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What Microscopy and Metabarcoding Reveal on a Coral Reef: Fish Biodiversity
and Toxic Benthic Dinoflagellates in French Polynesia

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

Doctor of Philosophy in Biology

by

Erick Ricardo Zerecero Marín

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2026

ABSTRACT OF THE DISSERTATION

What Microscopy and Metabarcoding Reveal on a Coral Reef: Fish Biodiversity

and Toxic Benthic Dinoflagellates in French Polynesia

by

Erick Ricardo Zerecero Marín

Doctor of Philosophy in Biology

University of California, Los Angeles, 2026

Professor Paul H. Barber, Chair

Coral reefs worldwide are shifting from coral to macroalgal dominance, expanding the substrate available to toxic benthic dinoflagellates whose biotoxins accumulate in reef food webs. Key knowledge gaps include which macroalgal hosts support the heaviest dinoflagellate loads, which species those loads consist of, and whether the dinoflagellates themselves reach fishes by the grazing pathway conventionally assumed. Field surveys resolve these dinoflagellates only to genus, though species within a genus can differ in toxicity by orders of magnitude. Monitoring these reefs also depends on tools that can inventory their communities quickly. I therefore pursued two aims in French Polynesia: to establish what environmental DNA can recover of reef fish biodiversity, and to resolve the toxic dinoflagellates on macroalgae and in the fishes that feed there. Overall, my dissertation shows that environmental DNA can characterize reef fish communities rapidly, and that the dinoflagellate genus of greatest public-health concern is the least visible in the fishes that carry its toxins.

I first evaluated environmental DNA metabarcoding in Mo'orea, where previous barcoding has provided a near-complete reference database of fishes. From 39 L of filtered

seawater across 13 sites, environmental DNA recovered 30.2% of all known Mo'orean fishes (257 of 880) using MiFish 12S and Leray CO1 markers, and reliably characterized backreef, forereef, and pelagic communities, a resolution supported by the rapid degradation of vertebrate DNA in two tropical mesocosm experiments.

Second, I counted toxic dinoflagellates on four macroalgal hosts at five sites in Mo'orea's north-shore lagoon across two sampling periods. Site mattered far more than host or sampling period, and shaped both. The genera differed: *Gambierdiscus* was most abundant on *Turbinaria* and *Dictyota*, a ranking that held at every site, while *Ostreopsis*, roughly 93% of all cells counted, showed little host preference. As *Turbinaria ornata* expands across Mo'orea's reefs, its growing standing stock may raise lagoon-wide *Gambierdiscus* abundance.

Third, I applied D1–D2 LSU metabarcoding to macroalgal substrate and reef-fish hindguts. *Ostreopsis* dominated the substrate community (78.5% of reads) rather than *Gambierdiscus* (7.0%), and feeding mode filtered exposure sharply, detritivores carrying the heaviest loads and corallivores loads orders of magnitude lower. Hindgut assemblages were nearly completely nested within substrate assemblages, yet of ten *Gambierdiscus* species on macroalgae only *G. pacificus* was recovered from any fish, in a single individual. The species-level view therefore qualifies what genus-level cell counts can be taken to mean about ciguatera risk.

The dissertation of Erick Ricardo Zerecero Marín is approved.

Peggy Marie Fong

Mindy Lou Richlen

Thomas Bates Smith

Monica Medina

Paul H. Barber, Committee Chair

University of California, Los Angeles

2026

DEDICATION

This is dedicated to the families of immigrant children who unconditionally support

their children's dreams

In memory of Eva Vergara

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BIOGRAPHICAL SKETCH

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- Gold, Z., Sprague, J., Kushner, D. J., Zerecero Marin, E. R., & Barber, P. H. (2020). eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PLoS ONE*, 16(2), e0238557.
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FELLOWSHIPS, AWARDS, AND HONORS

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2018	UCLA Eugene V. Cota-Robles Fellowship
2023	Entering Mentoring Training Program (CIMER curriculum), University of California, Los Angeles
2017, 2019	Association for the Sciences of Limnology and Oceanography (ASLO) Multicultural Program

Rapid eDNA Marine Biodiversity Survey of Mo'orea, French Polynesia

Abstract

Rapid assessments of marine biodiversity are vital for characterizing marine ecosystems and providing important baseline information. Environmental DNA (eDNA) holds promise as a simple to use, cost effective method for inventorying marine species. Here we evaluate the ability for eDNA approaches to characterize marine fish biodiversity in Mo'orea, French Polynesia where previous DNA barcoding efforts have provided a near complete reference database of fishes. In just 39 L of filtered sea water across 13 sites, eDNA approaches recovered 30.2% (257 out of 880) of all known fishes in Mo'orea using MiFish 12S and Leray CO1 markers. Furthermore, we demonstrate that eDNA methods can reliably characterize fish communities associated with backreef, forereef, and pelagic habitats. The ability to differentiate across coral reef habitats was supported by the rapid degradation of fish eDNA observed in two tropical mesocosm experiments. By comparing the proportion and community assemblage derived from MiFish 12S metabarcoding data, we found a half-life of 15.6 – 18.4 h and 93.6 – 99.5% decay of all vertebrate DNA reads across 84 hours. Together, these results highlight the capabilities of eDNA approaches to provide rapid assessments of marine fish biodiversity in coral reef habitats.

Introduction

Environmental DNA (eDNA) metabarcoding has rapidly emerged as a powerful tool for surveying biodiversity in aquatic systems. By isolating and sequencing genetic fragments shed by organisms into the environment, eDNA provides a non-invasive means to document species presence and community composition across broad spatial and temporal scales (Taberlet et al.,

2012; Deiner et al., 2017). Compared to traditional monitoring techniques, eDNA sampling requires less field effort and can be deployed in remote or resource-limited areas (Ruppert et al., 2019). Impressively, eDNA is effective across a diversity of ecosystems, including freshwater ecosystems like rivers and lakes (Thomsen et al., 2012; Valentini et al., 2016), and marine systems such as kelp forests, estuaries, and coral reefs, among others (Port et al., 2016; Closek et al., 2019), underscoring its potential as an efficient and scalable monitoring tool (Kelly et al., 2014; Qiu et al., 2023; Monuki et al., 2021; Nagarajan et al., 2022). Beyond efficiency, eDNA methods are also especially effective at identifying rare, invasive, and endangered taxa of critical conservation concern (Dejean et al., 2012; Valentini et al., 2016). This growing body of literature highlights the transformative role of eDNA in aquatic biodiversity assessment.

Coral reefs are among the most biodiverse and most threatened ecosystems on Earth, facing escalating pressure from warming, bleaching, overfishing, and habitat degradation (Eddy et al., 2021). Managing and conserving these systems depends on biodiversity data that can be collected quickly, at scale, and at a resolution fine enough to capture how communities are structured across the reef. Yet, traditional approaches most notably SCUBA based visual surveys are time-, labor-, and expertise-intensive, constraining how much of a reef can be assessed and how often (Muenzel et al., 2024). Reefs are also strongly zoned, with distinct fish assemblages partitioned across back reef, fringing reef, and pelagic habitats over short distances, so a useful monitoring tool must not only inventory species rapidly but also resolve these habitat-level differences. eDNA metabarcoding is a promising candidate on both fronts but realizing that promise in tropical reef systems requires understanding how well eDNA signals stay localized and how completely they can be assigned to the local fauna.

Studies in temperate marine ecosystems demonstrate that eDNA signatures can distinguish communities on fine spatial scales over 10–150 m (Port et al., 2016; Yamamoto et al., 2016), indicating that where water movement is limited, biological processes such as shedding and microbial degradation of eDNA often outweigh its physical transport (Port et al., 2016). However, in more dynamic coastal systems, ocean currents and advection can strongly influence eDNA distribution, with eDNA only distinguishing communities on the scale of kilometers (O'Donnell et al., 2017; Lamy et al., 2021; Xiong et al., 2025). Research demonstrates that community composition can vary significantly both with water depth and fine-scale habitat structure, with eDNA signatures varying across meters to tens of meters in vertical water columns (Dugal et al., 2023; Shea & Boehm 2024; Jeunen et al., 2020; Monuki et al., 2021; Port et al., 2016). Together, these studies suggest that eDNA can capture ecological patterns across multiple spatial scales, but that the resolution of eDNA signals is highly context dependent on degradation and transport processes.

Key to understanding the variation in spatial resolution of eDNA in marine ecosystems is the persistence and degradation of eDNA signals. Early studies in freshwater mesocosms showed detection limits ranging from less than a day to several weeks, influenced by temperature, light exposure, and microbial activity (Dejean et al., 2011; Pilliod et al., 2014; Strickler et al., 2015). Marine experiments in temperate environments similarly demonstrated rapid exponential decay, with detection typically lasting less than a week (Thomsen et al., 2012; Wood et al., 2020). However, most of these studies relied on laboratory aquaria or mesocosms where homogenized tissue was “spiked” into the water to simulate a natural eDNA signal, or animals were held in confined tanks to generate eDNA through natural shedding processes. While informative, these

approaches may not reflect natural eDNA dynamics, as artificially high concentrations could inflate apparent persistence times (Barnes & Turner, 2016; Andruszkiewicz Allan et al., 2021).

In addition to potentially unrealistic eDNA concentrations impacting eDNA signal persistence, most eDNA degradation studies focus on temperate rather than tropical ecosystems, where high levels of solar radiation, elevated sea surface temperatures, and oligotrophic nutrient regimes could accelerate DNA breakdown (Jo et al., 2019; Kasai et al., 2020; Andruszkiewicz Allan et al., 2021). In theory, shorter persistence of eDNA signals should limit diffusion and dispersion of eDNA, enhancing the resolution of eDNA surveys on smaller spatial scales in tropical ecosystems. In areas with such shorter persistence times, eDNA sampling would therefore provide a near real-time snapshot of community composition. Understanding tropical eDNA degradation dynamics *in situ* is therefore essential both for interpreting eDNA signals and for designing effective monitoring programs in tropical marine ecosystems.

A further challenge of eDNA metabarcoding in tropical ecosystems is that differences in eDNA community profiles can only be fully understood through accurate taxonomic assignment of ASVs, which requires complete and accurate reference sequence databases. Incomplete or biased databases limit species identification, potentially skewing community assessments (Machida et al., 2017; Cristescu 2014). In well-studied temperate systems, improving regional 12S reference libraries has been shown to substantially increase taxonomic resolution (Gold et al. 2021; Schenekar et al., 2020) and reveal overlooked species, including warm-season migrants undergoing climate-driven range shifts that traditional surveys failed to detect (Stoeckle et al. 2020).

For coral reefs ecosystems that harbor exceptional biodiversity, much of this diversity is still undescribed (Knowlton et al., 2010; Barber & Boyce, 2006), and a substantial number of

species remain absent from global genetic repositories such as GenBank or BOLD, limiting the effectiveness of eDNA surveys (Marwayana et al. 2022, Claver et al.,2023). This problem is exacerbated by the fact that comprehensive barcoding efforts have largely focused on the mitochondrial cytochrome oxidase I (COI) gene (Hebert et al. 2003; Ward et al. 2005; Ward et al. 2009), with sequences deposited in dedicated repositories such as the Barcode of Life Data System (BOLD; Ratnasingham & Hebert 2007). While COI metabarcoding loci have been found to be useful for invertebrate taxa (Leray et al. 2012), the application of this locus for eDNA metabarcoding is often less effective with vertebrates given the high number of non-vertebrate species simultaneously amplified (Collins et al. 2019). Instead, the 12S locus has become a widely used eDNA metabarcoding gene for fishes (Miya et al., 2015; Closek et al., 2019; Miya et al.,2020). Thus, although the previous efforts like the Mo'orea Biocode Project comprehensively barcoded marine metazoans on the island of Mo'orea using COI (Check 2006; Leray et al. 2012), the apparent mismatch in reference database and metabarcoding targets limits the efficacy of such sequencing efforts for eDNA based fish surveys. As such, recent initiatives emphasize the need for region-specific marker specific databases to maximize taxonomic assignments in tropical biodiversity hotspots (Juhel et al., 2020; Marques et al., 2021; Marwayana et al., 2022). Taken together, the above gaps highlight the need for studies that directly test eDNA degradation under natural tropical reef conditions while simultaneously developing reference databases tailored to the local fauna. Addressing both issues is critical for advancing eDNA from a promising tool into a reliable monitoring framework in coral reef ecosystems. Here, we investigate eDNA dynamics in Mo'orea, French Polynesia, with four complementary efforts: (1) quantify the persistence of natural eDNA signals under tropical conditions, *in situ*, (2) evaluating the spatial resolution of eDNA across tropical coral reef and pelagic habitats; (3) presenting and

applying a novel, region specific 12S reference database, built from near complete field collections of the Mo‘orea Biocode Project; and (4) testing the efficacy of the resulting analyses through comparison of eDNA metabarcoding and long-term SCUBA visual surveys on the island of Mo‘orea. By combining experimental degradation trials with region-specific reference database development, our study provides new insights into the ecological resolution, persistence, and applicability of eDNA monitoring in tropical coral reef systems.

Methods

Study Site

We conducted this study on the north shore of Mo‘orea, French Polynesia (17°30’S, 149°50’W), where the Mo‘orea Coral Reef Long-Term Ecological Research (LTER) project has conducted regular fish surveys for ~20 years, providing one of the most comprehensive datasets for fish diversity. In addition, Mo‘orea was the home of the Mo‘orea Biocode Project, an effort to collect, identify, voucher, and DNA-barcode all terrestrial and marine metazoans larger than ~2 mm in size (Smithsonian Institution, 2012; Gump South Pacific Research Station). As such, the marine ecosystems of Mo‘orea are among the best studied, sampled, and DNA barcoded coral reef ecosystems in the world.

