observations across all three sites. Total number of observations are grouped using an ordinal DACOR scale, with dominant (D) with >1,000 observations, abundant (A) with 101-1,000 observations, common (C) with 11-100 observations, occasional (O) with 2-10 observations, and rare (R) with only one observation. Trophic designations are based on literature, including reports from the region (Sandin & Williams 2010) and the expert opinion of the authors.

Species	Family	Trophic group	Relative abundance	Total # of Observations
Pycnochromis iomelas	Pomacentridae	Planktivore	D	19372
Pycnochromis acares	Pomacentridae	Planktivore	D	5261
Dascyllus flavicaudus	Pomacentridae	Planktivore	A	704
Ctenochaetus striatus	Acanthuridae	Herbivore	A	652
Acanthurus nigricans	Acanthuridae	Herbivore	A	569
Chaetodon pelewensis	Chaetodontidae	Lower carnivore (benthic invertivore)	A	329
Ctenochaetus flavicauda	Acanthuridae	Herbivore/Detritivore	A	327
Melicthys vidua	Balistidae	Planktivore	A	278
Pycnochromis xanthura	Pomacentridae	Planktivore	A	230
Plectroglyphidodon johnstonianus	Pomacentridae	Lower carnivore (benthic invertivore)	A	170
Pycnochromis margaritifer	Pomacentridae	Planktivore	A	144
Zebrasoma rostratum	Acanthuridae	Herbivore	A	142
Zebrasoma scopas	Acanthuridae	Herbivore	A	138
Odonus niger	Balistidae	Planktivore	A	137

Table 3.1. List of fish species observed across the three sites on the forereef of Moorea, with trophic group designations, sorted by number of observations across all three sites (continued)

Species	Family	Trophic group	Relative abundance	Total # of Observations
Cirrhilabrus scottorum	Labridae	Planktivore	A	114
Balistapus undulatus	Balistidae	Lower carnivore (benthic invertivore/piscivore)	A	111
Naso literatus	Acanthuridae	Herbivore	C	86
Acanthurus nigrofuscus	Acanthuridae	Herbivore	C	85
Chaetodon ornatissimus	Chaetodontidae	Lower carnivore (benthic invertivore)	C	84
Gomphosus varius	Labridae	Lower carnivore (benthic invertivore)	C	80
Cephalopholis argus	Serranidae	Top predator	C	79
Nematoeleotris magnifica	Microdesmidae	Planktivore	C	75
Chaetodon quadrimaculatus	Chaetodontidae	Lower carnivore (benthic invertivore)	C	70
Centropyge bispinosa	Pomacanthidae	Herbivore	C	61
Scarus oviceps	Scaridae	Herbivore	C	57
Paracirrhites arcatus	Cirrhitidae	Lower carnivore (benthic invertivore/piscivore)	C	55
Chlorurus sordidus	Scaridae	Herbivore	C	53
Epibulus insidiator	Labridae	Lower carnivore (benthic invertivore/piscivore)	C	49
Zebrasoma veliferum	Acanthuridae	Herbivore	C	45
Labroides rubrolabiatus	Labridae	Lower carnivore (benthic invertivore)	C	42
Cephalopholis urodeta	Serranidae	Top predator - piscivore	C	39
Chaetodon reticulatus	Chaetodontidae	Lower carnivore (benthic invertivore)	C	30
Centropyge flavissima	Pomacanthidae	Herbivore	C	25
Pseudocheilinus hexataenia	Labridae	Lower carnivore (benthic invertivore)	C	19

Table 3.1. List of fish species observed across the three sites on the forereef of Moorea, with trophic group designations, sorted by number of observations across all three sites (continued)

Species	Family	Trophic group	Relative abundance	Total # of Observations
Aphareus furca	Lutjanidae	Top predator (piscivore)	C	18
Scarus spp.	Scaridae	Herbivore	C	18
Parupeneus multifasciatus	Mullidae	Lower carnivore (benthic invertivore)	C	15
Lethrinus olivaceous	Lethrinidae	Top predator (apex)	C	14
Chaetodon unimaculatus	Chaetodontidae	Lower carnivore (benthic invertivore)	C	12
Acanthurus pyroferus	Acanthuridae	Herbivore	O	10
Plagiotremus tapeinosoma	Blenniidae	Lower carnivore (benthic invertivore)	O	9
Sufflamen bursa	Balistidae	Lower carnivore (benthic invertivore)	O	9
Balistoides viridescens	Balistidae	Lower carnivore (benthic invertivore)	O	8
Oxycheilinus unifasciatus	Labridae	Top predator (piscivore)	O	8
Pseudanthias pascalus	Serranidae	Planktivore	O	8
Scarus globiceps	Scaridae	Herbivore	O	8
Chaetodon lunula	Chaetodontidae	Lower carnivore (benthic invertivore)	O	6
Fistularia commersonii	Fistularidae	Lower carnivore (benthic invertivore/piscivore)	O	6
Parupeneus spp	Mullidae	Lower carnivore (benthic invertivore)	O	6
Acanthurus spp	Acanthuridae	Herbivore	O	5
Caranx melampygus	Carangidae	Top predator (apex)	O	5
Centropyge loriculus	Pomacanthidae	Herbivore	O	5
Parupeneus insularis	Mullidae	Lower carnivore (benthic invertivore)	O	5
Chromis vanderbilti	Pomacentridae	Planktivore	O	4

Table 3.1. List of fish species observed across the three sites on the forereef of Moorea, with trophic group designations, sorted by number of observations across all three sites (continued)

Species	Family	Trophic group	Relative abundance	Total # of Observations
Forcipiger longirostris	Chaetodontidae	Lower carnivore (benthic invertivore)	O	4
Halichoeres hortulanus	Labridae	Lower carnivore (benthic invertivore)	O	4
Naso spp	Acanthuridae	Planktivore/Herbivore	O	4
Scarus rubroviolaceus	Scaridae	Herbivore	O	4
Cantherhines dumerilii	Monacanthidae	Lower carnivore (omnivore)	O	4
Ctenochaetus binotatus	Acanthuridae	Herbivore/detritivore	O	4
Ctenochaetus spp	Acanthuridae	Herbivore/detritivore	O	4
Hemigymnus fasciatus	Labridae	Lower carnivore (benthic invertivore)	O	3
Naso hexacanthus	Acanthuridae	Planktivore	O	3
Scarus altipinnis	Scaridae	Herbivore	O	3
Zanclus cornutas	Zanclidae	Lower carnivore (benthic invertivore)	O	3
Acanthurus blochii	Acanthuridae	Herbivore	O	2
Acanthurus nigricauda	Acanthuridae	Herbivore	O	2
Cantherhines pardalis	Monacanthidae	Lower carnivore (omnivore)	O	2
Carcharhinus melanopterus	Carcharhinidae	Top predator (apex-shark)	O	2
Chaetodon ephippium	Chaetodontidae	Lower carnivore (benthic invertivore)	O	2
Halichoeres claudia	Labridae	Lower carnivore (benthic invertivore)	O	2
Lethrinus spp	Lethrinidae	Top predator (piscivore)	O	2

Table 3.1. List of fish species observed across the three sites on the forereef of Moorea, with trophic group designations, sorted by number of observations across all three sites (continued)

Species	Family	Trophic group	Relative abundance	Total # of Observations
Parupeneus cyclostomus	Mullidae	Lower carnivore (benthic invertivore)	O	2
Pseudocheilinus octotaenia	Labridae	Lower carnivore (benthic invertivore)	O	2
Thalassoma lutescens	Labridae	Lower carnivore (benthic invertivore)	O	2
Acanthurus olivaceus	Acanthuridae	Herbivore/Detritivore	R	1
Amanses scopas	Monacanthidae	Lower carnivore (omnivore)	R	1
Chaetodon vagabundus	Chaetodontidae	Lower carnivore (benthic invertivore)	R	1
Cheilinus undulatus	Labridae	Lower carnivore (benthic invertivore)	R	1
Cirrhilabrus exquisitus	Labridae	Planktivore	R	1
Forcipiger flavissimus	Chaetodontidae	Lower carnivore (benthic invertivore)	R	1
Gnathodentex aureolineatus	Lethrinidae	Lower carnivore (benthic invertivore)	R	1
Lethrinus xanthochilus	Lethrinidae	Top predator	R	1
Melicthys niger	Balistidae	Planktivore	R	1
Naso brevirostris	Acanthuridae	Planktivore	R	1
Naso vlamingii	Acanthuridae	Planktivore	R	1
Neocirrhites armatus	Cirrhitidae	Lower carnivore (benthic invertivore)	R	1
Pseudocheilinus tetrataenia	Labridae	Lower carnivore (benthic invertivore)	R	1
Scarus festivus	Scaridae	Herbivore	R	1
Scarus frenatus	Scaridae	Herbivore	R	1

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CHAPTER 4

PARADOX OF THE PLANKTIVORES: PATTERNS OF ABUNDANCE, PREY CONSUMPTION, AND GUT MICROBIAL COMMUNITIES IN ABUNDANT PLANKTIVOROUS FISHES FROM REEFS IN THE SOUTH AND CENTRAL PACIFIC

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Introduction

A fundamental question in ecology and evolution is the question of how the diversity of life that we observe on Earth came to be and how it is maintained and organized in biological communities (Hubbell, 2001; Hutchinson, 1961, 1959). One of the prevailing traditional mechanisms for the maintenance of diversity and coexistence theory is that of resource or niche-partitioning, or the idea that organisms partition resources from their environment in such a way that increases intraspecific competition while decreasing interspecific competition (Chesson, 2000; Roughgarden, 1976). A large body of theoretical work has validated initial assumptions that local coexistence can be promoted by species differing from each other in one or several ways (Chesson, 1991).

The food webs of coral reefs are strikingly complex, a result of the high species diversity of coral reefs and the complexity of basal sources, including multiple sources of primary productivity from oceanic and benthic production (Zgliczynski et al. 2019; Casey, Meyer, Morat, Brandl, and Casey 2019). At first glance, this complexity seems ideal for the development of fine-scale niche-partitioning. With multiple sources of primary productivity and high levels of diversity at many taxonomic levels (Plaisance et al., 2011), we might expect there to be an abundance of niches to fill and no lack of niche-partitioning. This is corroborated by studies that have found fine-scale dietary partitioning in closely related and ecologically similar species (Leray et al., 2019; McMahon et al., 2016). Other studies, however, have demonstrated remarkable dietary flexibility, high levels of trophic overlap, and dominance of dietary generalists on coral reefs (Bellwood et al., 2006; Frisch et al., 2016; Miller et al., 2019; Zgliczynski et al., 2019) that would seem to contrast with this view.

Accurate assignment of species into trophic groups and determinations of trophic niches have been challenging because many common approaches, such as stable isotope and visual stomach content analysis, have limitations, despite their invaluable contributions to our understanding of the feeding habits of consumers (Hyslop, 1980). Stomach content analysis (SCA), for example, is best interpreted as a snapshot of short-term feeding behavior, as identifiable prey remains are most often from recent meals. SCA is further complicated by the fact that prey organisms are subject to differential digestion, and partial digestion can prevent identification of contents to high taxonomic resolution (Hyslop, 1980). High proportions of empty stomachs in collected fishes, especially those fishes with rapid gut throughput times, also limit sample sizes and present high sample demands, which is not always practical for coral reef environments and especially for rare species. Stable isotope analysis (SIA) of tissue can provide longer timescale views of diet and is not plagued by the same issues as SCA, but hinges on accurate identification and collection of sources and cannot always provide the scale of detail required for fine-scale taxonomic differences in diet without often prohibitively extensive collections (Zgliczynski et al., 2019).

Integrating multiple methods that look at different timescales of integration from dietary behavior offers a way forward towards defining species' trophic niches. Metabarcoding and metagenomic sequencing have shown promise in providing information on possible prey sequences and the gut microbiome (French et al., 2020; Srivathsan et al., 2015) for interpretations of trophic niches and dietary behavior.

Still, determining that differences in axes of the "n-dimensional hypervolume" (Hutchinson, 1957) such as food or space use exist between species, is insufficient evidence for concluding that species can coexist, as MacArthur mentions in his original publication on the

population ecology of warblers in 1958 (MacArthur, 1958), and Siepielski and McPeek reminded us in 2010 (Siepielski and McPeek, 2007). Not all species differences will promote coexistence. A more rigorous test of the ability of species to persist together indefinitely is if each species will tend to increase when rare—the "invasibility" criterion for coexistence (Chesson and Ellner, 1989). Although tests of the invasibility criterion for species coexistence are most rigorously conducted through laboratory or experimental manipulations (Cothran et al., 2015), such experiments are difficult to conduct in practice, particularly for larger organisms with relatively long lifespans. However, we can integrate different sources of observational evidence from multiple techniques to interrogate the following questions for co-occurring species: (i) first, are there species differences in spatial distribution or trophic niches, and (ii) is there evidence for differential fluctuations of these species through time?

Here we incorporate information from multiple sources of evidence, including long-term underwater visual surveys (UVCs) from the well-studied location of Moorea and information from metagenomic sequencing from fishes collected from Moorea (French Polynesia) and the Southern Line Islands, to evaluate the evidence for possible coexistence in a guild of co-occurring planktivorous reef fishes.

Methods: Fish field collections

Specimen collections and dissections: Moorea, French Polynesia

Damselfishes (family: Pomacentridae) in the genus *Pycnochromis*[1] were collected from the island of Moorea in French Polynesia between October and December 2018. Collection efforts

[1] A note on species names: a recent taxonomic revision resurrected the name *Pycnochromis* for a group of fishes in genus *Chromis* sister to *Dascyllus* (Tang et al., 2021), including many of species herein. For readability, we move to *Pycnchromis* for the formerly *Chromis* species in this manuscript, noting that long term surveys currently retain the name *Chromis* spp. Chromis xanthura was the one exception, which has been delimited into a "*Chromis xanthura* species group" by Motomura et al. (2017) comprising *Chromis xanthura*, *Chromis opercularis* and *Chromis anadema*. Their topology was confirmed by Tang et al. 2021.

focused on *Pycnochromis margaritifer* (Fowler, 1946), *Pycnochromis iomelas* (Jordan and Seale, 1906), *Pycnochromis vanderbilti* (Fowler 1941), *Pycnochromis acares* (Randall and Swerdloff, 1973), *Pycnochromis pacifica* (Allen and Erdmann, 2020), and *Chromis xanthura* (Bleeker 1854). *Pycnochromis vanderbilti* is widespread throughout the central and western Pacific, often occurs in aggregations and is thought to feed on zooplankton, especially copepods. Images of the focal species highlighting morphological and coloration differences can be found in Figure 1. *Pycnochromis acares,* named for its diminutive size, is thought to be most closely related to the small-bodied *P. vanderbilti* and can often be found in the same region, but seems to stay within intraspecific groups within the mixed aggregations (Randall and Swerdloff 1973 and personal observations). In Moorea, they generally do not venture into the surge closer to the reef crest where *P. vanderbilti* can be found. They have also been observed feeding on zooplankton in the water column. *P. vanderbilti* has conspicuous stripes on the body and a black band on the lower caudal lobe. *Pycnochromis margaritifer* and *Pycnochromis iomelas* are another closely related pair of species. Both are found across the Pacific, with *Pycnochromis margaritifer* having a more widespread distribution according to current species records of collections and observations. The two species with the largest maximum body size, *Pycnochromis pacifica* (max SL: 7.6 cm) and *Chromis xanthura* (max TL: 17.0 cm), were observed to have larger ranges and were frequently observed as single individuals or in smaller schools (~5-6 individuals) in Moorea. Opportunistic sampling of *Pseudanthias pascalus* (family: Serranidae) was also conducted, for comparison of the *Pycnochromis* and *Chromis* with a more distantly related presumed zooplanktivore.

All fishes were collected from approximately 4-15m depth using spearguns or fish anesthetic with hand nets (1 mm mesh) and barrier nets (6 mm mesh) during daylight hours (0800-1700). Immediately after collection, fishes were placed on ice in individual ziplock bags

for transport to the lab. On return to the lab, morphometric data including weight (0.01 g precision) and length (total length, standard length, fork length, and body depth to 0.1 cm precision) were taken with corresponding images. All dissections were conducted the night of collection with bleach-sterilized equipment. All surfaces and equipment were bleached between dissections to prevent contamination between samples. Intestines from each fish were removed and the contents were carefully emptied into 1.5 ml Eppendorf tubes using sterile tools. Foregut and hindgut samples were separated for larger fish (*Pycnochromis xanthura*) with higher quantities of biomass in the intestine. For smaller fish with less material, foregut and hindgut samples were pooled. Otoliths were removed for later processing along with muscle tissue for stable isotopes (stored at -20°C). Fish intestinal contents were stored at -80°C until transport in liquid nitrogen to the United States, where they were immediately stored at -80°C.

Specimen collection and dissections: Southern Line Islands

Samples of *Pycnochromis margaritifer* were collected from the Southern Line Islands between October and November 2013 (Starbuck, Flint, Millennium, Malden, and Vostok). All fishes were collected during the daylight hours (08:00-17:00) using fish anesthetic with hand nets (1 mm mesh). Immediately after collection, fishes were frozen on ice and brought to the United States for further processing, including dissections of the intestines. Intestines were separated into foregut and hindgut and stored in separate 1.5mL Eppendorf tubes in RNAlater at -20°C. Ten fishes of approximately mean size given the collections from each island were selected for metagenomic sequencing. For comparability with the sample processing of the collections from French Polynesia, foregut and hindgut digesta were removed from intestines with sterilized tools and pooled prior to plating on 96-well plates for subsequent DNA extraction.

Specimen collections and dissections: La Jolla, California

Samples of *Chromis punctipinnis* were collected from an artificial reef in La Jolla, California on January 10th, 2019 between 9:30-11:30 during daylight hours. Fish were collected with barrier nets and hand nets. Immediately after collection, fishes were frozen on ice and brought to the laboratory for further processing. The fish were dissected following the same protocol for the fishes collected from French Polynesia. Briefly, intestinal contents were removed from the foregut and hindgut with sterile tools and pooled prior to storage at -80°C until subsequent processing.

DNA Extraction and Library Preparation

DNA extraction was conducted according to standard Earth Microbiome Project protocols (Thompson et al., 2017). DNA was extracted in single tubes to avoid well-to-well contamination using the ThermoFisher MagMAX Microbiome Ultra Nucleic Acid Isolation Kit with a modification in which the lysis step was performed in single 2 ml tubes, while the cleanup was performed on a KingFisher Flex robot using a magnetic bead cleanup. As well-to-well contamination is a known issue in plate-based lysis using vortexing for DNA extractions, and is particularly frequent in low biomass samples, this modification was introduced to reduce contamination at this step (Minich et al., 2019). A serial dilution of two positive controls (*Paracoccus spp.* and *Bacillus subtilis*) was included to estimate the limit of detection for the assay (Minich et al., 2018). Fish species were randomized on the DNA extraction plates according to species identity to prevent plate effects of species.