Experimental Design

To evaluate the power of eDNA for biodiversity assessment in tropical marine ecosystems, we carried out four complementary efforts. First, to quantify eDNA persistence under tropical conditions, we conducted a time-series degradation experiment using both a land-based mesocosm and a unique *in situ* seawater enclosure to assess natural degradation of eDNA signals. Second, we conducted eDNA surveys across reef and pelagic habitats to test whether

eDNA resolves fish assemblage differences at fine spatial scales. Third, we developed and applied a novel, region-specific 12S reference database built from the Mo‘orea Biocode Project collections to maximize taxonomic assignment of the local fauna. Fourth, we compared eDNA detections against long-term SCUBA visual surveys conducted by the Mo‘orea Coral Reef LTER to assess the efficacy of eDNA relative to traditional monitoring.

Common eDNA Methods

Sample handling and molecular processing followed common protocols across all efforts, with effort-specific exceptions noted below. In all cases, seawater samples were collected in sterile one-liter enteral feeding bags (Kendall-Covidien 702500; “pouches”), similar to intravenous drip bags, and gravity-filtered immediately after collection.

eDNA Sample Collection and Filtration

We gravity filtered seawater samples immediately after collection using 0.2 μm PES Sterivex™ cartridges (Millipore SVGPL10RC), attached to the drip bags using Luer-lock-to-tubing adapters made from ~6 inches (~15 cm) of sterilized Nalgene tubing fitted with a ¼-inch male Luer-lock adapter (Cole-Parmer EW-30800-26). This approach allowed samples from each collection point to be filtered simultaneously. After filtration, all remaining seawater was expelled with a 3 mL syringe, and cartridges were capped with Luer-lock caps and stored at –20 °C until extraction.

DNA Extraction

Laboratory processing of eDNA samples followed the same protocols with minor exceptions. For the degradation experiment, filtered Sterivex cartridges were processed in the field and transported to UCLA at –20 °C for DNA extraction. In contrast, extractions of the field-based

community surveys were performed on-site at the Gump Molecular Biology Laboratory within 10 h of filtration. In both cases, we performed DNA extractions using a modified Qiagen DNeasy Blood & Tissue protocol optimized for enclosed Sterivex filters (Spens et al. 2017). Modifications included performing all lysis steps inside the sealed 0.22 μ m Sterivex cartridges rather than in microcentrifuge tubes. Specifically, 720 μ L ATL buffer and 80 μ L Proteinase K were added directly through the Luer opening, both ends were capped, and filters were incubated overnight (≥ 12 h) at 56 °C on a rotating mixer to ensure continuous washing of the lysis solution across the membrane. Lysates were then recovered by attaching sterile 3 mL syringes to the cartridge outlet and pushing the digest into 1.7 mL tubes; samples exceeding 550 μ L were split into multiple tubes to remain within column loading limits. Prior to column binding, recovered lysates were mixed 1:1:1 with buffer AL and ice-cold 99–100% ethanol to facilitate DNA adsorption. Lysates were then loaded onto DNeasy spin columns in sequential aliquots. Washing and elution steps followed the manufacturer protocol, except that DNA was eluted twice with 100 μ L of pre-heated (70 °C) AE buffer to maximize yield.

PCR Amplification and Library Preparation

To characterize diversity within the mesocosms, we used the MiFish universal teleost primers, targeting approximately 163–185 bp of 12S (Miya et al. 2015). To characterize community marine biodiversity, we used the MiFish universal teleost primers as well as COI primers mlCOIintF / jgHCO2198 targeting approximately 313 bp of COI (Leray et al. 2013). Both primer sets incorporated Illumina Nextera adapters for two-step indexing following the modifications described in Curd et al. (2019). To account for variation related to PCR bias and minimize false positives, we amplified each eDNA sample in triplicate (Alberdi et al. 2018; Ficetola et al.

2016). All PCRs included a negative control where molecular-grade water replaced the DNA extract, and positive controls consisting of Grass carp (*Ctenopharyngodon idella*) DNA.

PCR reactions were prepared in 25 µL volumes using the Qiagen Multiplex 2× Master Mix, consisting of 12.5 µL of master mix, 0.5 µL each of forward and reverse primers (2 mM), 10.5 µL of ddH₂O, and 1 µL of DNA template. Thermocycling followed a touchdown protocol modified from Curd et al. (2019) and included an initial denaturation at 95 °C for 15 min to activate the polymerase. Denaturation was followed by 13 touchdown cycles consisting of 94–95 °C for 30 s, with an annealing step starting at 69.5 °C and decreasing by 1.5 °C per cycle for 30 s, and an extension at 72 °C for 1 min. After the touchdown phase, an additional 35 cycles were run with denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, and extension at 72 °C for 1 min, ending with a final extension at 72 °C for 10 min. To determine PCR efficacy, all PCR products were electrophoresed on a 2% agarose gel stained with SybrGreen in TBE buffer.

To create sequencing libraries, we first pooled 5 µL of each replicate PCR for each of the two barcodes and then cleaned the pooled products using Serapure magnetic beads (Faircloth & Glenn 2014). We then quantified the pooled amplicons using the Quant-iT™ dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA, USA) on a Victor3 plate reader (PerkinElmer, Waltham, MA, USA) to prepare libraries with equal molar representation of all samples, replicates, and barcodes. To uniquely index each sample, we conducted a secondary indexing PCR using KAPA HiFi HotStart ReadyMix (Kapa Biosystems, Wilmington, MA, USA) with Nextera i5/i7 indexes from the Nextera Index A and B kits (Illumina, San Diego, CA, USA), following the workflow described in Curd et al. (2019). This indexing PCR was carried out in 25 µL reactions containing 12.5 µL of KAPA HiFi HotStart ReadyMix, 0.625 µL each of the i5 and i7 primers, and approximately 10 ng of template DNA, using thermocycling conditions of 95 °C

for 5 min; 5 cycles of 98 °C for 20 s, 56 °C for 30 s, and 72 °C for 3 min; followed by a final extension at 72 °C for 5 min. Indexed PCRs were verified on 2% agarose gels to confirm the correct product size, bead-cleaned, and re-quantified using the same Quant-iT dsDNA assay. Final indexed libraries were pooled in equimolar concentrations and sequenced on an Illumina MiSeq (PE 2×300 bp) at the Technology Center for Genomics & Bioinformatics (University of California, Los Angeles) using a MiSeq V3 reagent kit with 20% PhiX added to each run.

Effort 1: eDNA Degradation Experiment

Experimental Design and Time-Series Collection

To determine the rate of eDNA degradation in a tropical marine ecosystem, we employed two parallel approaches during fieldwork in July 2018. First, we used a pump without filtration to fill a land-based 2000 L plastic tank that was unshaded, receiving full, natural lighting. Second, because the sides of the land-based mesocosm were opaque, limiting light transmission from the sides and bottom, we created an *in situ* water enclosure constructed from a PVC frame ($\approx 180 \times 180 \times 110$ cm) wrapped in Sun Selector UVA greenhouse film (agricultural-grade plastic, Agriculture Solutions SSCL-w12-len), creating a UV transparent enclosure with a volume of approximately 3,240 L. We then filled this enclosure, in the ocean, with seawater using a pump without filtration, and affixed the filled enclosure to a stationary dock (Figure 1-1). This latter approach effectively isolated a portion of ocean, allowing us to monitor the decay of natural eDNA signals, *in situ*, under ambient conditions (sunlight, temperature, microbial activity) characteristic of the natural ocean environment.

We filled both mesocosms sequentially with a high-volume pump and then initiated time-series sampling. To establish a baseline for natural eDNA signals, we collected three 1 L water samples from the ocean, immediately next to the pump inlet as we filled the mesocosms. These three

samples are hereafter referred to as the Ocean Baseline ($T = -1$) and serve as a reference community representing the natural source water prior to experimental enclosure. Then, to test for degradation within the mesocosms and to test for variation among replicates, using the same sterile bags we collected two 1 L replicates from each mesocosm at 0 h, 1 h, 4 h, 12 h, and 20 h. To obtain information on degradation on a finer temporal scale, we also sampled 1 L of water at 2 h, 3 h, 6 h, 8 h, 10 h, 14 h, 28 h, 38 h, 46 h, 54 h, 62 h, and 84 h (the final time point, due to storm damage to the seawater enclosure).

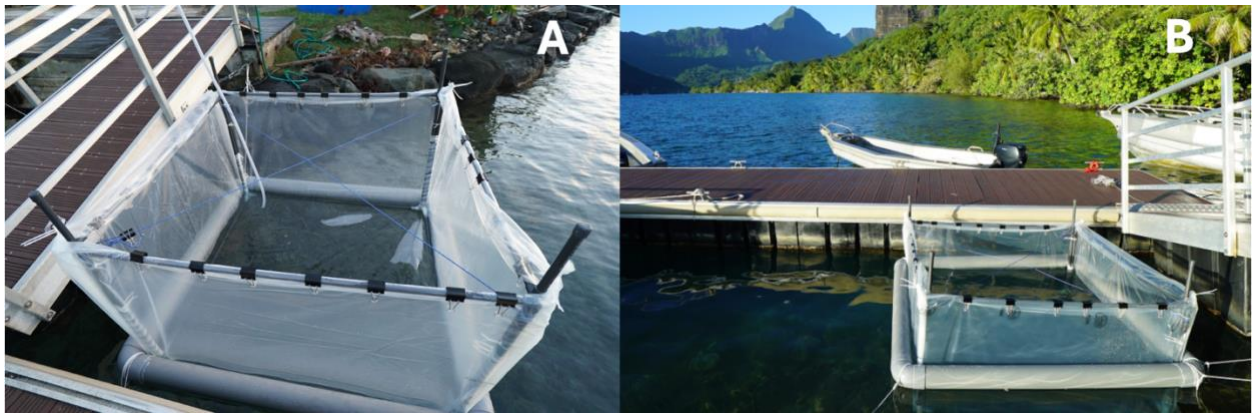


Figure 1-1. UV transparent in situ mesocosm enclosure A) before and B) after filling with seawater

Wind and Weather Data

To characterize wind conditions during the eDNA degradation experiment (5–9 July 2018), we obtained three-hourly wind speed, wind gust, and precipitation data for Moorea-Temae Airport (MOZ) from World Weather Online historical weather records. MOZ is located approximately 7 km east of the experimental site at the UC Berkeley Richard B. Gump South Pacific Research Station (Cook’s Bay, Moorea) and was the nearest weather station with publicly accessible historical records. Daily mean wind speeds were calculated by averaging the eight three-hourly observations recorded each day (00:00, 03:00, 06:00, 09:00, 12:00, 15:00, 18:00, and 21:00 local

time), daily peak gusts were taken as the maximum gust value recorded across these observations, and daily rainfall totals were calculated as the sum of precipitation at each three-hourly interval. All wind speed and gust values are reported in kilometers per hour (km/h), and rainfall is reported in millimeters (mm). Data were visualized using the ggplot2 package in R.

Bioinformatic Processing

Raw sequence data from the degradation experiment were processed using the Anacapa Toolkit (Curd et al. 2019), which performs primer trimming, quality filtering, and amplicon sequence variant (ASV) assignment under default parameters. Anacapa performed taxonomic assignments using a MiFish Universal 12S CRUX database, supplemented with the newly generated 12S sequences described in Effort 3. Potential contaminants were identified using the DECONTAM package (Davis et al. 2018) based on frequency and prevalence profiles; all ASVs flagged as contaminants were removed. We also removed all singleton ASVs prior to downstream analyses. Because the MiFish primers are known to amplify certain microbial taxa, we classified ASVs as vertebrate (Chordata: Vertebrata) or bacterial (domain Bacteria) to quantify temporal changes in read composition within each mesocosm over time. Specifically, we calculated the proportion of total reads classified as Teleost (Class: Actinopteri) per sample at each time point and visualized these as bar plots to characterize the rate of eDNA degradation. We computed mean percent Teleost reads per mesocosm type (Mesocosm and Ocean Enclosure) across biological replicates, with standard deviation where multiple replicates were available. All statistical analyses were performed in R (R Core Team, 2024) using the tidyverse (Wickham et al. 2019) and vegan (Oksanen et al. 2022) packages.

Bacterial Community Composition

To characterize the bacterial community composition captured by the MiFish 12S primers, we extracted non-vertebrate amplicon sequence variants (ASVs) from the Anacapa toolkit output and queried them against the NCBI nucleotide database using BLAST (Altschul et al., 1990). Briefly, ASV sequences were obtained from the detailed taxonomy output file generated during the Anacapa pipeline and filtered to exclude sequences assigned to Chordata. The remaining non-vertebrate ASVs were ranked by total read abundance across all samples, and the 50 most abundant sequences were exported in FASTA format for BLAST analysis. Sequences were queried against the NCBI core nucleotide (core_nt) database restricted to Bacteria (taxid:2) using the BLASTn algorithm optimized for highly similar sequences (megablast), with a maximum of five target sequences per query. The top BLAST hit per ASV was selected based on highest bit score, with percent identity ranging from 96.1% to 100%. Organism-level taxonomy for each matching accession was retrieved programmatically from NCBI GenBank using the rentrez package (Winter, 2017) in R (R Core Team, 2024). Bacterial community composition was summarized at the organism level and visualized as relative abundance stacked bar plots across mesocosm types and time points. Additionally, principal component analysis (PCA) ordinations were performed on Hellinger-transformed read counts using the vegan package (Oksanen et al., 2022) to examine shifts in bacterial community composition over time. We followed this procedure because the MiFish 12S primers are known to amplify bacterial 12S rRNA sequences, particularly when concentrations of vertebrate eDNA are low, resulting in an increasing proportion of bacterial reads as vertebrate eDNA degrades over time.

Effort 2: Spatial Community Surveys

Sampling Design

To test for the ability of eDNA to distinguish among unique fish communities across distinct coral reef environments, we sampled along a habitat transect that included both lagoonal and oceanic ecosystems. In July 2016, we sampled backreef habitats in the lagoon and then along the reef slope of the forereef, selecting these habitats because of documented differences in fish community composition from LTER visual survey data. In addition, we sampled at pelagic stations 500 m, 1 km, 2 km, and 4 km offshore, perpendicular from the reef crest. To maximize comparability of reef-associated sites, we chose sites proximal to LTER fish survey sites, with pelagic sampling sites being directly offshore from these sites. At each of these 13 sampling sites, we collected triplicate 1 L seawater samples at ~1 m depth while snorkeling, except for the forereef sites where we used SCUBA to collect samples one meter above the benthos at 10 m and 30 m, using enteral feeding pouches. To minimize degradation during transportation, we placed water samples on ice immediately after removal from the ocean and then transported them back to the Gump Research Station within one hour of collection. We then simultaneously isolated eDNA from all samples, employing gravity filtration through 0.2 μm PES Sterivex™ cartridges (Millipore SVGPL10RC) as described in Common eDNA Methods.

Bioinformatic Processing

For spatial community surveys, raw sequence data from all loci were processed using the Anacapa Toolkit (Curd et al. 2019), as described above. We performed taxonomic assignments separately for each locus using CRUX-generated reference databases built from the Leray COI and MiFish Universal 12S primer sets, built on the December 2019 NCBI nt database download. For COI, ASVs were matched against a CRUX-constructed metazoan COI database generated from GenBank and BOLD sequences (December 2019 download) corresponding to the Leray primer region (Leray et al. 2013), supplemented with COI reference sequences from the Moorea

Biocode Project to improve taxonomic resolution for local fauna. For 12S, taxonomy was assigned using the CRUX-generated reference database supplemented with the newly constructed 12S reference sequences presented in Effort 3. As with the degradation data, potential contaminants were identified using the DECONTAM package (Davis et al. 2018) based on frequency and prevalence profiles, and ASVs flagged as contaminants were removed. Similarly, singleton ASVs (represented by a single read across the dataset) were excluded prior to downstream analyses. All amplified microbial samples were excluded from downstream spatial analyses.

Statistical Analysis

We analyzed final ASV tables and associated metadata in R using *ranacapa* (Kandlikar et al. 2018), *phyloseq* (McMurdie & Holmes 2013), and *vegan* (Oksanen et al. 2022). Rather than rarefying to even sequencing depth — which discards data and reduces statistical power (McMurdie & Holmes, 2014) — we retained all reads and analyzed fish communities using presence-absence data. After filtering ASVs to Chordata only, 39 (12S) and 26 (COI) samples retained sufficient fish detections for downstream analysis. We compared species richness across habitats using one-way ANOVA with Tukey post hoc tests. We assessed beta diversity using Jaccard dissimilarities, visualized with NMDS and PCoA ordinations, and tested for group-level differences using PERMANOVA (*adonis2* with 999 permutations; Anderson 2001), with pairwise comparisons for all habitat pairs. We then evaluated homogeneity of group dispersion with *BETADISPER* prior to PERMANOVA interpretation. To identify species significantly associated with each habitat, we ran indicator species analysis using the *multipatt* function in the *indicspecies* R package (De Cáceres & Legendre 2009) with 999 permutations. To estimate true species richness, we used Chao1 richness estimators via *specpool* in *vegan*, and species

accumulation curves were generated to assess whether sampling effort was sufficient to capture community diversity across habitats and markers (Oksanen et al. 2022).

Effort 3: Reference Database Development

The Mo‘orea Biocode project comprehensively barcoded all marine vertebrates on Mo‘orea (Leray et al. 2012), following COI-based Barcode of Life (BOLD) protocols (Ratnasingham & Hebert 2007). However, because the MiFish 12S primer set (Miya et al. 2015) has become the standard for marine vertebrate metabarcoding, we constructed a novel, location-specific 12S barcoding database. First, we determined which marine vertebrates from Mo‘orea were represented in GenBank and DNA barcoding databases, such as BOLD. For the 706 species from Mo‘orea without a 12S DNA barcode, we used DNA extractions from the Mo‘orea Biocode project and amplified each of these with the MiFish 12S primer set, using the amplification protocols described in Common eDNA Methods. We then sent the PCR amplicons to the Smithsonian National Museum of Natural History for Sanger sequencing. Lastly, we combined existing and new barcode sequences into a single database, creating the most comprehensive 12S DNA barcoding database possible for Mo‘orea’s marine vertebrates. This reference database, designated bio_12S, will be made publicly available.

Effort 4: Comparison with Visual Surveys

Visual Survey Data Compilation and Processing

To compare eDNA-based biodiversity patterns with long-term visual monitoring, we compiled fish community records from the Mo‘orea Coral Reef Long-Term Ecological Research (MCR LTER) program. We downloaded the Annual Fish Survey dataset (knb-lter-mcr.6; MCR LTER, 2006–2019) from the MCR data portal (<http://mcrlter.msi.ucsb.edu>) and extracted the subset of

years and sites corresponding to our eDNA sampling region. Using the full 2006–2019 dataset enabled us to generate both cumulative species inventories and time- and site-specific comparisons that reflect long-term patterns at habitats sampled with eDNA. To assess whether long-term species accumulation in visual surveys inflated apparent differences with eDNA, we conducted a secondary comparison restricting LTER data to 2016 surveys only, corresponding to the year of eDNA collection.

LTER visual censuses follow a standardized belt-transect protocol described in the dataset metadata (MCR LTER, 2006–2019). At each site, divers survey four $50\text{ m} \times 5\text{ m}$ transects to count mobile fishes, followed by four $50\text{ m} \times 1\text{ m}$ transects to record attached and semi-cryptic species. Surveys are conducted annually during the first week of August. As noted by the survey coordinators (A. Brooks, *pers. comm.*), the same three divers completed transects 1, 2, and 4 from 2006–2019, whereas transect 3 was performed by a rotating set of trained observers. Diver initials were retained in the dataset but were not used for filtering because analyses relied on presence-absence rather than abundance data.

We aligned LTER survey locations with eDNA sampling stations based on habitat type and geographic proximity following guidance from LTER researchers. Forereef 1 and Forereef 2 corresponded to LTER1-FOR and LTER2-FOR, respectively. Backreef 1 and Pavement Nui matched LTER1-BAK and LTER2-BAK, and the Tema'e Beach site aligned with LTER3-BAK. The MRB site is adjacent to LTER1-BAK and was therefore compared to that species pool. Pelagic stations had no direct visual analogs and were instead compared to the regional Mo'orea species inventory.

Because species names in the LTER dataset predate several taxonomic revisions, we standardized all identifications using the authoritative checklist of French Polynesian fishes

published by Siu et al. (2017). All names were harmonized prior to analysis to ensure consistency across LTER records, eDNA assignments, and regional species inventories. For analytical comparisons with eDNA, raw LTER transect counts were converted to presence-absence at each site. This approach allowed us to generate species lists matching eDNA sampling locations and to derive cumulative species inventories across all available survey years. We chose to use only presence-absence data because eDNA yields relative detections that cannot be directly compared with visual abundance estimates (Muenzel et al., 2024).

Statistical Comparisons

We conducted comparisons between eDNA and visual surveys using several complementary approaches. First, we generated species accumulation curves with the *vegan* package in R (Oksanen et al. 2022) to assess sampling completeness and evaluate how quickly eDNA and visual surveys approached asymptotic richness. Second, we quantified species overlap among eDNA detections, cumulative LTER detections, and the regional French Polynesia checklist (Siu et al. 2017) using set-based comparisons visualized with Venn diagrams (*ggvenn*; Yan 2021). Lastly, when appropriate, we compared richness and community structure at matched eDNA–LTER sites to assess concordance across backreef, forereef, and pelagic habitats. We conducted all data processing and statistical analyses in R using *phyloseq* (McMurdie & Holmes 2013), *vegan* (Oksanen et al. 2022), and *ranacapa* (Kandlikar et al. 2018).

Results

eDNA Degradation

DNA sequencing resulted in ~25 million reads, with 21,886,074 reads retained following primer trimming, quality filtering, and taxonomic assignment using the *Anacapa Toolkit*. After

subsequent contaminant removal with DECONTAM and elimination of singleton ASVs, 6,859,700 reads remained for downstream analysis. Total read counts per sample in the Ocean Enclosure ranged from 167,803 at T-0 to 128,361 at T-84, while the Mesocosm ranged from 279,062 at T-0 to 57,114 at T-84.

Ocean Baseline samples ($n = 3$) collected from source water prior to experimental setup showed a vertebrate read proportion of $92.7 \pm 5.4\%$, closely matching the T-0 values observed in both experimental systems (92.5% Mesocosm; 87.9% Ocean Enclosure). This demonstrates that mesocosm systems initially captured a near-natural vertebrate eDNA community.

In both mesocosms, vertebrate reads declined sharply over time (Figure 1-2). At T-0, vertebrate reads comprised $92.5 \pm 0.5\%$ of total reads in the Mesocosm and $87.9 \pm 1.1\%$ in the Ocean Enclosure, declining to $32.1 \pm 7.0\%$ and $23.6 \pm 7.9\%$, respectively, by T-20, with vertebrate reads approaching zero by T-46 in the Ocean Enclosure (0.2%) and by T-84 in the Mesocosm (0.4%). As teleosts (Actinopteri) comprised 98.8% of all vertebrate reads, the teleost eDNA degradation curve is effectively representative of total vertebrate eDNA dynamics in both mesocosm types. Teleost eDNA declined exponentially over time in both mesocosm types (Mesocosm: $k = 0.045 \text{ hr}^{-1}$, half-life = 15.6 hrs; Ocean Enclosure: $k = 0.038 \text{ hr}^{-1}$, half-life = 18.4 hrs), with teleost reads starting at approximately 90% of total reads at T-0 and declining to near zero by T-84 hrs in both systems (Figure 1-3). Concurrent with the decrease in vertebrate sequences was a corresponding increase in the proportion of bacterial reads as fish eDNA degraded over time. Sampling ended at 84 hours due to storm damage to the seawater enclosure. Full vertebrate and teleost read proportions at each time point are provided in Appendix Tables 1-A1 and A2.

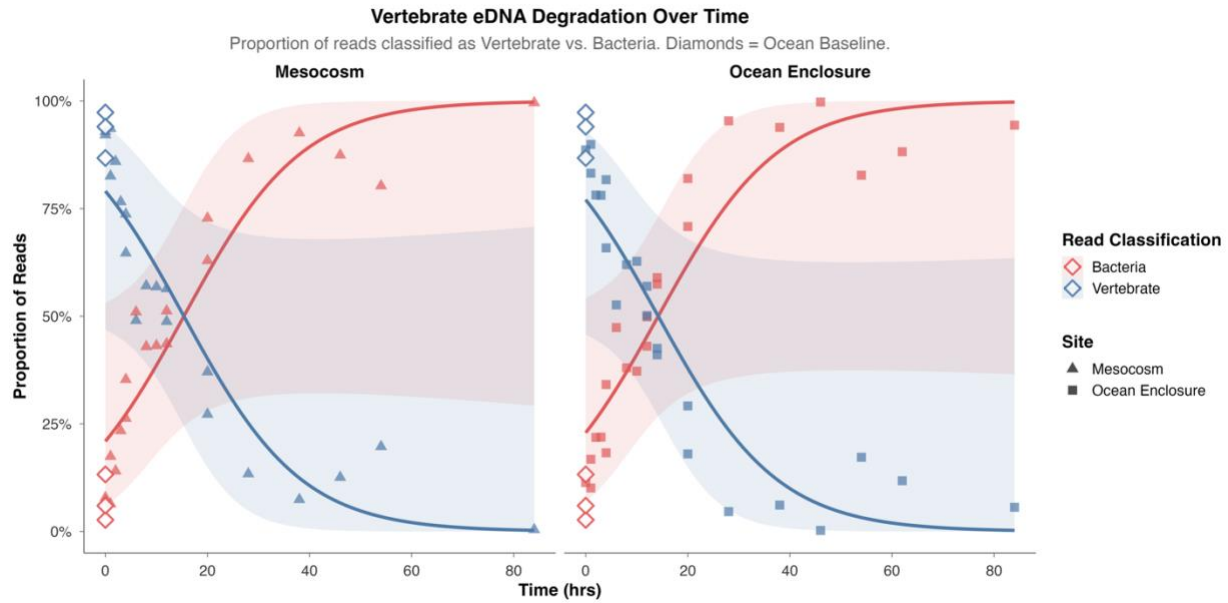


Figure 1-2. Proportion of sequencing reads classified as vertebrate (blue) versus bacteria (red) over 84 hours in a land-based mesocosm and an open ocean enclosure in Mo'orea, French Polynesia. Open diamonds at $T = 0$ represent Ocean Baseline samples ($n = 3$) collected from the source water prior to experimental setup and are overlaid on both panels for reference. Lines show binomial GLM fits with 95% confidence intervals (shaded). Both experimental systems show a consistent inverse relationship between vertebrate and bacterial read proportions, reflecting rapid tropical eDNA degradation.