Sequencing libraries were prepared using a high-throughput version of the HyperPlus library chemistry (Kapa Biosystems) as optimized for nanoliter-scale liquid-handling robotics. Detailed protocols and methods can be found in (Sanders 2019 and Shaffer). DNA from each

sample was quantified using the Quant-iT PicoGreen dsDNA Assay kit and normalized to 5 ng in molecular-grade water with an Echo 550 acoustic liquid handling robot (Labcyte, San Jose, CA, USA). Reagents for each step of library preparation were added in 1:10 the recommended volumes using a Mosquito HTS micropipetting robot (SPT Labtech, Tokyo, Japan). Fragmentation was performed at 37°C for 4 minutes and A-tailing was performed at 65°C for 30 minutes.

To add sequencing adaptors, the Echo 550 robot was used to add universal adaptor "stub" adaptors and ligase mix to the end-repaired DNA, with the ligation reaction performed at 20°C for 60 minutes. AMPure XP magnetic beads and a BlueCat purification robot (BlueCat Bio, Concord, Massachusetts, USA) were used to clean adaptor-ligated DNA by adding magnetic bead solution to the sample, washing twice with 70% EtOH and resuspending in molecular-grade water. The Echo 550 robot was used to add i7 and i5 indices to adaptor-ligated samples without repeat barcodes. Amplification was conducted by adding PCR master mix to the cleaned, adaptor-ligated DNA and running for 15 cycles, followed by an additional purification with magnetic beads and a BlueCat robot. The Mosquito HTS robot was used to transfer all samples to a 384-well plate after elution into molecular-grade water and libraries were quantified using the Quant-iT PicoGreen dsDNA Assay kit. After PCR, a normalized pooled amount of DNA was pooled from all libraries using the Echo 550 robot, following the Katharoseq protocol (Minich et al. 2018), in order to enable downstream quantification across libraries. The library pool was run on an Illumina NovaSeq6000 (Illumina, San Diego, California, USA).

Bioinformatic Analyses

For the first workflow, all sequences were uploaded, demultiplexed and processed in Qiita (Gonzalez et al., 2018) according to Earth Microbiome processing recommendations. The study

ID on Qiita is 13903 and the analysis artifact IDs are 52183 and 50087. First, raw sequence data was converted to FASTQ files and demultiplexed to produce per-sample FASTQ files. Trimmed reads after adaptor and host removal were mapped to the Web of Life database of microbial genomes (Zhu et al., 2019) with Woltka classification (Zhu et al., 2021) to produce a BIOM table of taxonomic predictions for exploratory analysis of microbial communities. The Web of Life database has been curated to represent the phylogenetic breadth of Bacteria and Archaea. Previous work has compared mapping from the Web of Life to a curated database of NCBI reference microbial genomes (Rep200) and found little difference across environments (Shaffer et al., 2022). Sequences were rarified to 5000 sequences per sample for downstream analyses and explored in Emperor (Vázquez-Baeza et al., 2013). While rarefaction of samples does result in a loss of power, due to the discarded fractions of the original libraries (McMurdie and Holmes, 2014), we do this for comparability for downstream analyses given the high levels of variability in number of sequences for samples in this dataset.

For the second workflow, Illumina paired-end reads were joined, retaining the non-overlapping ends, and sequences were uploaded to the metagenomics analysis server MG-RAST (Meyer et al., 2008). The sequenced reads were translated *in silico* into predicted protein sequences and compared with the SEED database to provide taxonomic identifications and information on organism abundance using Blastx (Altschul et al., 1990) with a maximum e-value cutoff of 1 × 10-5, a minimum alignment cutoff of 15, and a minimum percent identity of 80%. In order to assess differences between the species from French Polynesia, a similarity profile routine (SIMPROF) test was conducted on relative abundance of bacterial taxonomic groups at the phylum level (taxonomic level 2) from the Qiita workflow using the *clustsig* package (Whitaker & Christman, 2010) implemented in R (R Development Core Team, www.r-

project.org). SIMPROF is a permutation procedure, which tests for the presence of sample groups in a priori unstructured sets of samples (Clarke et al., 2008). Analyses were based on 10,000 random permutations of the data.

Underwater visual surveys (Moorea): CRIOBE

Annual underwater visual surveys were conducted on Moorea (17°30'S, 149°50'W), a high relief volcanic island in the Society Islands archipelago of French Polynesia. The coral reef ecosystem at Moorea can be divided into three major reef types: a fringing reef, a barrier reef separated by a narrow sandy channel, and an outer reef slope, or forereef. The outer reef slopes on the north shore are characterized by spur-and-groove formations that run approximately perpendicular to the reef slope isobaths. Fish and benthic communities on the forereef, barrier reef, and fringing reef environments of Moorea have been monitored annually since 1987 by researchers at Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) at the site of Tiahura, with the first observation conducted in 1983 from the inception of the Tiahura radial (Galzin, 1987; Galzin and Legendre, 1988). Tiahura has been a designated a fully-protected, no-take marine protected area since the establishment of the Plan de Gestion de l'Espace Maritime (PGEM) of Moorea in 2004. The fish community is monitored by visual census using a 2 x 50 m benthic transect located at 12 m depth, in which fishes are systematically counted and identified to species level between the months of September and October. Fish abundance is averaged from four temporal replicates which are sampled during two days of counting by trained observers. We constrained our analyses for species abundance to October, for seasonal comparability with collections.

Results

Underwater visual surveys

Underwater visual surveys conducted at the site of Tiahura on the forereef of Moorea show differential abundance of focal planktivores through time (Fig. 2, 3, 4). Not all focal species were observed from the beginning of the surveys conducted by CRIOBE in 1983; for example, *P. margaritifer* was not observed until 1993 and *Pycnochromis acares* did not appear in the survey records until 2012. There was evidence for persistent spatial structuring especially between one similar species pair (*P. iomelas* and *P. margaritifer). P. margaritifer* were found consistently in shallower habitats (barrier reef) where *P. iomelas* are not observed. This spatial structuring was so strong that despite high levels of abundance from integrated surveys, video recordings conducted at slightly deeper isobaths (~12-14m depth) in 2018 only identified a few observations of *P. margaritifer*, compared to the abundant *P. iomelas.* Further, *P. margaritifer* were collected from the barrier reef in 2018 for metagenomic sequencing at ~2-3m depths, but no *P. iomelas* were observed in these habitats on the northern shore of Moorea. Personal observations also suggest that there is fine-scale spatial structuring on the forereef where these two species co-occur. While there is occasional mixing of these two species in schools, *P. margaritifer* is more abundant closer to the reef crest on the forereefs of the northern shore of Moorea and tends to stay closer to the benthos, while *P. iomelas* is found at deeper depths closer to the open ocean. *P. iomelas* was consistently more abundant than *P. margaritifer* in the forereef surveys on Tiahura (Fig. 3). *Pycnochromis acares* did not appear in the surveys into 2012, but quickly became the numerically dominant species at the survey site (Fig. 3). The shift coincides with a drop in abundance of *P. vanderbilti*, which suggests either that these species had been mis-identified in earlier surveys or that a true competitive shift in dominance occurred with the appearance of *P. acares*. *Chromis xanthura* was frequently more abundant than *Pycnochromis pacifica* on the forereef surveys, but also had higher overall and within-survey variability (Fig.

4). *Chromis xanthura,* especially juveniles of the species, were observed in the barrier reef habitat in 2018, but were not identified in this habitat in the Tiahura transect surveys.

Metagenomic sequencing

We used metagenomic sequencing to sequence the gut contents of six species of fishes in the genus *Pycnochromis* from the island of Moorea, French Polynesia, and included a comparison of another planktivore from French Polynesia (*Pseudanthias pascalus*), 50 individuals of *Pycnochromis margaritifer* from five islands in the Southern Line Islands for a comparison of intraspecific spatial variability, and a temperate species (*Chromis punctipinnis*) from La Jolla, California for phylogenetic comparability. DNA from 188 samples were successfully sequenced, including 16 dilution controls, yielding a total of 172 fish metagenomes available for downstream analysis. Sequencing depth was variable by sample with a minimum of 4,334 sequences (*Pycnochromis margaritifer*) and a maximum of 22,857,975 sequences (*Pycnochromis acares*).

The highest diversity α-diversity, defined here as the number of distinct species annotations with the RAST pipeline, was observed in *Pycnochromis iomelas* ($\alpha = 631$) from Moorea and the lowest was observed in *Pycnochromis margaritifer* from Jarvis ($\alpha = 26$). The mean α-diversity across all samples was 194.88. This includes both metazoan sequences (host and prey) and microbial sequences. While α-diversity would be expected to scale with sequencing depth, this was not what we found. Looking at the sequences that were successfully annotated, we found that α-diversity was not correlated with the number of sequences ($\rho = 0.137$; $p = 0.3475$). Investigation of the data suggests that α-diversity corresponded more strongly to the percentage of eukaryotic sequences in the metagenome, and in particular, to high levels of fish sequences (match to Actinopterygii or lower taxonomic levels).

We found differences in coarse microbial taxonomic structure by high-level region (e.g., archipelago) and islands, with samples clearly separating by archipelagos (Line Islands and Society Archipelagos) as demonstrated in the principal coordinate analysis (PCoA) in Figure 5. This axis of variation explained 22.5 percent of the total variance in the dataset.