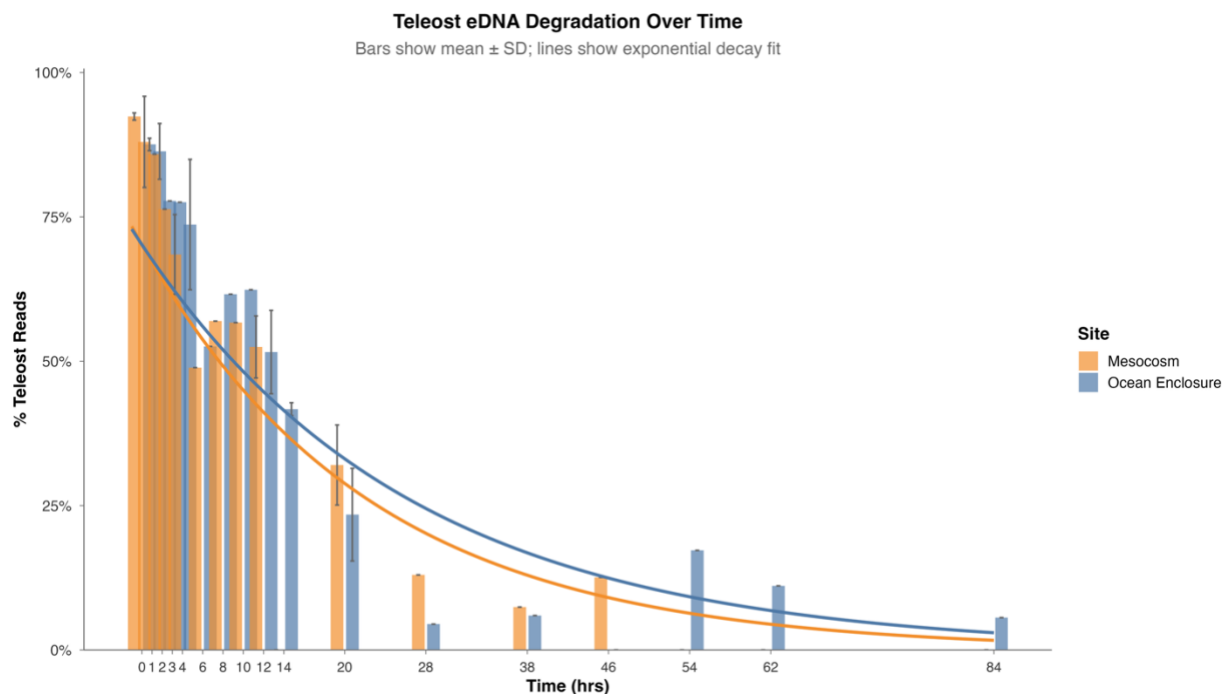


Figure 1-3. Percentage of sequencing reads classified as Teleost (Actinopteri) over 84 hours in a land-based mesocosm and ocean enclosure in Mo'orea, French Polynesia. Bars represent mean values across biological replicates $\pm$ standard deviation. Curves show best-fit exponential decay models for each experimental system (Mesocosm: $k = 0.045 \text{ hr}^{-1}$; Ocean Enclosure: $k = 0.038 \text{ hr}^{-1}$). Teleost read proportions declined consistently across both systems, mirroring the broader vertebrate degradation pattern shown in Figure 1-2.

Daily mean wind speeds at Moorea-Temae Airport (MOZ) increased steadily throughout the eDNA degradation experiment, rising from 6.8 km/h on 5 July to 12.5 km/h on 6 July and 20.1 km/h on 7 July, before reaching a daily mean of 24.5 km/h on 8 July (Figure 1-4). The last sample was collected at 10:43 local time on 8 July 2018. Later that day, wind conditions intensified sharply, with a peak gust of 64 km/h recorded at 21:00 and 11.6 mm of rainfall accumulating over the afternoon and evening hours. Elevated winds persisted into 9 July (daily mean 31.6 km/h), with peak gusts reaching 59 km/h by 09:00, consistent with a storm event that damaged the ocean water enclosure and precluded further sampling.

Daily Average Wind Speed During eDNA Degradation Experiment

Moorea-Temae Airport (MOZ) Historical Weather Data, July 2018

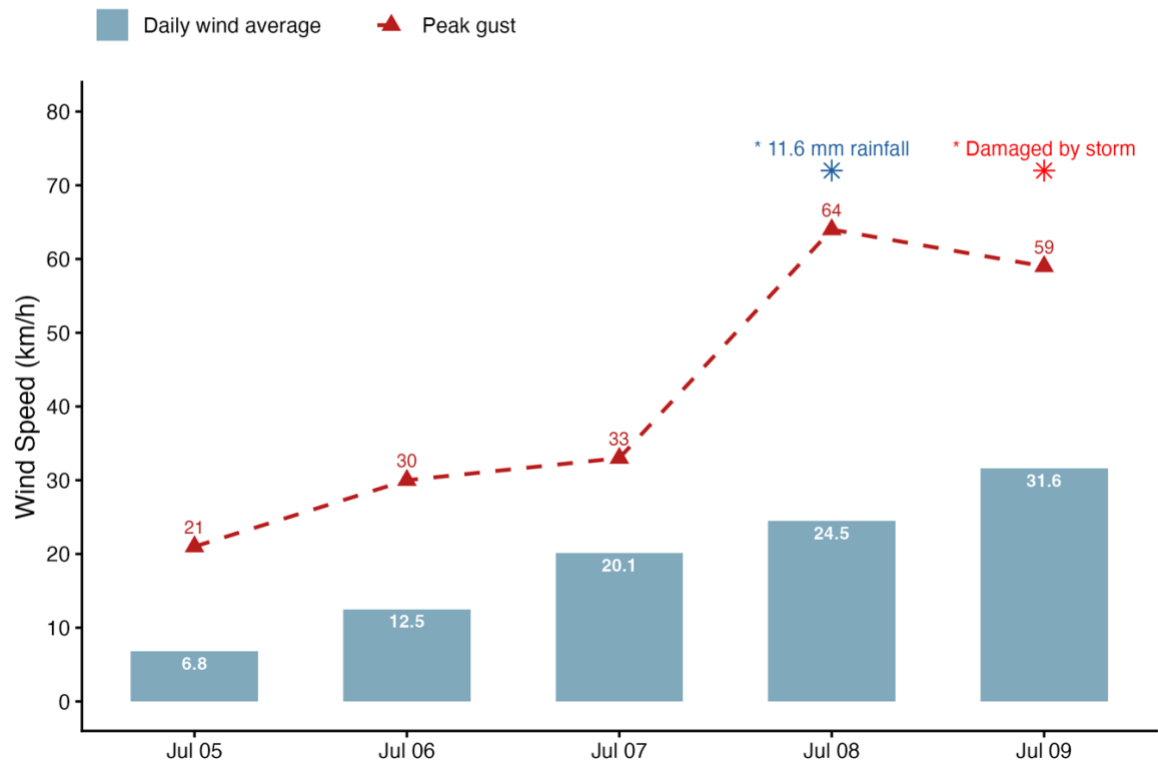


Figure 1-4. Daily mean wind speed (blue bars) and peak wind gust (red dashed line) at Moorea-Temae Airport (MOZ), ~7 km from the experimental site, during the eDNA degradation experiment (5–9 July 2018). Blue asterisk indicates total daily rainfall on 8 July; red asterisk marks storm damage to the ocean enclosure on 9 July. Data from World Weather Online.

Fish Community Composition Over Time

Fish community composition at the Family level changed markedly over time in both mesocosms (Figure 1-5). The Ocean Baseline community was dominated by *Mullidae*, *Engraulidae*, and *Holocentridae*, and T-0 samples in both experimental systems closely resembled this baseline composition, confirming that the mesocosms initially captured natural source-water communities. Although there were broad similarities across early sampling times,

the proportions of recovered families varied considerably within each mesocosm, and notable variation was observed between the two experimental systems. As vertebrate eDNA signals began to wane, family proportions shifted greatly and idiosyncratically, with some families disappearing completely (Figure 1-5). Although both mesocosms began from a near identical baseline community, each followed a distinct degradation trajectory, with different families being lost in different sequences and at different rates, consistent with eDNA decay proceeding as a stochastic, system specific process rather than a uniform decline. PCA of Hellinger-transformed family-level read counts revealed that fish communities shifted progressively over time, with early time points (T-0 to T-3) forming a distinct cluster separate from later degradation phases and communities converging toward a similar impoverished composition as vertebrate eDNA degraded (Figure 1-6A). The two systems differed in magnitude of this shift, the land based tank departed more strongly from the shared T-0 baseline than the in situ ocean enclosure, driven largely by a marked increase in the relative abundance of *Engraulidae* (anchovies), whereas the ocean enclosure tracked the source water community more closely over time (Figure 1-5). Disaggregating data by both mesocosm type and time showed substantial variation between communities in the two mesocosms at the same time periods (Figure 1-7A-D). Time-window PCA revealed that fish communities were most distinct between mesocosm types when eDNA was fresh (T 0–3 hrs) and converged as degradation progressed, while bacterial communities showed the opposite pattern, diverging over time as the vertebrate eDNA signal was replaced by locally distinct microbial assemblages (Figure 1-7E-H).

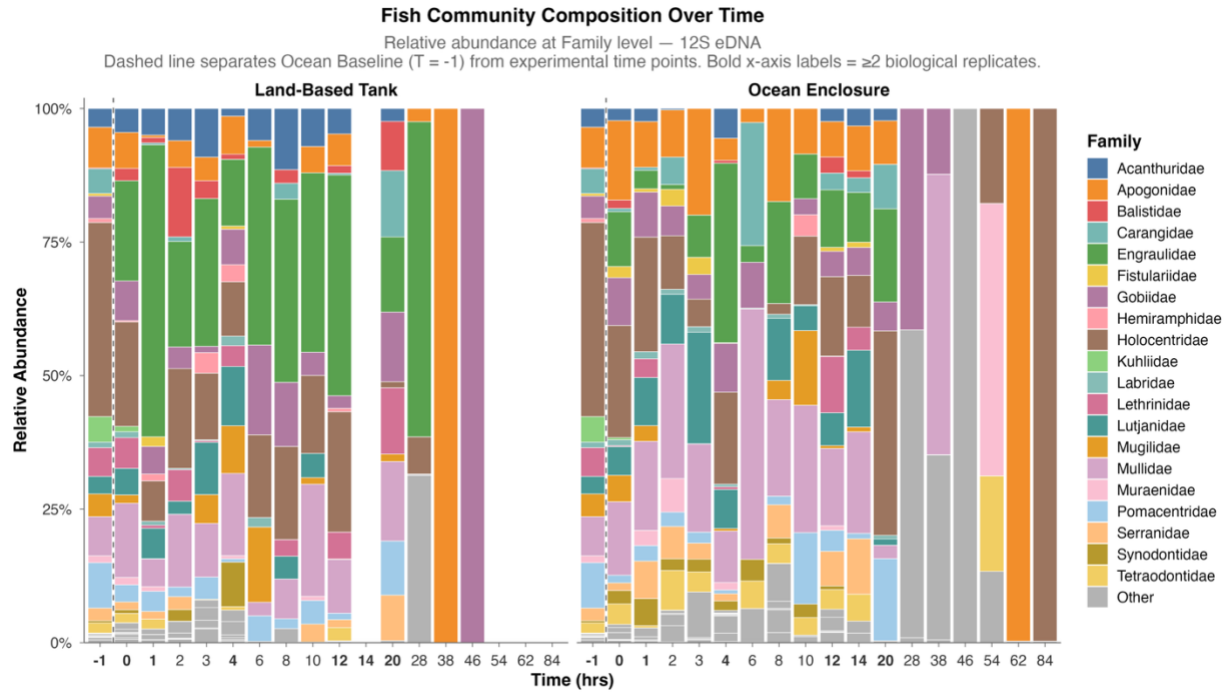


Figure 1-5. Relative abundance of fish families detected by 12S eDNA metabarcoding in a land-based mesocosm and ocean enclosure in Mo'orea, French Polynesia. Each bar represents the proportional composition of sequencing reads at Family level at a single time point. Ocean Baseline samples (T = -1; $n = 3$) were collected from the source water prior to experimental setup and are shown to the left of the dashed line in both panels as a reference community. Experimental sampling spanned 0–84 hours, with early time points (T-0 to T-12) reflecting a diverse assemblage of Mo'orea reef fish families and later time points showing a progressive loss of family-level diversity as vertebrate eDNA degraded below detectable levels. Bold x-axis labels indicate time points with ≥ 2 biological replicates; all other time points had a single 1 L sample.

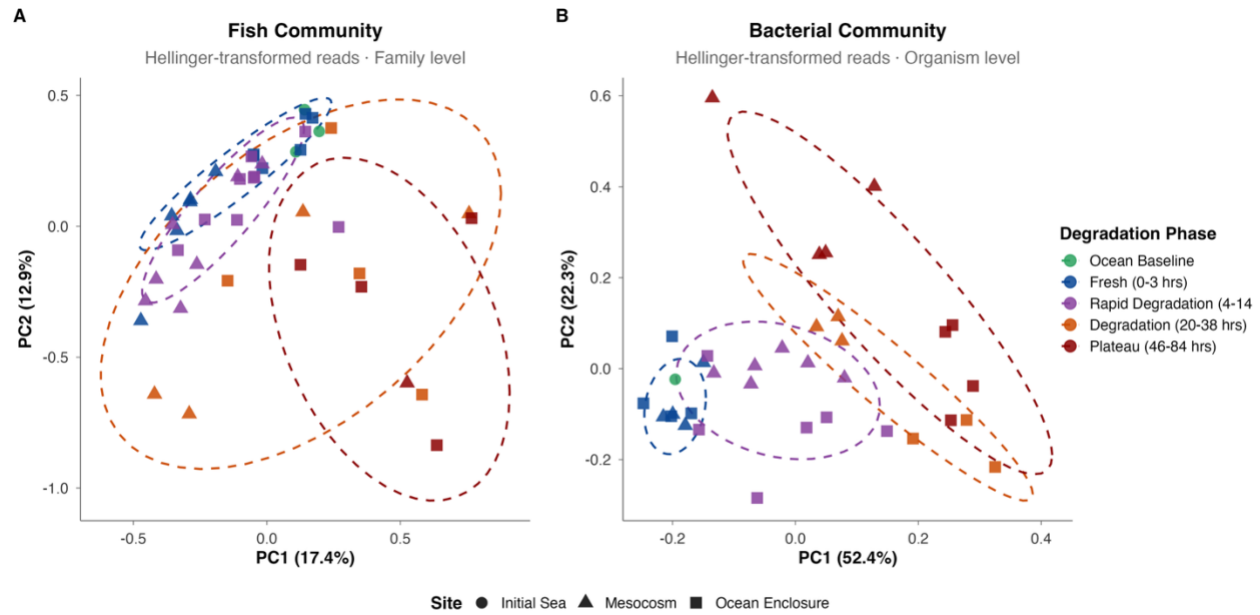


Figure 1-6. PCA of Hellinger-transformed read counts for fish (A) and bacterial (B) communities across eDNA degradation time points in Mo'orea, French Polynesia. Ocean Baseline samples (green circles, $n = 3$) were collected from source water prior to experimental setup. Dashed ellipses represent 80% confidence intervals per degradation phase. In both communities, samples at T-0 closely resemble the Ocean Baseline, indicating that the experimental systems initially captured a near-natural community composition. Over time, fish communities diverge from this baseline and show increasing compositional variability, while bacterial communities shift progressively along the first principal axis as vertebrate eDNA is replaced by a more variable microbial signal.

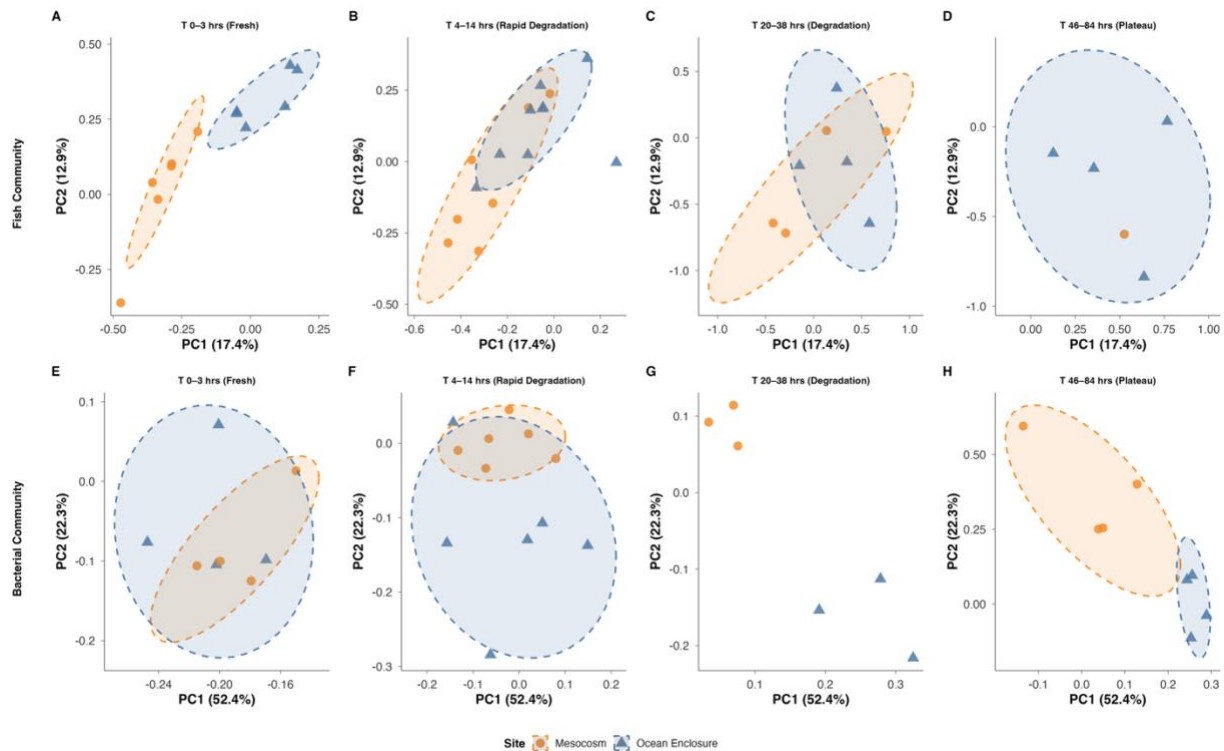


Figure 1-7. Time-window PCA of fish (A–D) and bacterial (E–H) community composition across four eDNA degradation phases in Mo'orea, French Polynesia. Ocean Baseline samples are not shown in this figure to preserve the phase-based comparison between experimental systems. Fish communities were most distinct between sites when eDNA was fresh (T 0–3 hrs) and converged as degradation progressed, while bacterial communities diverged over time. Dashed ellipses represent 80% confidence intervals. Circles = Mesocosm; Triangles = Ocean Enclosure. Ellipses are not shown for panel G (T 20–38 hrs, Bacterial Community) due to insufficient sample size per site.

Bacterial Community Composition Over Time

Community composition of the 50 most abundant bacterial ASVs was dominated by uncultured bacteria at all time points, including in the Ocean Baseline samples. Of the 49 ASVs that returned BLAST hits (96.1–100% identity), the majority matched uncultured bacterium, with

additional matches to uncultured Cyanobacteria, *Pseudoalteromonas* sp., Gammaproteobacteria, and Oceanospirillales, consistent with a typical tropical marine reef bacterial community (Nelson et al., 2011) (Figure 1-8). The Ocean Baseline samples (T = -1) showed a bacterial community composition closely resembling T-0 samples in both experimental systems, further supporting that the mesocosms initially captured a near-natural microbial community. Proportions of uncultured bacterium in both mesocosms increased over time initially but then decreased in the later stages of the experiment. PCA of Hellinger-transformed bacterial read counts revealed increasing spread in community composition over time, a pattern opposite to that observed for fish communities, as the vertebrate eDNA signal was replaced by an increasingly variable bacterial signal (Figure 1-6B, 7E-H).

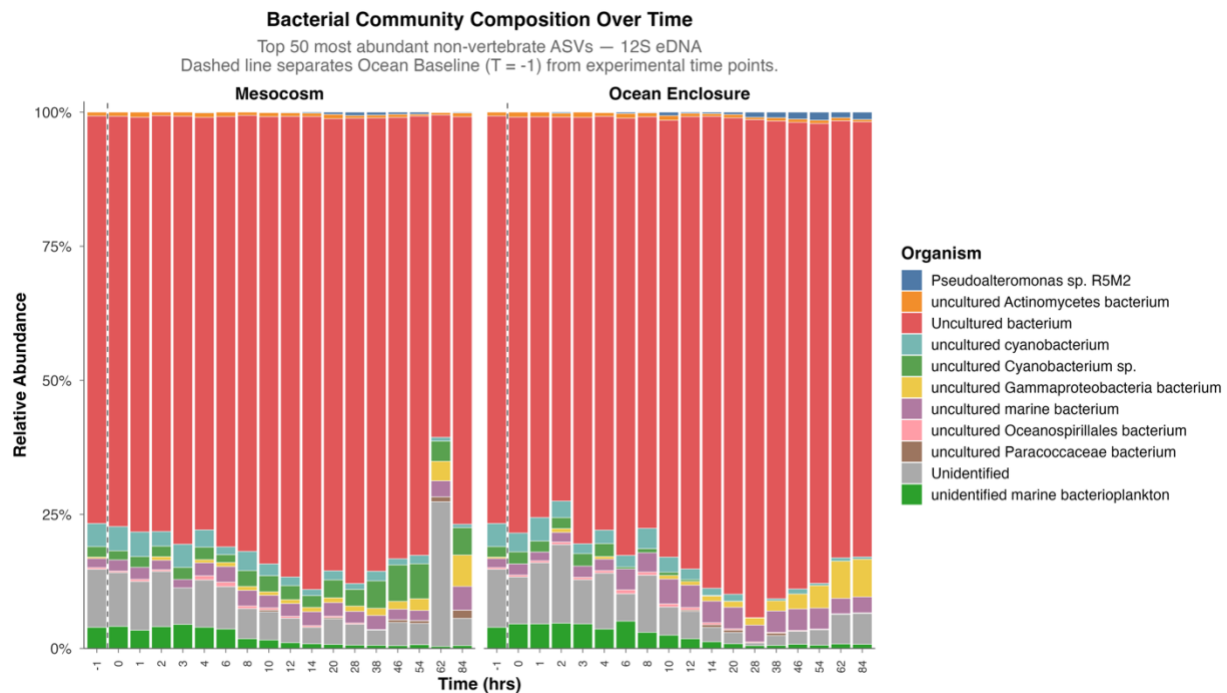


Figure 1-8. Relative abundance of the top 50 non-vertebrate ASVs across a land-based mesocosm and ocean enclosure in Mo'orea, French Polynesia. Taxonomy assigned via BLASTn against NCBI Bacteria (taxid:2; 96.1–100% identity). Ocean Baseline samples (T = -1; $n = 3$)

collected from source water prior to experimental setup are shown to the left of the dashed line in both panels as a reference community. Uncultured bacterium dominated throughout, with contributions from Cyanobacteria, *Pseudoalteromonas* sp., Gammaproteobacteria, and Oceanospirillales.

Spatial Community Composition Across Habitats

Species Detection and Richness

For the spatial survey, eDNA was collected as three 1 L replicates at each of the 13 sites. The 12S marker yielded 5,199,226 reads across 39 samples and the COI marker yielded 6,091,176 reads across 38 samples, following quality filtering with DECONTAM and removal of singletons. Rather than rarefying to even sequencing depth, which discards valid data and reduces statistical power (McMurdie & Holmes, 2014), we retained all reads and analyzed fish communities using presence absence data. After filtering to Chordata only ASVs, 39 (12S) and 26 (COI) samples retained sufficient fish detections for downstream analysis. The 12S marker detected 261 teleost species across these 39 samples, while COI detected 73 teleost species across 26 samples, a 3.6-fold difference in detection (Appendix Figure 1-A1). Combined eDNA detected 266 unique teleost species, with the two markers sharing 68 species in common. Beyond teleosts, eDNA additionally detected five *elasmobranchs* (two reef sharks, a spotted eagle ray, a sicklefin devil ray, and a stingray) and three marine mammals (spinner dolphin *Stenella longirostris*, rough-toothed dolphin *Steno bredanensis*, and a beaked whale, *Ziphiidae*), which were excluded from teleost-based habitat and visual-survey comparisons.

Species richness differed significantly among habitats for both markers (Figure 1-9; full ANOVA results in Appendix Table 1-A3). For 12S, backreef sites harbored the highest mean richness (74.8 ± 18.7 species per sample), followed by forereef (44.2 ± 20.8) and pelagic ($7.4 \pm$

4.2; ANOVA: $F_{2,36} = 55.49$, $p < 0.0001$). All pairwise Tukey HSD comparisons were significant ($p < 0.05$). COI richness was substantially lower across all habitats (backreef: 12.6 ± 5.4 ; forereef: 6.0 ± 2.4 ; pelagic: 1.7 ± 0.6 ; ANOVA: $F_{2,23} = 12.45$, $p < 0.001$), though the forereef–pelagic comparison was not significant (Tukey $p = 0.249$). Species accumulation curves indicated 12S sampling was 85.4% complete for backreef, 84.2% for forereef, and 60.1% for pelagic, while COI completeness was lower across all habitats (42.9–70.1%; full Chao1 estimates in Appendix Table 1-A4).

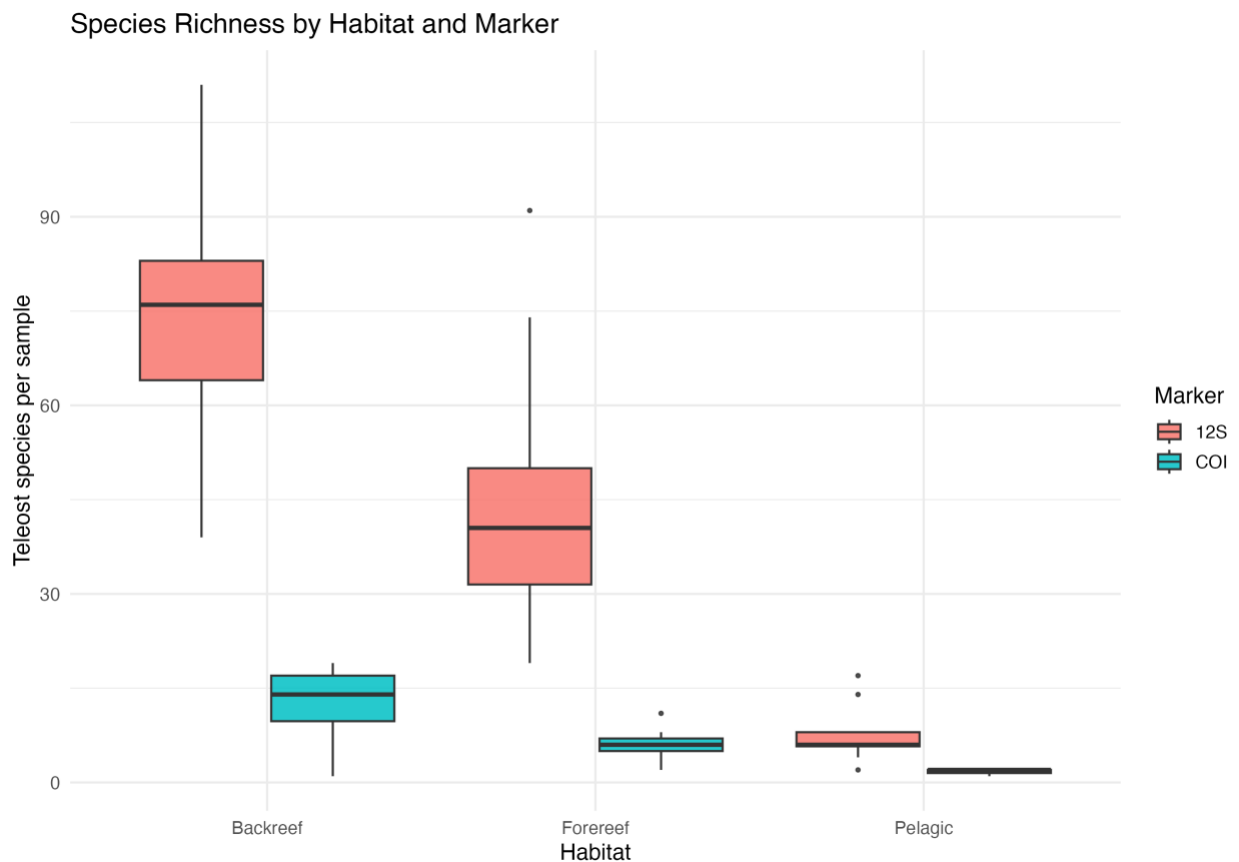


Figure I-9. Species richness per sample by habitat and marker (12S and COI) across backreef, forereef, and pelagic sites in Mo'orea. The 12S marker consistently detected higher richness than COI across all habitats.