Interestingly, when looking at only those samples collected from the island of Moorea (n=112), which were collected at the same point in time and stored under the same conditions, species do not cluster into significant groupings by their taxonomic identity (Fig. 6). There is significant structure as determined by a SIMPROF analysis (39 unique clusters with $p<0.05$), but surprisingly, it is not related to host species identity. None of the investigated sample parameters suggest a cause for the separation, with the exception of clustering of the four *Pycnochromis margaritifer* samples collected on the barrier reef instead of the forereef habitat. Like the other samples from French Polynesia, these metagenomes had high relative frequencies of feature matches to the bacterial phyla *Firmicutes*, *Proteobacteria* and *Bacteroides*, but lower frequencies of *Cyanobacteria* when compared to the fishes collected on the forereef (Fig. 5).

Discussion

Altogether, we found mixed evidence for ecological differences between species in a guild of co-occurring planktivorous reef fishes. At Moorea, fine-scale spatial differences and differences in abundance for the survey years between 1987-2019 were observed among *Pycnochromis iomelas*, *Pycnochromis margaritifer*, *Pycnochromis acares*, *Pycnochromis vanderbilti*, *Pycnochromis pacifica* and *Chromis xanthura*. Some of the variability in abundance observed in the surveys could be due to differences in temporal clustering (*B. French unpublished data; see Chapter 3*) of this group. It would be advantageous for species within this trophic guild to follow episodic environmental events of upwelling and nutrient input.

The evidence for trophic differences from the metagenomic sequencing demonstrated limited evidence for intraspecific clustering of the gut microbial taxa for the entire dataset or even among only those species collected from Moorea, but strong evidence for clustering by broad geographic location (Fig. 5, 6, 7). Intriguingly, several of the metagenomes contained sequences that were a match to *Cnidaria*, and in particular, sequences that were a match to known coral taxa at these coral reefs, including *Pocillopora*. It is quite possible that these are the result of secondary predation, incidental ingestion during predation attempts on the coral, or that coral polyps or mucus could even be the target of foraging by the fishes directly. Regardless of the mechanism of ingestion resulting in coral sequences in the guts of these zooplanktivores, this suggests—contrary to expectation—that these fishes are not solely relying upon allochthonous inputs of pelagic zooplankton and may be supplementing their diets with, or even primarily consuming, benthic prey. Laboratory studies have demonstrated that planktivorous reef fishes will feed on energy-rich coral larvae (Quimpo et al., 2019), and other studies have shown that predation by planktivorous fishes is a significant source of mortality for the coral larvae, with observations of feeding during the release of coral propagules during mass spawning events (Babcock et al., 1986; Pratchett et al., 2001).

Strong vertical stratification of zooplankton has been observed on reefs (Alldredge and King, 2009, 1977) and we had expected to see signatures of vertical zonation associated with the species foraging position in the water column. This led us to hypothesize that the species that were observed to stay closer to the benthos (*P. margaritifer* and *P. vanderbilti*) would have a stronger benthic signature, but sequences with a match to Cnidaria and Scleractinia were also found in species that forage high in the water column, including *P. acares* (12.4 percent of sequences were a match to the family Pocilloporidae for one individual) and *P. iomelas*. This

suggests that these species may opportunistically forage in both benthic and water column habitats. It is possible that some of the differences in diet and gut microbial structure that we observe may be due to ontogenetic shifts, as is known for fishes. Size ranges were constrained as much as possible to avoid such effects, but age differences between these samples are currently unquantified. Although this approach has strengths in the level of detail provided and the ability to look at genetic function in addition to identity, it also has limitations similar to some of the traditional methods for dietary analysis.

Overall, we revealed partitioning of space and evidence for both stable and dynamic differences in population abundance that may represent possible solutions to coexistence in a guild of closely-related and co-occurring group of zooplanktivorous reef fishes. Gut microbiome communities and presumed prey remains are less suggestive of dietary differences, and there may even be some unexpected overlap in benthic and water column resources that point to opportunistic foraging and an unrecognized connectivity to benthic sources of prey in this trophic guild.

We close by highlighting the importance of long-term monitoring for resolving relationships between species in a community setting. Coral reefs, like many ecological communities, are dynamic environments. Inferences of stable coexistence from a snapshot of resource partitioning at one point in time are misleading. While investigation of various axes within a single season can shed light on the mechanisms underlying community function, only monitoring through time can provide the perspective necessary to resolve the paradox of the planktivores.

Acknowledgements

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Chapter Four, in part, is currently being prepared for submission for publication of the material: B. J. French, J. J. Minich, N. M. D. Schiettekatte, G. Siu, B. J. Zgliczynski, M. Bryant, R. Knight, E. E. Allen, S. Planes, and S. A. Sandin. "Paradox of the planktivores: patterns of abundance, prey consumption, and gut microbial communities in abundant planktivorous fishes from reefs in the south and central Pacific." The dissertation author was the primary investigator and author of this material.

FIGURES

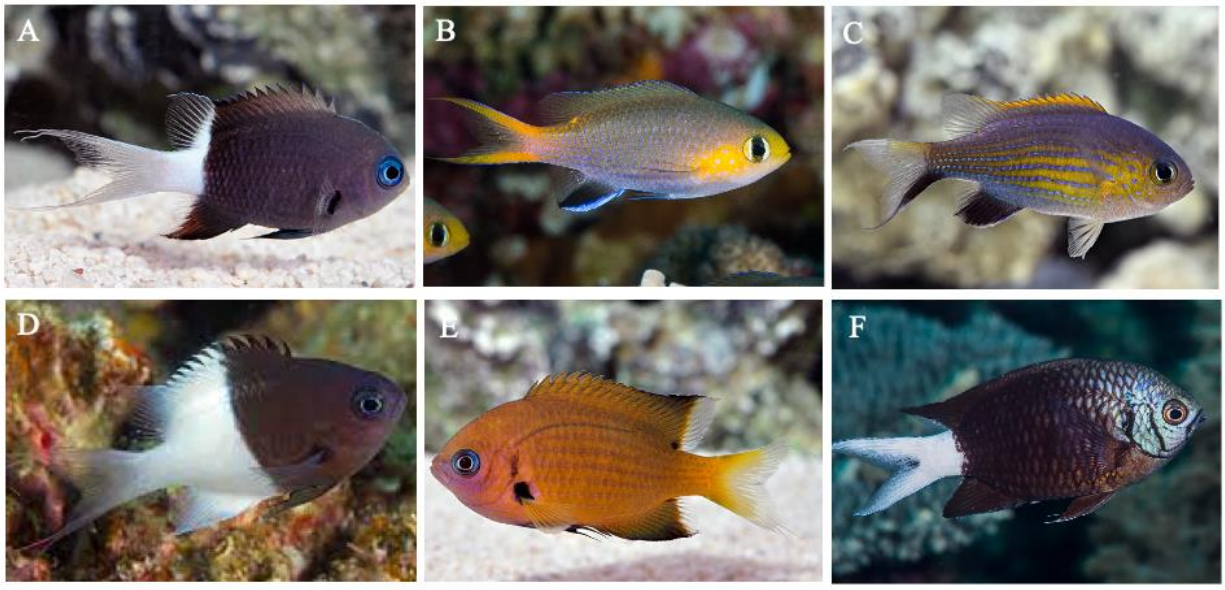


Figure 4.1. **Damselfishes (family: Pomacentridae) in the genus *Pycnochromis* from Moorea, French Polynesia.** From the top left (A) to the bottom right (F); (A) *Pycnochromis margaritifer*; (B) *Pycnochromis acares*; (C) *Pycnochromis vanderbilti*; (D) *Pycnochromis iomelas*; (E) *Pycnochromis pacifica*; and (F) *Chromis xanthura*. Images of *Pycnochromis acares* and *Chromis xanthura* are provided by Francois Libert and are included under a CC Attribution-NonCommercial-ShareAlike (CC BY-NC-SA 3.0) license. Images of *Pycnochromis iomelas*, *Pycnochromis margaritifer*, *Pycnochromis vanderbilti*, and *Pycnochromis pacifica* are courtesy of LiveAquaria.