Community Ordination and Composition

NMDS ordination of the 12S presence-absence data (stress = 0.153) revealed clear separation of fish communities among the three habitat types (Figure 1-10A & 10B). Backreef samples formed a tight cluster indicating homogeneous fish communities, forereef samples showed moderate spread, and pelagic samples were widely dispersed, reflecting high variability in open-water eDNA detections. PCoA on Jaccard distances corroborated this pattern, with the first two axes explaining 26.7% of total variation (PCo1 = 17.9%, PCo2 = 8.8%), separating communities along a nearshore-to-offshore gradient. PERMANOVA confirmed significant differences in community composition among habitats ($R^2 = 0.212$, $F = 4.84$, $p = 0.001$), with all pairwise habitat comparisons significant ($p = 0.001$; full pairwise results in Appendix Table 1-A5). BETADISPER indicated significant heterogeneity of dispersions ($F = 20.43$, $p = 0.001$), with pelagic communities showing the greatest within-habitat variability, suggesting that both compositional differences and variability differences contribute to the PERMANOVA result. Pelagic samples at all distances (500 m, 1 km, 2 km, and 4 km) clustered separately from reef-associated communities, with no consistent ordination pattern by distance from shore (Figure 1-10C). For COI, PCoA on Jaccard distances showed weaker but discernible habitat separation, with substantial overlap between backreef and forereef, further underscoring the superior

discriminatory power of 12S for fish community characterization.

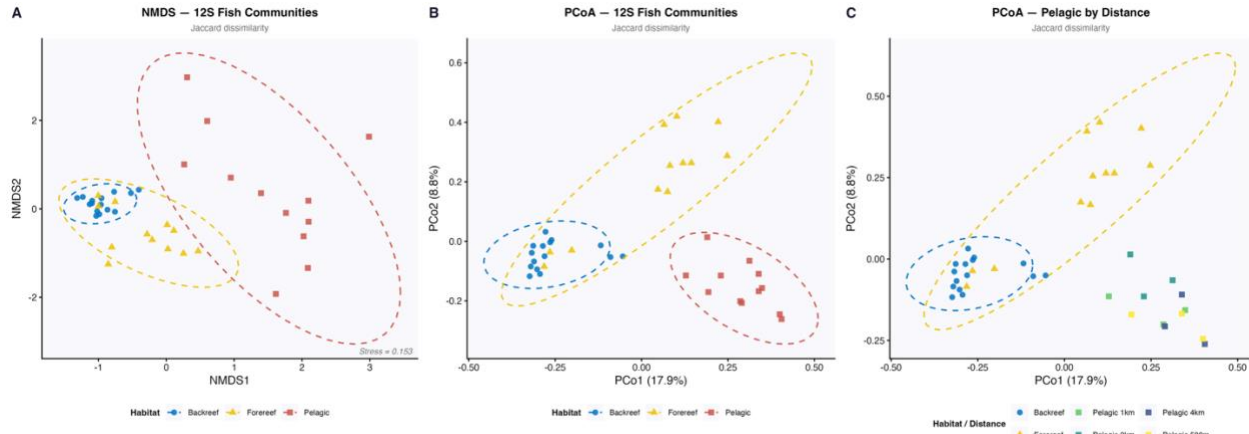


Figure 1-10. NMDS (A) and PCoA (B) ordinations of 12S fish communities based on Jaccard dissimilarity across backreef, forereef, and pelagic habitats in Mo'orea, French Polynesia. Panel C shows the PCoA with pelagic samples coloured by distance from the reef (500 m, 1 km, 2 km, 4 km); backreef and forereef ellipses are retained for reference. Dashed ellipses represent 95% confidence intervals. Stress = 0.153.

At the family level, *Labridae* (wrasses) and *Acanthuridae* (surgeonfishes) were the most frequently detected families across all habitats (Figure 1-11). Backreef and forereef communities were dominated by *Labridae*, with *Acanthuridae* increasing in relative detection frequency toward the forereef and pelagic. Pelagic samples showed a simpler composition with a higher proportion of pooled rare families. Indicator species analysis identified 91 species significantly associated with one or more habitats ($p < 0.05$). Backreef had the most habitat-specific indicators (49 species), including *Mulloidichthys flavolineatus* (IndVal = 0.866), *Leptoscarus vaigiensis* (0.858), and *Thalassoma trilobatum* (0.853), while a further 31 species were indicators of combined backreef-and-forereef (reef-generalist) habitat, including *Chlorurus sordidus* (0.941) and *Ctenochaetus striatus* (0.926). Forereef had 11 indicators, including *Centropyge bispinosa*

(0.769) and *Pterocaesio tile* (0.724). No species were indicators of pelagic habitat alone, consistent with the high community variability observed. Full indicator species results are provided in Appendix Table 1-A6.

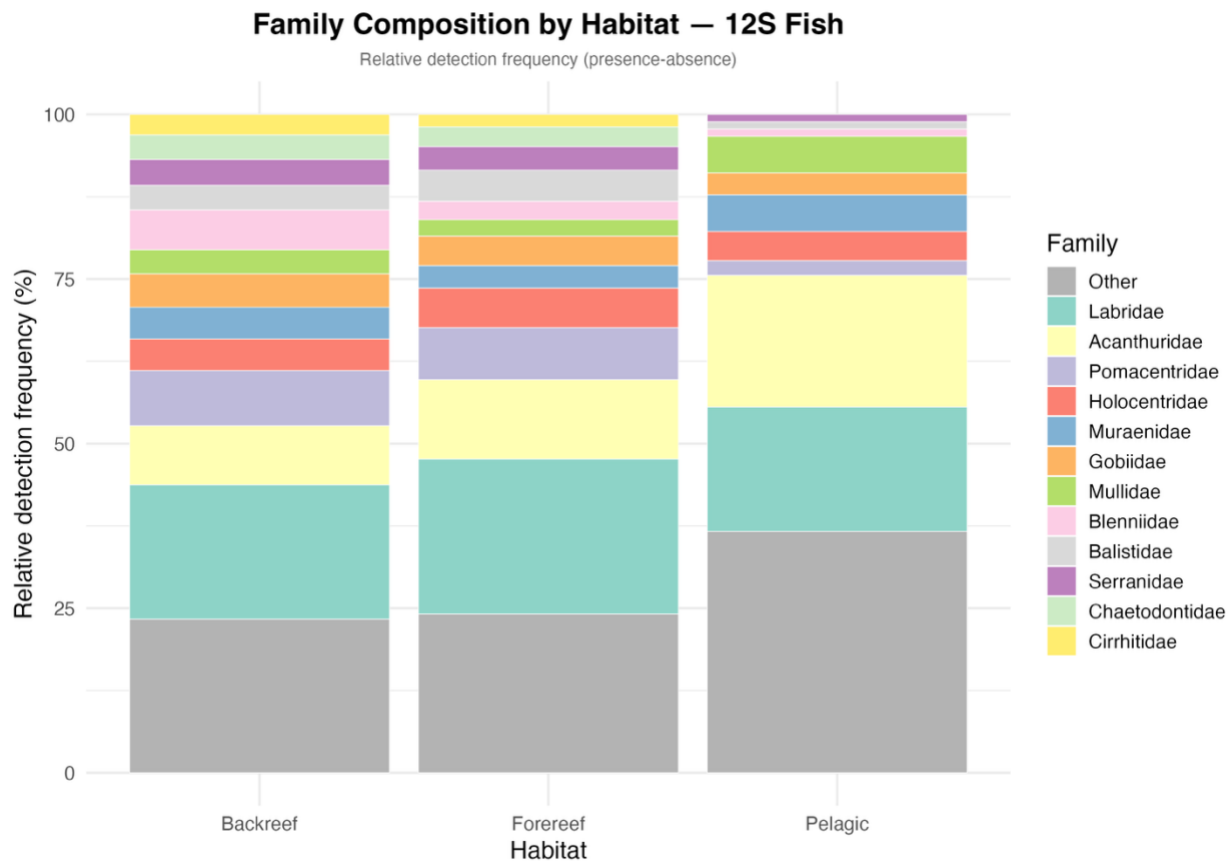


Figure 1-11. Family-level composition of 12S fish communities across backreef, forereef, and pelagic habitats in Mo'orea, French Polynesia. Bars show the relative detection frequency of the 12 most frequently detected families, calculated as each family's share of total species detections (sample $\times$ species presences) within a habitat; all remaining families are grouped as "Other." Because the analysis is presence-absence, values reflect detection frequency rather than sequence read abundance.

Comparison with Visual Surveys

Combined eDNA (12S + COI) detected 266 teleost species, of which 30 were not recorded in either the Mo‘orea LTER visual surveys or the Siu et al. (2017) checklist (Figure 1-12A). These novel detections included cryptobenthic and reef-associated fishes (e.g., moray eels, scorpionfish), mesopelagic species (lanternfish, hatchetfish), and pelagic taxa (flying fish, halfbeaks). In addition, eDNA detected three marine mammal species not captured by fish-targeted visual surveys: spinner dolphin (*Stenella longirostris*), rough-toothed dolphin (*Steno bredanensis*), and a beaked whale (*Ziphiidae*) (Nikolic et al., 2025). Of the combined eDNA detections, 215 teleost species were shared across all three datasets (eDNA, LTER, and Siu), while 11 species were shared between eDNA and the Siu checklist only, and eDNA detected 30 species found by neither survey method.

When compared against the full 2006–2019 LTER dataset (807 species), eDNA shared 225 species with visual surveys, while 287 species were detected exclusively by LTER and 296 were shared between LTER and the Siu et al. (2017) checklist only. However, when restricted to 2016 surveys only, the year of eDNA collection the LTER dataset contained 224 species, compared to 266 teleost species detected by combined eDNA (12S + COI), with eDNA recovering 42 more species than visual surveys conducted in the same year. Of these, 124 species were shared between both methods, while only 7 species were detected exclusively by LTER 2016 visual surveys and 39 species were detected by combined eDNA alone (Figure 1-12B).

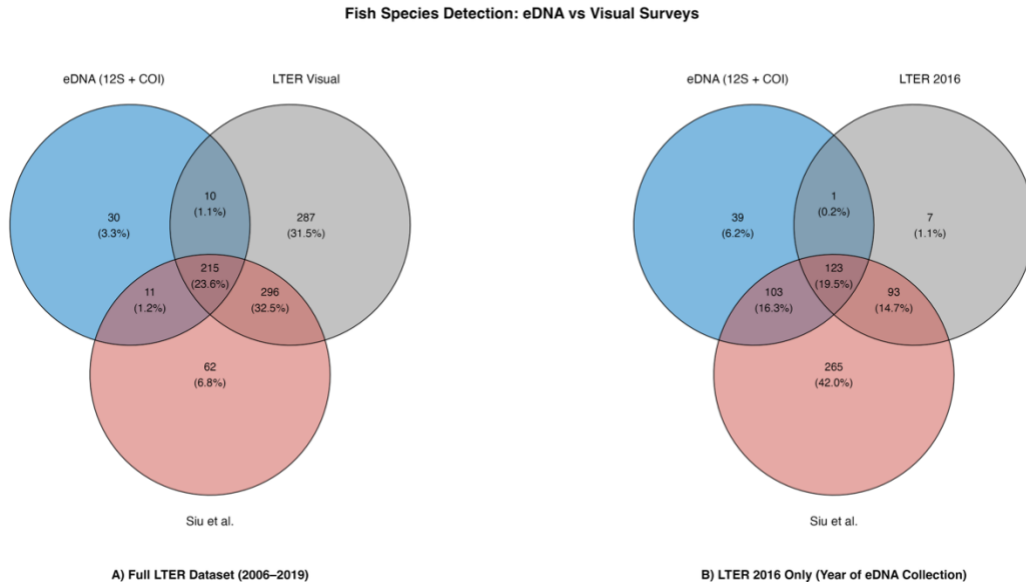


Figure 1-12. Venn diagrams comparing fish species detected by combined eDNA metabarcoding (12S + COI) with visual survey records in Mo‘orea, French Polynesia. (A) Comparison against the full MCR-LTER visual survey dataset (2006–2019) and the Siu et al. (2017) regional checklist; eDNA recovered 27.9% of the cumulative LTER fauna (225/807 species) and 38.7% of the Siu checklist (226/584 species). (B) Comparison restricted to LTER surveys from 2016, the year of eDNA collection; against this matched-year dataset, eDNA recovered 55.4% of LTER fishes (124/224 species) and 38.7% of the Siu checklist. Both panels show combined 12S + COI detections. Percentages denote each region's share of the total across all datasets.

Species accumulation curves showed that eDNA surveys recovered 266 teleost species with fewer than 40 samples, while Mo‘orea LTER visual surveys required over 2,000 surveys to approach similar cumulative richness (Figure 1-13).

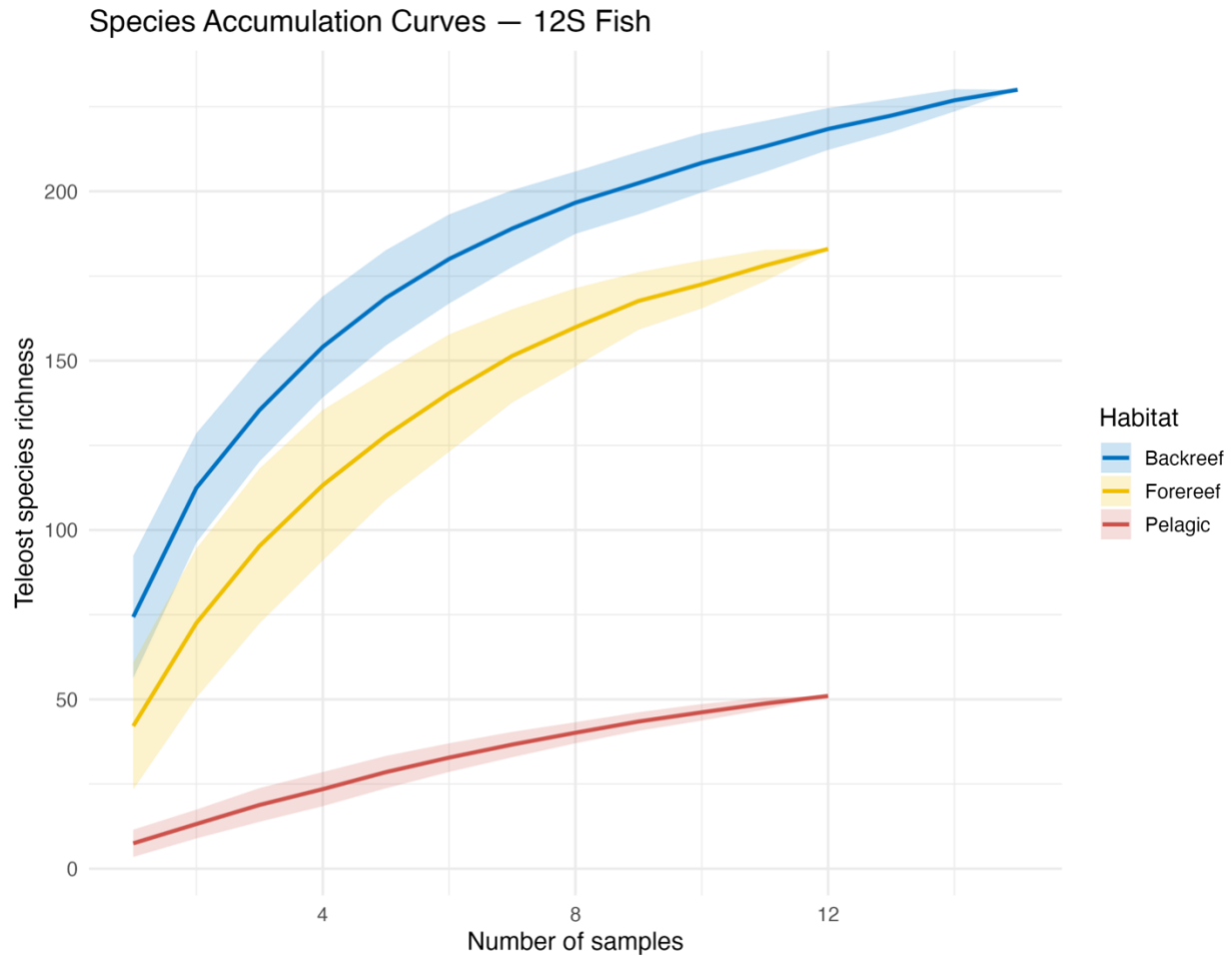


Figure 1-13. Species accumulation curves for 12S eDNA across backreef, forereef, and pelagic habitats in Mo'orea, French Polynesia. Lines show cumulative teleost species richness with increasing sample number; shaded areas represent 95% confidence intervals from 100 random permutations.

Discussion

Environmental DNA is a powerful tool for characterizing marine communities (Taberlet et al., 2012; Deiner et al., 2017; Miya et al., 2020), but questions about the longevity and potential transport of eDNA signals remain, particularly in tropical marine environments. Using naturally occurring eDNA under ambient tropical field conditions, this study demonstrates very rapid

degradation of vertebrate eDNA signals, with teleost eDNA declining exponentially with a half-life of approximately 15–18 hours and approximately two-thirds of the vertebrate eDNA signal lost within just 20 hours. Decline in vertebrate signal was accompanied by a corresponding increase in bacterial signals, highlighting an important relationship between vertebrate and microbial DNA during the degradation process. Rapid eDNA degradation suggests important limits to advection of eDNA, which should allow the discrimination of tropical reef fish communities on fine spatial scales, consistent with results seen in Port et al. (2016).

Consistent with rapid eDNA degradation, field eDNA surveys successfully distinguished teleost fish communities across unique habitat types on relatively small spatial scales. Backreef and forereef communities formed distinct but partially overlapping clusters in ordination space, both clearly separated from pelagic fish communities, while all pelagic distances (500m–4km) clustered together, remaining distinct from reef-associated habitats (Figure 1-10C). The discriminatory power of eDNA on Mo'orea coral reef environments is similar to previous studies that discriminated habitats on scales of 50–150m (Port et al., 2016; Yamamoto et al., 2016) and stands in contrast to studies showing differences only at the scale of kilometers (O'Donnell et al., 2017; Lamy et al., 2021). Given that Mo'orea back reefs experience high rates of water transport and relatively low residence times (Hench et al., 2008), the differences in eDNA signatures across such small spatial scales is likely due to rapid degradation. From just 39 L of seawater, eDNA metabarcoding recovered 55.4% of the teleost species documented by LTER divers during the matched 2016 survey year, and detected more species overall than the same-year visual surveys despite a fraction of the sampling effort a level of detection that the LTER program accumulated only across more than 2,000 visual surveys. Moreover, eDNA captured taxa not observed visually, including marine mammals (Nikolic et al., 2025), mesopelagic, and

pelagic species. These results add to a growing number of studies demonstrating the complementary nature of eDNA and visual surveys (Gold et al., 2021; Valdivia-Carrillo et al., 2021; Muenzel et al., 2024).

Rapid Degradation of Tropical eDNA Signals

Studies of eDNA persistence report a wide range of detection times, from less than a day to several weeks in freshwater mesocosms (Dejean et al., 2011; Pilliod et al., 2014; Strickler et al., 2015), to typically less than a week in temperate marine environments (Thomsen et al., 2012; Wood et al., 2020). More specifically, studies of marine eDNA persistence using fish held in enclosures report persistence times ranging from 7 to 46 hours (Sassoubre et al., 2016; Collins et al., 2018). However, a common feature of these studies is introducing eDNA by confining fish in an enclosure and then tracking degradation after removal, making it unclear whether starting concentrations reflect natural levels. Field studies face an additional complication: foreign eDNA introduced into a protected Southern California rocky reef was undetectable after just 7.5 hours (Ely et al., 2021), though rapid signal loss likely reflects both true degradation and physical dispersal by waves and currents rather than degradation alone. These distinctions matter because, given the exponential rate of eDNA decline, unnaturally high (or low) starting concentrations would artificially extend (or reduce) apparent persistence times and inflate (reduce) estimates of advection distances. Similarly, advection of signals introduced to natural environments can artificially decrease perceived degradation time.

In contrast, our mesocosm studies used large volumes of seawater with naturally occurring eDNA signatures exposed to ambient light and temperature. This approach revealed sharp declines in vertebrate eDNA within 20 hours, with signals virtually disappearing by 48 hours, considerably faster than rates reported for temperate systems. For example, Collins et al.

(2018) estimated ~48-hour detectability using inshore English Channel water spiked with eDNA from local species in a controlled laboratory setup, and Sassoubre et al. (2016) reported 3–4 day persistence for marine fish held in mesocosms; in both cases, starting concentrations likely influence persistence estimates up or down. The accelerated degradation we observed is likely driven by the combination of high solar radiation, elevated sea surface temperatures (27–29°C in Mo‘orea), and highly active tropical microbial communities, all of which promote enzymatic and photolytic DNA breakdown (Barnes & Turner, 2016; Andruszkiewicz et al., 2021). The slightly slower decay rate in the Ocean Enclosure compared to the land-based Mesocosm suggests that natural *in situ* conditions may modestly slow eDNA degradation relative to a land-based tank environment, perhaps by buffering maximum temperatures. By capturing the decay of natural eDNA signatures rather than spiked or artificially elevated concentrations, this study provides realistic degradation rate estimates under field-relevant conditions.

The rapid decay of eDNA signals observed in this study has important implications for the interpretation of tropical eDNA surveys. Rapid degradation means that species detections within any given sample are more likely to reflect very recent biological presence. Moreover, assuming diffusion of eDNA is passive, concentrations will be highest closest to the source. Combined, these two processes reduce the risk of false positives from older, advected signals. Rapid degradation in tropical systems give eDNA an advantage over temperate systems, where longer persistence may introduce temporal ambiguity into community reconstructions. However, rapid degradation also means that sampling and sample processing must be timely, with sample processing occurring immediately or cooled during transport to minimize loss of signal. Additionally rapid degradation means that low-abundance taxa may fall below detection thresholds if degradation outpaces shedding. The low species richness observed in pelagic

samples (7.4 ± 4.2 species per sample for 12S) likely reflects both genuine ecological sparsity and the compounding effect of dilution and rapid degradation in the open water column.

Although we observed rapid degradation in eDNA signals, there was an unexpected increase in teleost signal toward the end of the experiment, most pronounced in the ocean enclosure. This increase is likely the result of increasing wind speeds near the end of the experiment (Figure 1-4), which may have aerosolized and transported fresh eDNA from surrounding surface waters into the open ocean enclosure via sea spray. Additionally, the storm event on the night of July 8–9 brought rainfall that may have further introduced external eDNA into the enclosure via water droplet deposition. Recent work demonstrates that eDNA routinely crosses the air-water boundary, with aquatic fish eDNA detectable in passive air samples collected near spawning streams, despite air concentrations being tens of thousands of times more dilute than water (Ip et al., 2025). Combined with evidence that airborne eDNA signals covary with wind speed (Johnson et al., 2023), these findings suggest that wind-driven sea spray and storm-associated precipitation may have introduced external fish eDNA into our open ocean enclosure, artificially elevating teleost read counts at later time points.

Interestingly, there was substantial variation in fish communities among the two mesocosm at the same sampling times, and across sampling times within the same mesocosm. This idiosyncratic heterogeneity is surprising given that each enclosure contained approximately 3,000–4,000 liters of water and that both mesocosms were filled from the same location at nearly the same time. This variation suggests that eDNA decay is stochastic and that initial shift in decay across taxa after starting conditions will result in different eDNA communities over time. Another likely mechanism driving differences in observed mesocosm communities was the challenge of sampling low biomass eDNA samples. Here, we amplified all samples in triplicate

to minimize the impacts of PCR bias (Alberdi et al. 2018; Ficetola et al. 2016), however, there was still substantial variation among 1 liter samples collected from the mesocosms suggesting that both biological and technical replicates are important in reducing stochastic variation. Given both mesocosms varied in their community composition at time T-0, despite being filled from the same location at nearly the exact same time suggests that there is substantial heterogeneity in natural eDNA signatures. Notably, substantial variation in fish community composition was observed even among time points with two biological replicates (T-0, T-1, T-4, T-12, T-20), suggesting that stochasticity in eDNA community composition is high even when replicate sampling is employed, highlighting the need for potentially increased biological replication in future eDNA degradation studies. This between-system divergence was also asymmetric: the land-based tank departed more strongly from the shared T-0 baseline than the *in-situ* ocean enclosure, driven largely by a pronounced increase in *Engraulidae*. We interpret this cautiously. Because the land-based tank was a static, open system, we cannot exclude external eDNA inputs such as airborne deposition, runoff, or activity of birds or insects contributing to its departure from the source-water community. At the same time, because these data are compositional, the apparent rise of any one taxon is amplified by the relative-abundance inflation that occurs as the dominant reef-fish signal degrades. These explanations are not mutually exclusive, and the ocean enclosure which more closely tracked the source-water community through most of the time series likely provides the more reliable record of natural eDNA decay.

Power of eDNA to Differentiate Coral Reef Habitats

The ability of eDNA to resolve fine-scale habitat differences, whether between reef and seagrass (Port et al. 2016) or inside and outside of marine protected areas (Gold et al. 2021), is an important attribute of eDNA, yet studies of the discriminatory power of eDNA in tropical reef

systems is limited (but see Yamamoto et al., 2016). Ordination analyses demonstrate clear and significant (PERMANOVA, $p = 0.001$) separation of fish communities among backreef, forereef, and pelagic habitats. These results are particularly notable given the active wave-driven circulation within Mo'orea's backreef lagoon, where oceanic swell drives water over the reef crest and through the lagoon before exiting through reef passes (Hench et al., 2008; Leichter et al., 2013). Despite this regular flushing, lagoonal/backreef waters retain chemically and biologically distinct characteristics from offshore waters (Leichter et al., 2013), suggesting that eDNA degradation is sufficiently rapid to preserve habitat-specific signals before advection can homogenize them across habitat types. These findings are consistent with Port et al. (2016), who demonstrated that eDNA could differentiate fish communities at scales of tens to hundreds of meters in temperate kelp forests, and with Yamamoto et al. (2016), who showed fine-scale community variation in Japanese coastal waters, suggesting that eDNA degradation processes must act more rapidly than transport processes in the Mo'orea coastal ecosystem.

Ordination analyses show substantial overlap in backreef and forereef fish communities, a pattern that is expected given that these habitats contain unique species that are habitat specialists as well as habitat generalists that are found in both locations. The tight clustering of backreef/forereef samples in NMDS ordination contrasts sharply with the wide dispersion of pelagic samples, a pattern that reflects both the ecological homogeneity of structured reef fish communities and the stochastic, low-density nature of open water eDNA detections.

BETADISPER confirmed that habitats differ not only in community centroid but also in within group variability, an important distinction given that PERMANOVA results can be influenced by heterogeneous dispersions (Anderson & Walsh 2013). The indicator species analysis identified 49 backreef indicators dominated by goatfishes (*Mulloidichthys flavolineatus*), parrotfishes

(*Leptoscarus vaigiensis*), and small site-attached reef fish, with a further 31 species characterizing combined backreef-and-forereef (reef-generalist) habitat, and 11 forereef indicators including deeper-water specialists such as *Centropyge bispinosa* and *Pterocaesio tile*. These findings align with visual survey data from the Mo‘orea LTER, which consistently document distinct fish assemblages between backreef, forereef, and fringing reef habitat zones (MCR LTER, Brooks et al. 2025), validating that eDNA captures ecologically meaningful community structure.

Several reef-associated species detected in pelagic samples (e.g., *Gymnothorax buroensis*, *Thalassoma quinquevittatum*) and there are two likely explanations for these results. First, these detections may represent eDNA transported from adjacent reefs by currents rather than true pelagic fauna, consistent with findings by Ellis et al. (2022) showing that eDNA can be transported over hundreds of meters in dynamic coastal environments. Second, this signal could be eDNA shed by dispersive larvae, which are common of marine teleosts, including the aforementioned species. In either case, the presence of these reef-associated families alongside higher proportions of uncharacterized taxa in pelagic samples suggests that even in advective, open-ocean environments, gradients in eDNA concentration preserve meaningful spatial information about source communities.