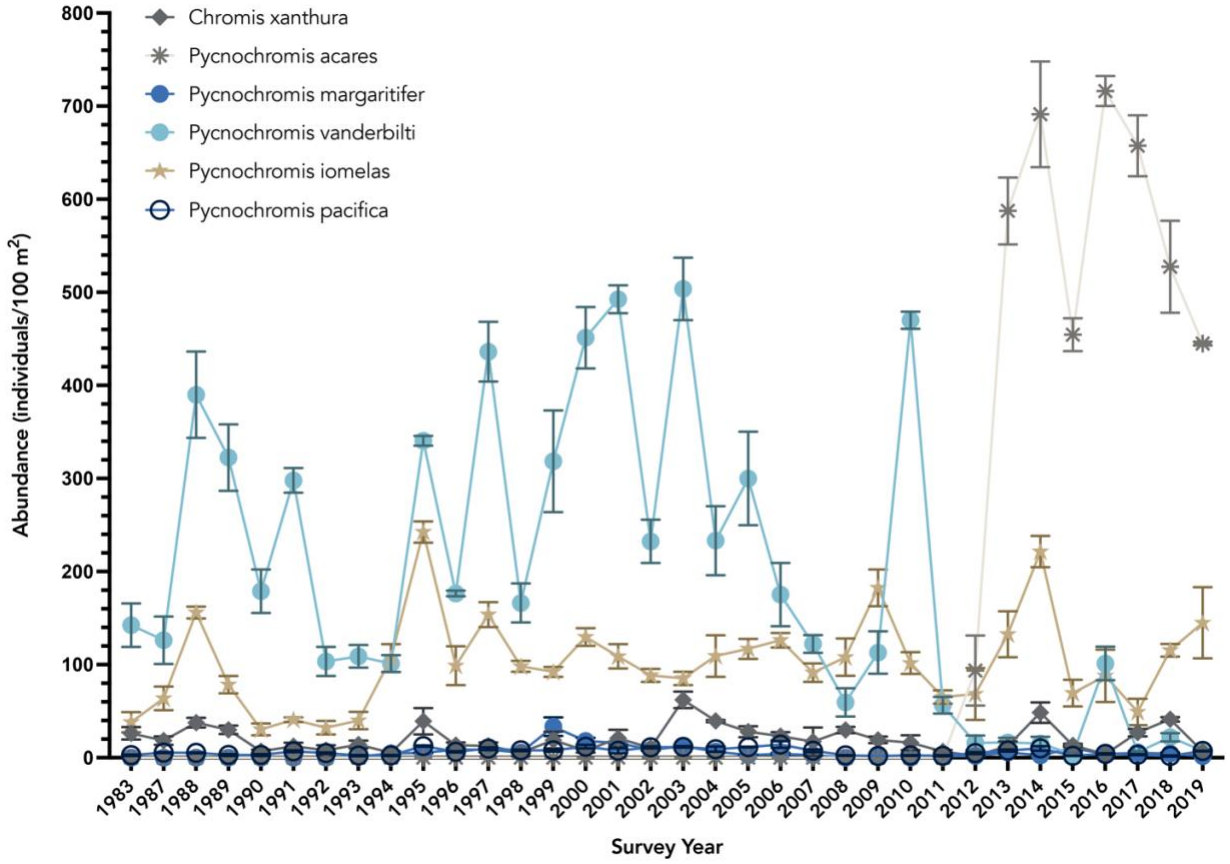


Figure 4.2. Time series of abundance (individuals/100 m^2) in the focal species of *Pycnochromis* at Tiahura in Moorea from underwater visual surveys. Annual surveys were conducted beginning in 1987, with the first timepoint collected in 1983 (Galzin et al., 2016; Galzin and Legendre, 1987).

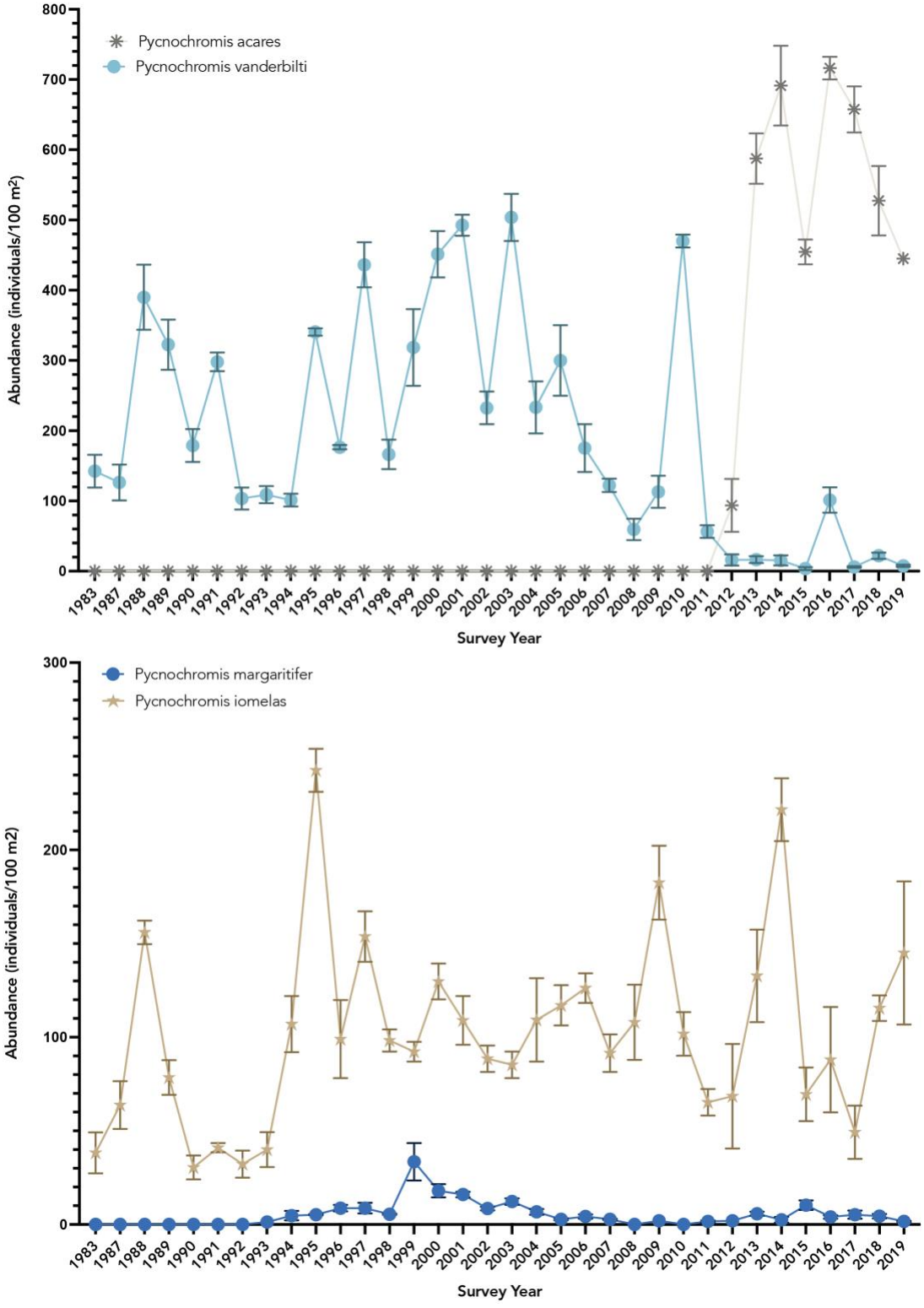


Figure 4.3. Time series of abundance (individuals/100 m^2) in the closely related species pairs of *Pycnochromis* at Tiahura in Moorea from underwater visual surveys. Top figure – *P. acares* and *P. vanderbilti*; bottom figure – *P. iomelas* and *P. margaritifer*.

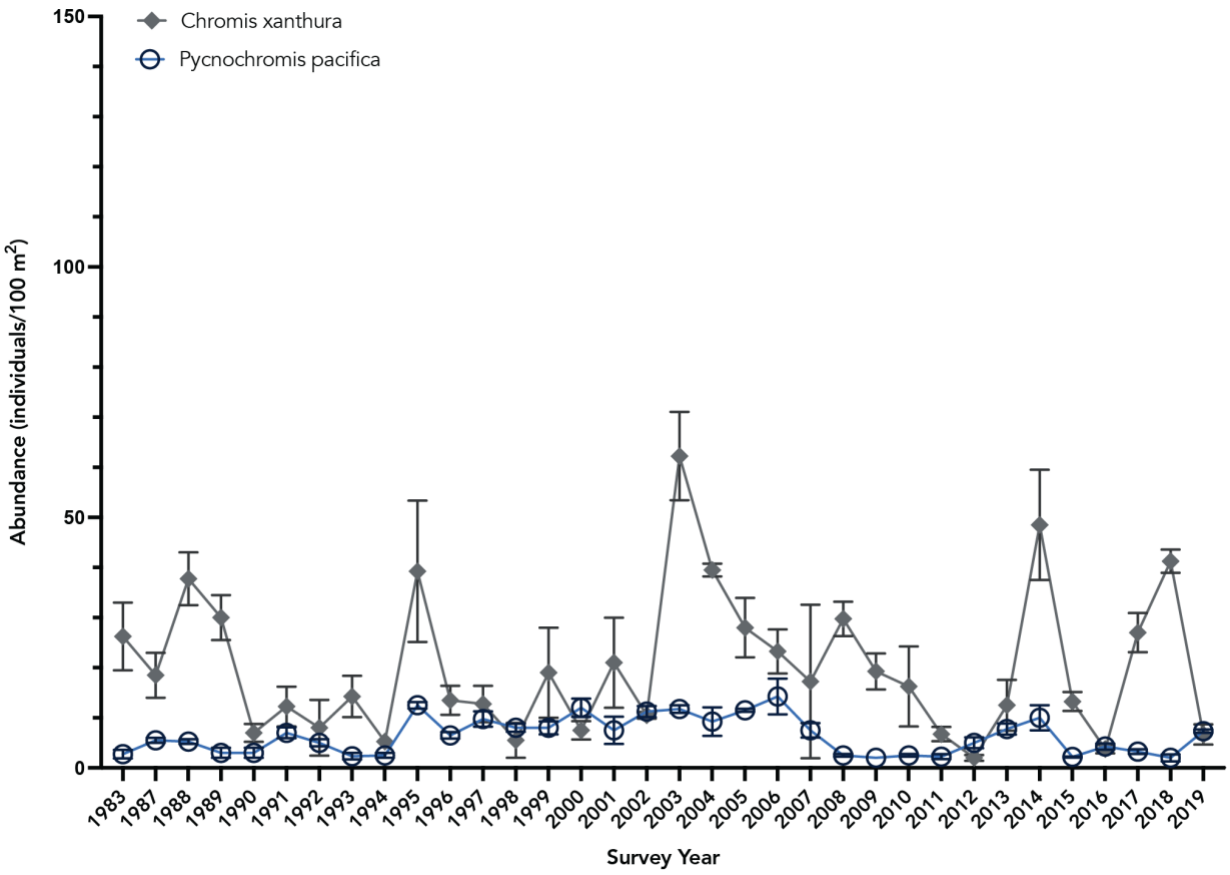


Figure 4.4. Time series of abundance (individuals/100 m^2) of *Chromis xanthura* and *Pycnochromis pacifica* at Tiahura in Moorea from underwater visual surveys.

Figure 4.5. Relative frequency of per-gene features at the phylum level from fish gut metagenomes using the Web of Life database with Woltka classification. The legend (next page) is organized in descending frequency of identified features.

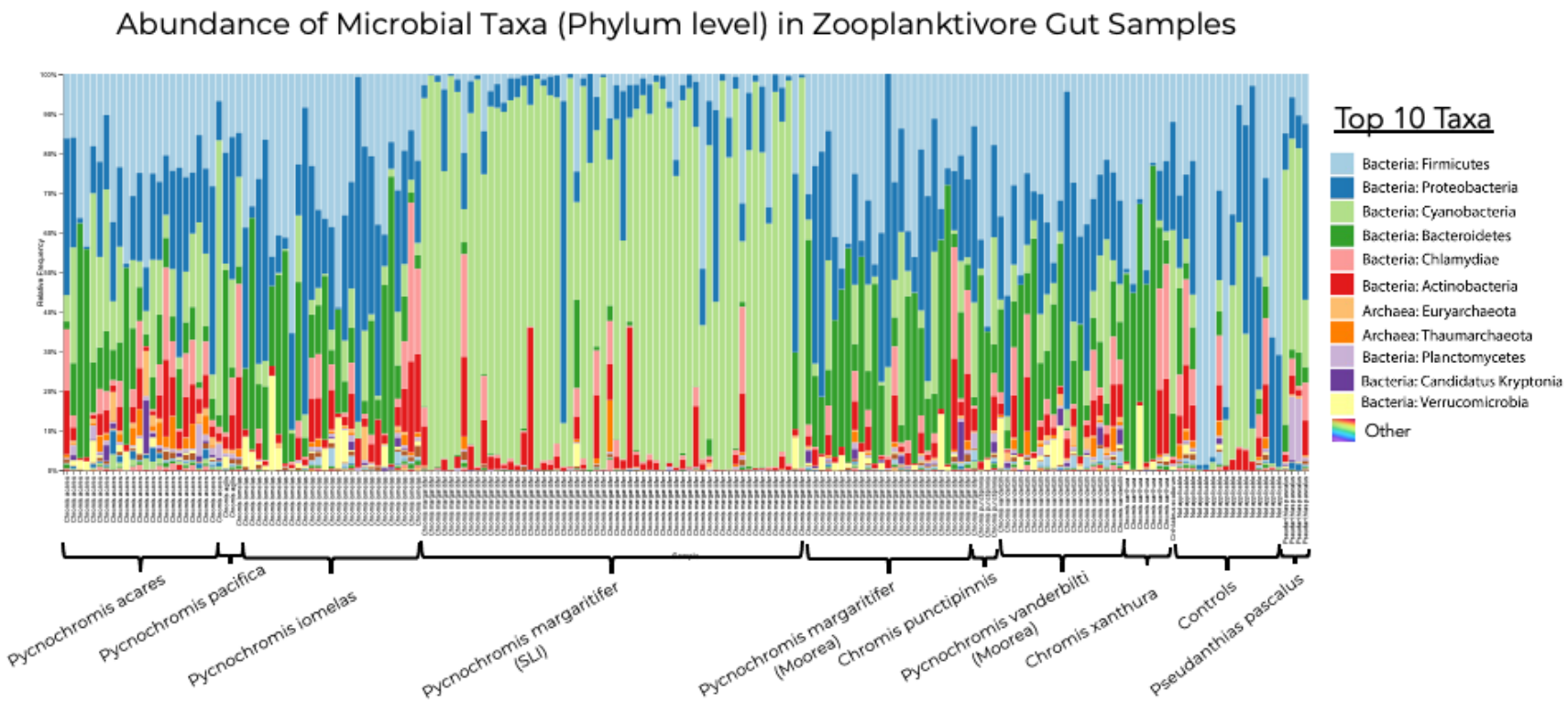

Abundance of Microbial Taxa (Phylum level) in Zooplanktivore Gut Samples
Top 10 Taxa
Bacteria: Firmicutes
Bacteria: Proteobacteria
Bacteria: Cyanobacteria
Bacteria: Bacteroidetes
Bacteria: Chlamydiae
Bacteria: Actinobacteria
Archaea: Euryarchaeota
Archaea: Thaumarchaeota
Bacteria: Planctomycetes
Bacteria: Candidatus Kryptonia
Bacteria: Verrucomicrobia
Other
Pycnochromis acares
Pycnochromis pacifica
Pycnochromis iomelas
Pycnochromis margaritifer (SLI)
Pycnochromis margaritifer (Moorea)
Chromis punctipinnis
Pycnochromis vanderbilti (Moorea)
Chromis xanthura
Controls
Pseudanthias pascalus

Figure 4.6. PCoA plots depicting the results of a Jaccard index of similarity for β-diversity on the rarified (feature count: 5000) metagenomes. Samples are colored by broad geographic region (archipelago or island chain) in the top panel; by finer-scale region (island, atoll, or specific region) in the middle panel; and by host species name in the bottom panel depicts.

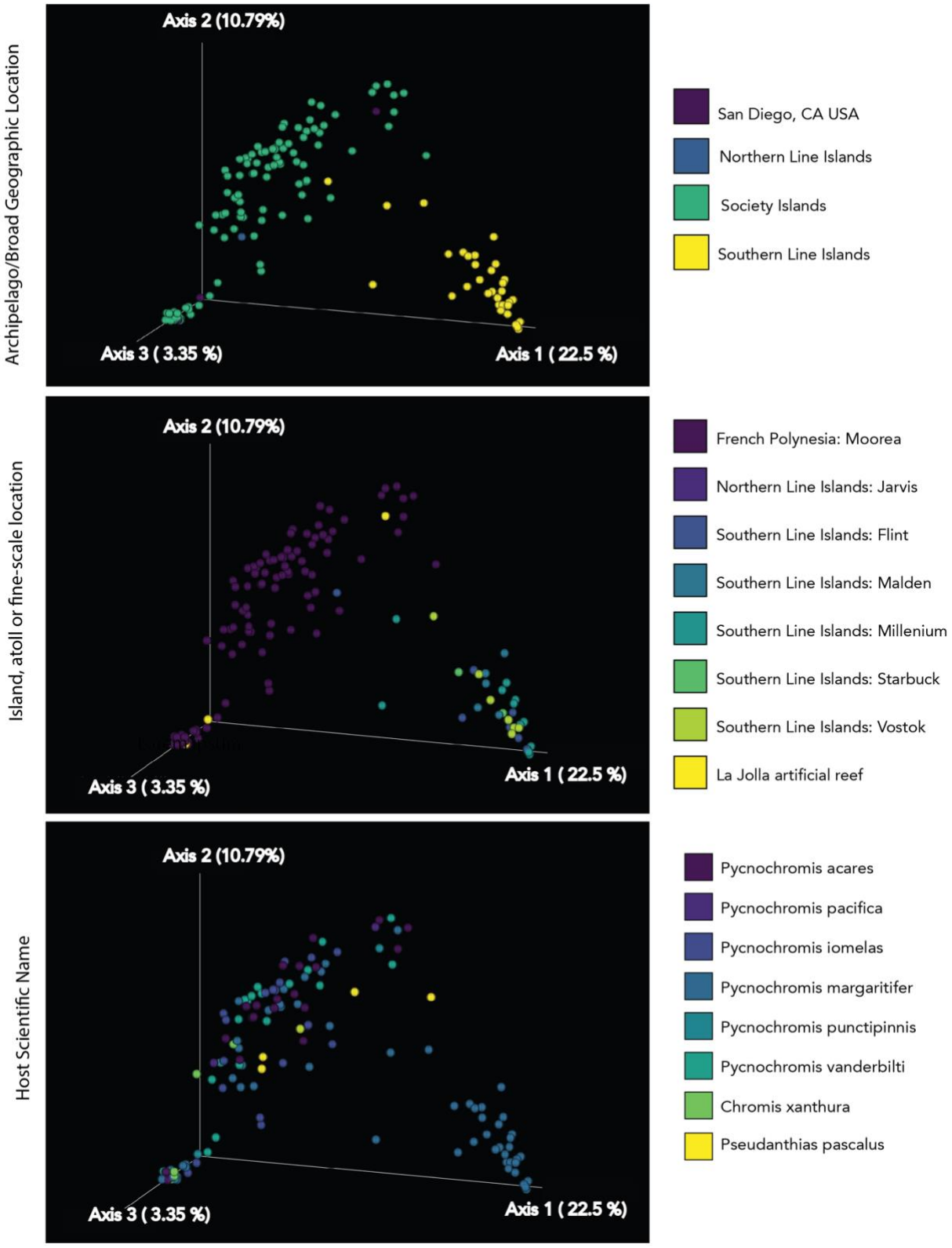
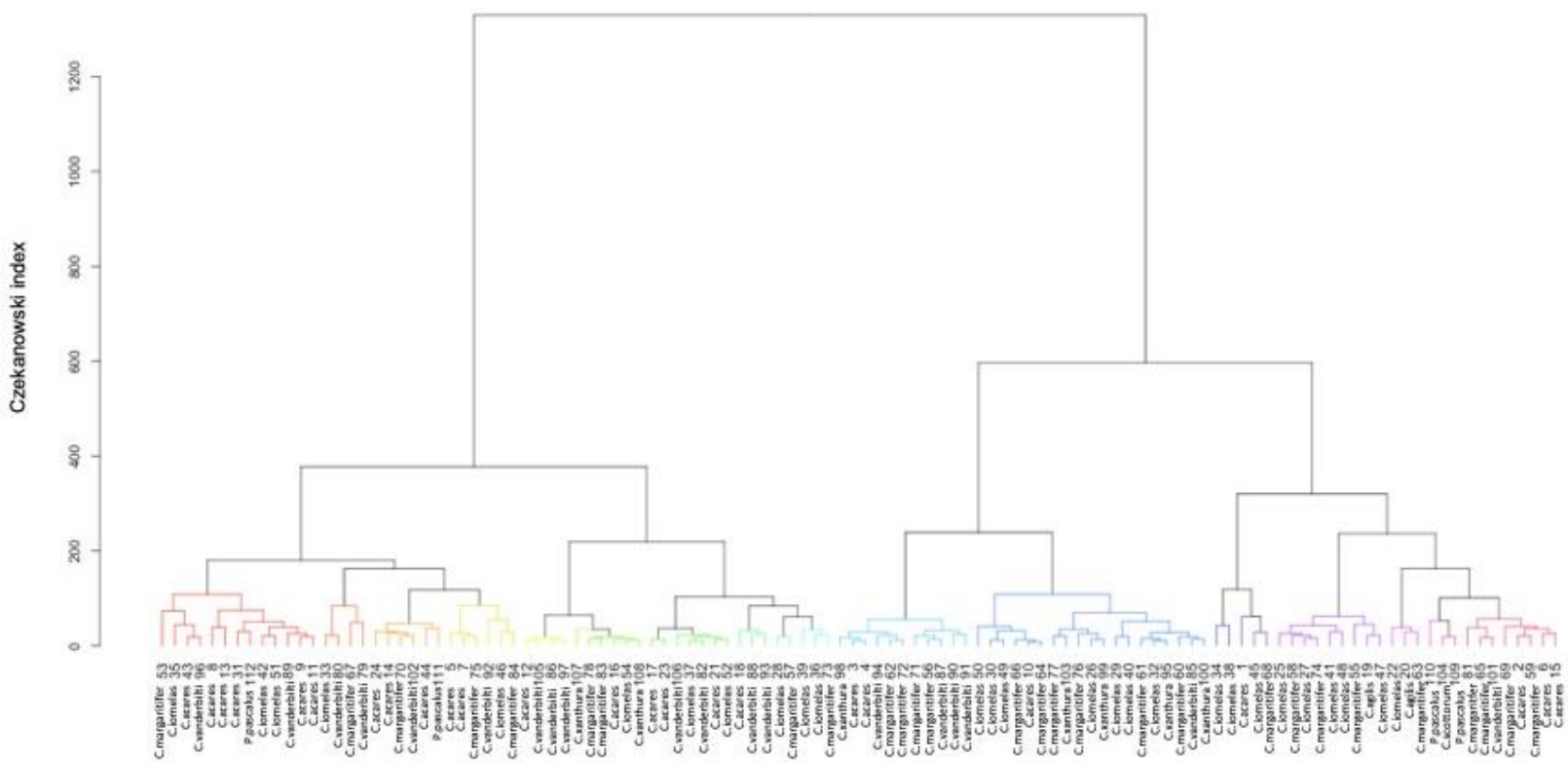

Archipelago/Broad Geographic Location
Axis 2 (10.79%)
Axis 3 (3.35 %)
Axis 1 (22.5 %)
San Diego, CA USA
Northern Line Islands
Society Islands
Southern Line Islands
Island, atoll or fine-scale location
Axis 2 (10.79%)
Axis 3 (3.35 %)
Axis 1 (22.5 %)
French Polynesia: Moorea
Northern Line Islands: Jarvis
Southern Line Islands: Flint
Southern Line Islands: Malden
Southern Line Islands: Millenium
Southern Line Islands: Starbuck
Southern Line Islands: Vostok
La Jolla artificial reef
Host Scientific Name
Axis 2 (10.79%)
Axis 3 (3.35 %)
Axis 1 (22.5 %)
Pycnochromis acares
Pycnochromis pacifica
Pycnochromis iomelas
Pycnochromis margaritifer
Pycnochromis punctipinnis
Pycnochromis vanderbilti
Chromis xanthura
Pseudanthias pascalus

Figure 4.7. Hierarchical dendrogram from the phylum level feature table (Bacteria and Archaea only) for all species collected from Moorea, French Polynesia. Colors show significant clustering with a SIMPROF analysis.

Hierarchical clustering of zooplanktivorous fish species from Moorea, French Polynesia



SUPPLEMENTARY MATERIAL

Supplementary Methods:

Underwater visual surveys: Society Islands

Additional underwater visual census surveys were conducted by at five islands (Moorea, Huahine, Raiatea, Tetiaroa, and Tahiti) in the Society Islands in 2019 in the form of standardized 25 m belt transects as described in more detail in (Friedlander et al. 2010; Friedlander and DeMartini 2002). Briefly, belt transects were completed along the 10-15 m isobath parallel to shore by a pair of divers. For each survey, the team of divers made two passes along the transect, counting and identifying all species within the survey area to the lowest possible taxonomic level. Visually estimated lengths for total length (TL) were recorded for each individual fish. On the outbound pass along the transect, as one diver lay the transect line, both divers quantified large-bodied fishes (≥20cm) within a 4-m wide strip. On the return swim, divers identified all small-bodied fishes (<20cm) in adjacent 2-m wide strips on either side of the transect line. Transect dimensions (8m x 25m for large-bodied fishes and 4m x 25m for small-bodied fishes) were selected to optimize data precision and accuracy while maximizing field effort. Three transects were conducted by each diver pair at each station. Divers maintained regular in-water and post-dive communication to avoid issues of double counting and observer drift.

Figure 4.S1. Fish biomass (in g/m^2) of all trophic groups from underwater visual surveys across islands in French Polynesia from surveys in 2019. Individual columns correspond to island and survey station number. HUA = Huahine, MOO = Moorea, RAI = Raiatea, TAH = Tahiti, and TTR = Tetiaroa.

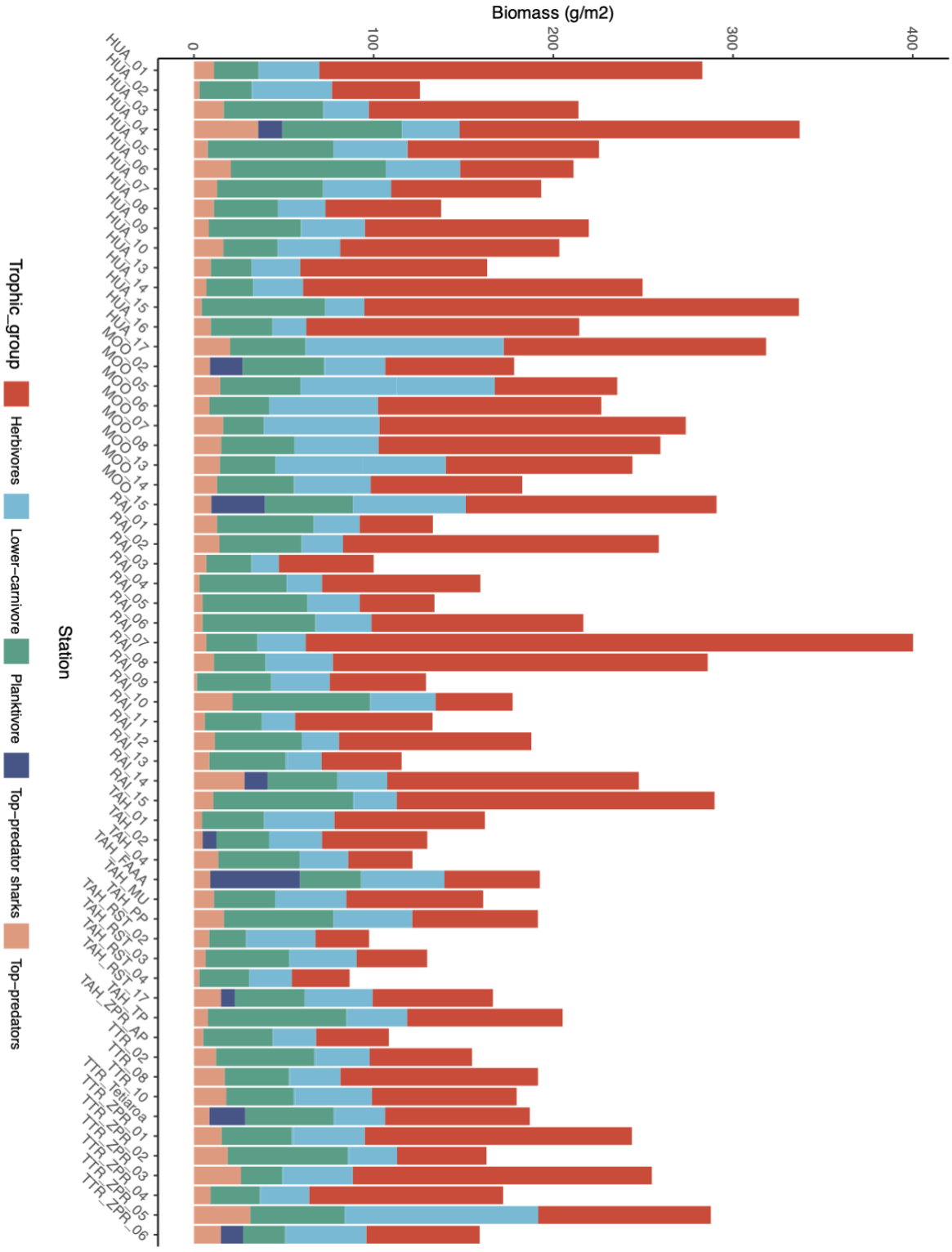
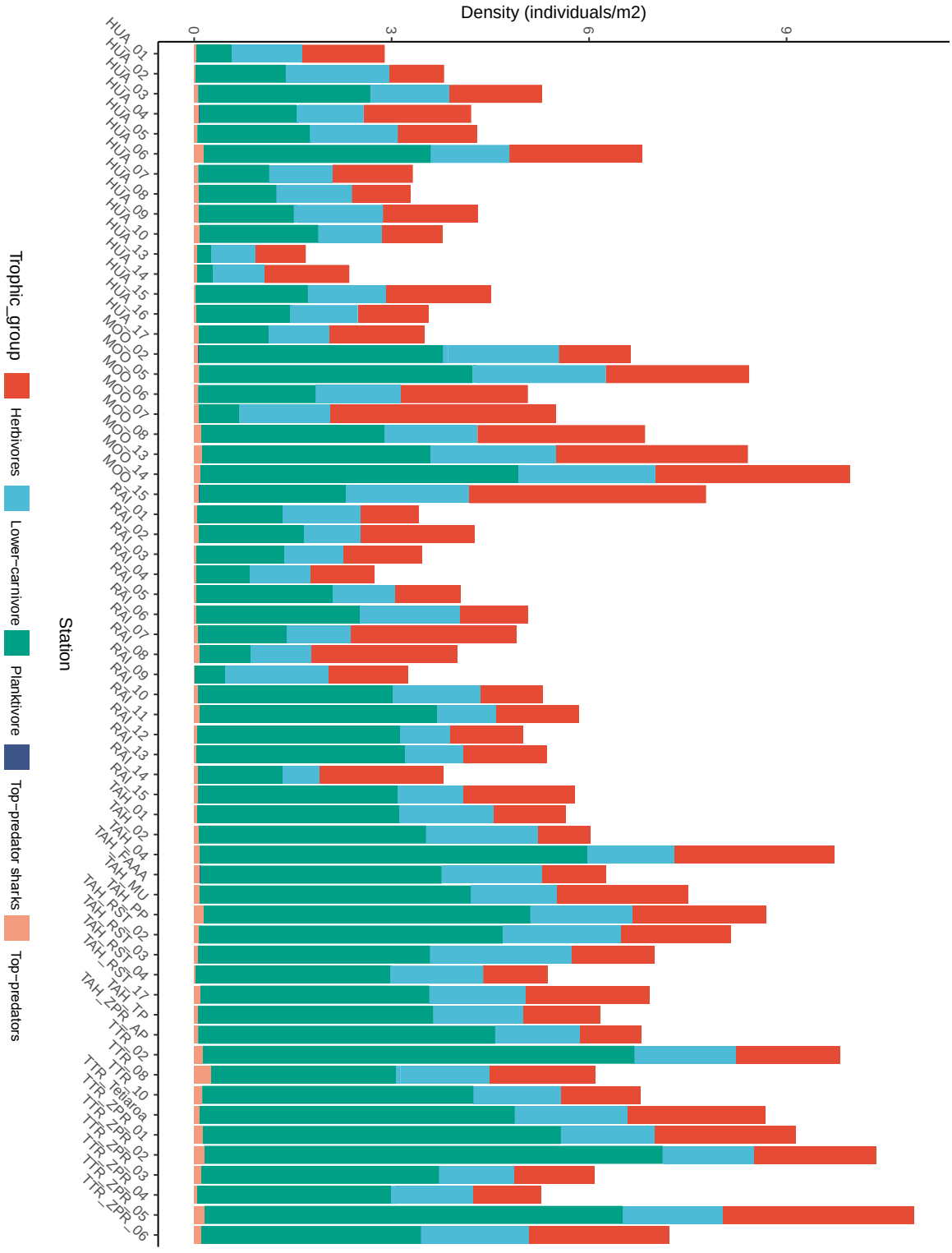

Biomass (g/m2)
0
100
200
300
400
Station
HUA_01
HUA_02
HUA_03
HUA_04
HUA_05
HUA_06
HUA_07
HUA_08
HUA_09
HUA_10
HUA_13
HUA_14
HUA_15
HUA_16
HUA_17
MOO_02
MOO_05
MOO_06
MOO_07
MOO_08
MOO_13
MOO_14
MOO_15
RAI_01
RAI_02
RAI_03
RAI_04
RAI_05
RAI_06
RAI_07
RAI_08
RAI_09
RAI_10
RAI_11
RAI_12
RAI_13
RAI_14
RAI_15
TAH_01
TAH_02
TAH_04
TAH_FAAA
TAH_MU
TAH_PP
TAH_RST_02
TAH_RST_03
TAH_RST_04
TAH_RST_17
TAH_TP
TAH_ZPR_AP
TTR_02
TTR_08
TTR_10
TTR_Tetiaroa
TTR_ZPR_01
TTR_ZPR_02
TTR_ZPR_03
TTR_ZPR_04
TTR_ZPR_05
TTR_ZPR_06
Trophic_group
Herbivores
Lower-carnivore
Planktivore
Top-predator sharks
Top-predators

Figure 4.S2. Fish density (individuals/m^2) by trophic group from underwater visual surveys across islands in French Polynesia (2019). Individual columns correspond to island and station number. HUA = Huahine, MOO = Moorea, RAI = Raiatea, TAH = Tahiti, and TTR = Tetiaroa.


Density (individuals/m2)
0
3
6
9
Station
HUA_01
HUA_02
HUA_03
HUA_04
HUA_05
HUA_06
HUA_07
HUA_08
HUA_09
HUA_10
HUA_13
HUA_14
HUA_15
HUA_16
HUA_17
MOO_02
MOO_05
MOO_06
MOO_07
MOO_08
MOO_13
MOO_14
MOO_15
RAI_01
RAI_02
RAI_03
RAI_04
RAI_05
RAI_06
RAI_07
RAI_08
RAI_09
RAI_10
RAI_11
RAI_12
RAI_13
RAI_14
RAI_15
TAH_01
TAH_02
TAH_04
TAH_FAAA
TAH_MU
TAH_PP
TAH_RST_02
TAH_RST_03
TAH_RST_04
TAH_RST_17
TAH_TP
TAH_ZPR_AP
TTR_02
TTR_08
TTR_10
TTR_Tetiaroa
TTR_ZPR_01
TTR_ZPR_02
TTR_ZPR_03
TTR_ZPR_04
TTR_ZPR_05
TTR_ZPR_06
Trophic_group
Herbivores
Lower−carnivore
Planktivore
Top−predator sharks
Top−predators

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CONCLUSIONS

Community Ecology of Fishes on Coral Reefs in the South and Central Pacific

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As I hope that I have highlighted in this dissertation, coral reef fish communities are complex. For a discipline that seeks to explain patterns and processes of organisms and their interaction with the environment, the diversity of coral reefs provides a considerable, and considerably exciting, challenge. This dissertation conducts an exploration into a small percentage of the unknowns in our understanding of how coral reef fish communities operate, including what mechanisms promote their diversity and coexistence, what elements can be improved from our current methods of observation based on new insights into temporal variability, and how we might incorporate both classical methods and ways of "seeing" ecological communities with newer approaches, such as metagenomic sequencing, for the purposes of increased ecological understanding and better management of these ecosystems. I also provide region-specific biological parameters of growth and weight that demonstrate differences in the important ecological trait of body size, and which are important for monitoring and managing coral reef fish communities. I include members of the fish community that are valuable in the aquarium trade and which are often overlooked and understudied in such contexts.

Returning to our original question regarding the mechanisms underlying the incredible diversity of fishes on coral reefs, we can assess the balance of the evidence provided in this dissertation. The strong patterns of clustering observed in the planktivorous reef fishes suggest potential ecological equivalence and perhaps a lack of niche-partitioning. How can a group of fishes that are all doing the same thing at the same time—including foraging—be substantially engaged in partitioning resources? These observations are consistent with the results of the metagenomic sequencing. The microbial taxa observed in the gut microbiome of planktivorous reef fishes show a strong signal of geographic region broadly but a lack of intraspecific

clustering within a single region. This in contrast with the results from the metagenomic sequencing in hawkfishes in the genus *Paracirrhites* at Flint Island in the Southern Line Islands, which revealed fine-scale niche-partitioning between the abundant *Paracirrites arcatus* and the less cosmopolitan members of the species complex. The observations of length, weight, and calculation of growth parameters for abundant members of the fish community in Moorea, French Polynesia also suggest differences in ecological traits which could indicate resource partitioning. Together, a picture emerges of a community driven by both niche and neutral factors. Evidently, and in keeping the emerging consensus in the field, both stochastic processes and resource partitioning are important mechanisms involved in community assembly in natural ecosystems. This fits more broadly with patterns that we might observe with other famously competing hypotheses in ecology in the increased recognition that many processes operate on a continuum, including diversity and convergence as well as niche and neutral models, and it is interesting to consider the influence of spatial and temporal scales on the conclusions that are drawn.

Additionally, this dissertation sought to look beyond the organism to the microbial partners. I believe that future ecological studies looking at metazoans as holobionts rather than as simple Darwinian individuals hold much promise for our understanding of our world's complex ecosystems, despite the technical challenges that follow along this path. Truly, we—and by we, I mean all metazoans—contain multitudes. Many stories on the level of both microbe and macrobe remain in both the video and sequencing data. I hope that I and others will continue to learn from the stories that these data streams and their focal organisms hold, and I believe that we will, if we look closely enough, with patience, curiosity, and an eye towards seeing like those beings that exist in realms beyond the human.