Importance of Marker Selection and Region-Specific Reference Databases

Accurate taxonomic identification from eDNA is fundamentally constrained by the completeness of available reference databases, and this problem is particularly acute in highly diverse marine fish communities (Juhel et al., 2020; Marwayana et al., 2022). Global databases such as NCBI GenBank and BOLD contain strong representation for commercially important and well-studied taxa but remain incomplete for the high diversity of tropical reef fishes, many

of which are rarely sequenced. Our development of a region specific 12S reference database anchored to the Moorea Biocode Project substantially improved taxonomic resolution in our dataset, enabling species level identification for a large proportion of detections. This finding echoes Juhel et al. (2020) and Collins et al. (2019), both of whom showed that database gaps can cause 30–50% of tropical eDNA reads to remain unassigned or misassigned at the species level, and with Marwayana et al. (2022) who demonstrated a 2.5fold increase in ASV identification with improved databases.

Data from 12S greatly outperformed COI in our study, with 261 vs. 73 teleost species detected and 91 indicator species identified by 12S. Importantly, this result cannot be attributed to discrepancies in database completeness. Every sample for which we generated 12S sequences has a corresponding COI sequence, meaning both databases share identical taxonomic coverage. Instead, the disparity reflects non-specific amplification by the COI primers: only 0.3% of COI reads were assigned to fish, compared to 65.6% for 12S, with the remaining COI reads amplifying non-target metazoans (Appendix Figure 1-A1). These performance differences therefore reflect the superiority of the MiFish primer set (Miya et al. 2015) for barcoding teleost fishes. Although COI was developed as the standard locus for DNA barcoding (Hebert et al., 2003), its utility for fishes is relatively limited compared to 12S. COI, while widely and successfully used for barcoding invertebrates (Hebert et al., 2003; Leray et al., 2013), amplifies a much broader range of taxa (Stat et al., 2019) and is subject to greater primer bias in mixed community samples (Deagle et al., 2014; Stat et al., 2017), particularly in tropical environments with high invertebrate eDNA backgrounds (Stat et al., 2017). Our results join a growing body of evidence supporting 12S as the preferred marker for marine vertebrate eDNA surveys (Miya et al. 2015; Closek et al. 2019) and reinforce that investment in regional barcode libraries (e.g.

Gold et al., 2021) is essential to maximize ecological returns from metabarcoding programs in tropical biodiversity hotspots.

eDNA as a Rapid and Powerful Complement to Long-Term Visual Surveys

Combined eDNA (12S + COI) detected 266 teleost species and discriminated among fish communities characteristic of Mo‘orea's distinct reef-associated habitats. Against the matched year 2016 LTER surveys, the appropriate like-for-like comparison; eDNA recovered 55.4% of the visually documented fish fauna and detected 42 more species than the divers (266 vs. 224), with only 7 species recorded exclusively by visual survey. On a per-effort basis, eDNA was substantially more efficient: the 39 eDNA samples yielded an average of 6.8 species each, compared to 4.7 species per LTER transect, roughly 1.5 times the per-unit species yield of diver surveys. 12S eDNA detected 261 teleost species and COI 73, with 68 species shared between markers. These results are consistent with prior work showing eDNA matching or exceeding the per-sample detection efficiency of diver surveys (Gold et al., 2021; Closek et al., 2019; Valdivia-Carrillo et al., 2021; Juhel et al., 2021). At the same time, our sampling was deliberately modest: as is common practice in lower-diversity temperate ecosystems, we collected three 1 L replicates per site (Port et al., 2016; Gold et al., 2021; Closek et al., 2019). This effort is likely insufficient to fully capture the exceptional diversity of tropical coral reef fish communities, where work from the Coral Triangle indicates that comprehensive eDNA inventories may require sampling hundreds of liters of water (Marwayana et al., 2022); greater sampling volume would therefore be expected to further widen eDNA's advantage.

This efficiency reflects the contrast in sampling effort underlying the two methods. The 39 eDNA samples recovered more species than the LTER 2016 surveys, which required four divers conducting 48 transects over six days (August 1–6, 2016) substantially greater field effort that

nonetheless recovered fewer species. Comparable per-sample advantages have been reported elsewhere: Juhel et al. (2021) recovered over 200 fish species from a small number of water samples in Raja Ampat, substantially outperforming paired SCUBA surveys in per-sample species yield.

Impressively, even when comparing eDNA to the full 20 years of visual survey data, eDNA recovered 41 teleost species never observed in over 2,000 LTER visual surveys. These species include mesopelagic fishes (lanternfishes, hatchetfishes) and cryptobenthic reef taxa (moray eels, scorpionfishes) that are rarely or never encountered during daytime visual surveys. This pattern is well established across reef systems worldwide. Boussarie et al. (2018) found that eDNA detected 44% more shark species than underwater visual census and baited video across the New Caledonian archipelago, despite two orders of magnitude less sampling effort, revealing "dark" diversity of elusive megafauna that traditional surveys missed entirely. Bessey et al. (2020) similarly reported that the notable additions from eDNA on a tropical reef in the Timor Sea were cryptic and nocturnal fishes overlooked by conventional surveys. Hayes et al. (2025) found that eDNA detected 44 more unique tropical reef-fish species than visual surveys across a 2000-km latitudinal gradient in eastern Australia, including cryptic species not previously recorded by long-term visual monitoring. Beyond these, eDNA also detected three marine mammal species (spinner dolphin *Stenella longirostris*, rough-toothed dolphin *Steno bredanensis*, and a beaked whale, *Ziphiidae*) whose presence would not be captured by fish-targeted survey methods regardless of methodology (Nikolic et al., 2025), representing a category of biodiversity that visual surveys are structurally unable to recover. Together, these results suggest that eDNA does not merely replicate visual survey data with greater efficiency, but captures a fundamentally

broader ecological signal, detecting cryptic, deep-dwelling, and incidentally present taxa that visual methods are not designed to observe.

While there was substantial overlap in taxa recovered from both methods, eDNA and visual surveys capture fundamentally different aspects of fish biodiversity and should be viewed as complementary rather than competing approaches. The 7 species recorded by LTER in 2016 that were missed by eDNA reflect the strength of diver-based approaches, which excel in direct observation of large, mobile, and diurnally active species, species present at low abundance, or species that are strongly site-attached with highly localized eDNA signals. Moreover, visual surveys provide abundance and size data that are critical for assessing the health of marine fish communities information that eDNA cannot currently provide.

Although species accumulation curves indicate that eDNA sampling was not fully complete particularly for pelagic habitats (60.1% Chao1 completeness) compared to backreef (85.4%) and forereef (84.2%), these values should be interpreted in the context of sampling effort. Our spatial sampling employed three 1L replicates per site, a protocol common in temperate marine ecosystems but likely conservative for highly diverse tropical coral reef communities, where previous work suggests that larger water volumes may improve detection (Marwayana et al. 2022). Despite this conservative sampling effort, combined eDNA (12S + COI) detected 42 more teleost species than LTER visual surveys conducted in the same year by 4 trained divers over 6 days. This result suggests that even modest eDNA sampling effort can rival or exceed the detection power of intensive visual survey programs in tropical reef ecosystems. Increasing water volume or replicate number per site would likely further improve eDNA performance, particularly in pelagic and open-water habitats where fish density and eDNA concentrations are lower. Together, these results support eDNA as a powerful and efficient complement to

traditional visual surveys, capable of capturing broad biodiversity patterns rapidly and cost-effectively across diverse reef habitats (Stat et al. 2019; Nguyen et al. 2020).

Conclusion

This study adds to a growing body of evidence that eDNA metabarcoding is a powerful, efficient, and ecologically informative tool for tropical reef biodiversity assessment. Natural eDNA signals showed very rapid degradation, a result that ensures eDNA detections reflect near recent biological presence, a property that underlies the ability of eDNA to distinguish among spatially proximate, but ecological distinct coastal tropical marine habitats. Data from 12S consistently outperformed COI across all metrics of species detection, habitat discrimination, and indicator species identification. Although COI is a widely used DNA barcoding marker, 12S should be considered the standard for marine vertebrate eDNA surveys, and efforts to expand DNA barcoding databases for marine vertebrates should focus on 12S rather than COI. Remarkably, combined (12S + COI) eDNA detected 42 more fish species than LTER visual surveys conducted in the same year, despite requiring only three days of sampling compared to 6 days of intensive survey effort by 4 trained divers, underscoring the exceptional efficiency of eDNA as a biodiversity monitoring tool in tropical coral reef ecosystems.

From a conservation and management perspective, our results highlight the value of eDNA as a rapid, cost-effective, and scalable complement to long-term visual monitoring programs. The development of a region-specific 12S reference database anchored to the Mo‘orea Biocode Project substantially improved taxonomic resolution, demonstrating the value of investing in local barcode libraries for tropical biodiversity hotspots. The detection of marine mammals and mesopelagic species alongside reef fish further demonstrates that a single eDNA survey design

can simultaneously capture biodiversity across multiple ecological guilds, expanding what is achievable within constrained field budgets.

Several priorities emerge for future work. More extensive field sampling across multiple seasons would improve characterization of pelagic and forereef communities, which showed lower completeness in this single-season dataset, and would allow assessment of temporal variability in eDNA-based community composition. Continued expansion of region-specific reference databases, ideally through collaborative tissue vouchering programs linked to visual survey efforts, will be essential to fully realize the taxonomic potential of 12S metabarcoding in species-rich tropical systems. Finally, direct integration of eDNA surveys with concurrent visual transects at the same sites and time points would provide a rigorous side-by-side comparison of detection efficiency, enabling clearer quantification of the unique contribution each method makes to total biodiversity inventories.

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Appendix

Supplementary tables

This appendix contains the supplementary tables referenced in the main text, including temporal composition data, richness analyses, diversity completeness estimates, PERMANOVA results, and indicator species analysis.

Appendix Table 1-A1. Mean vertebrate percentage by time and site

Percentage of vertebrate reads through time at the initial sea, mesocosm, and ocean enclosure sites. SD and n are reported per time point; “—” indicates SD could not be computed (n = 1).

Time (hrs)	Site	Mean vertebrate (%)	SD (%)	n
-1	Initial Sea	92.69	5.41	3
0	Mesocosm	92.51	0.55	2
1	Mesocosm	88.08	7.81	2
2	Mesocosm	85.94	—	1
3	Mesocosm	76.58	—	1
4	Mesocosm	69.20	6.39	2
6	Mesocosm	49.01	—	1
8	Mesocosm	57.06	—	1
10	Mesocosm	56.83	—	1
12	Mesocosm	52.59	5.39	2
20	Mesocosm	32.14	6.97	2
28	Mesocosm	13.39	—	1
38	Mesocosm	7.42	—	1
46	Mesocosm	12.57	—	1
54	Mesocosm	19.71	—	1
84	Mesocosm	0.43	—	1
0	Ocean Enclosure	87.87	1.09	2
1	Ocean Enclosure	86.56	4.72	2
2	Ocean Enclosure	78.14	—	1

Time (hrs)	Site	Mean vertebrate (%)	SD (%)	n
3	Ocean Enclosure	78.08	—	1
4	Ocean Enclosure	73.80	11.21	2
6	Ocean Enclosure	52.63	—	1
8	Ocean Enclosure	61.98	—	1
10	Ocean Enclosure	62.76	—	1
12	Ocean Enclosure	53.55	4.82	2
14	Ocean Enclosure	41.78	1.07	2
20	Ocean Enclosure	23.59	7.88	2
28	Ocean Enclosure	4.62	—	1
38	Ocean Enclosure	6.13	—	1
46	Ocean Enclosure	0.25	—	1
54	Ocean Enclosure	17.24	—	1
62	Ocean Enclosure	11.79	—	1
84	Ocean Enclosure	5.62	—	1

Appendix Table 1-A2. Mean teleost percentage by time and site

Percentage of teleost reads through time at the initial sea, mesocosm, and ocean enclosure sites.

Same conventions as Appendix Table 1-A1.

Time (hrs)	Site	Mean teleost (%)	SD (%)	n
-1	Initial Sea	92.55	5.46	3
0	Mesocosm	92.38	0.63	2
1	Mesocosm	88.00	7.89	2
2	Mesocosm	85.85	—	1
3	Mesocosm	76.35	—	1
4	Mesocosm	68.51	6.90	2
6	Mesocosm	48.90	—	1
8	Mesocosm	56.95	—	1
10	Mesocosm	56.69	—	1
12	Mesocosm	52.48	5.37	2

Time (hrs)	Site	Mean teleost (%)	SD (%)	n
20	Mesocosm	32.03	6.93	2
28	Mesocosm	13.00	—	1
38	Mesocosm	7.42	—	1
46	Mesocosm	12.57	—	1
54	Mesocosm	0.00	—	1
84	Mesocosm	0.00	—	1
0	Ocean Enclosure	87.55	1.08	2
1	Ocean Enclosure	86.35	4.83	2
2	Ocean Enclosure	77.77	—	1
3	Ocean Enclosure	77.54	—	1
4	Ocean Enclosure	73.68	11.29	2
6	Ocean Enclosure	52.57	—	1
8	Ocean Enclosure	61.62	—	1
10	Ocean Enclosure	62.39	—	1
12	Ocean Enclosure	51.60	7.22	2
14	Ocean Enclosure	41.70	1.11	2
20	Ocean Enclosure	23.43	8.02	2
28	Ocean Enclosure	4.47	—	1
38	Ocean Enclosure	5.97	—	1
46	Ocean Enclosure	0.00	—	1
54	Ocean Enclosure	17.24	—	1
62	Ocean Enclosure	11.11	—	1
84	Ocean Enclosure	5.60	—	1

Appendix Table 1-A3a. One-way ANOVA, species richness by habitat. Effect of habitat on per-sample species richness for the 12S and COI markers (non-rarefied, presence-absence).

Marker	Source	df	Sum Sq	Mean Sq	F	p-value
12S	Habitat	2	30,273.10	15,136.55	55.49	<0.0001
12S	Residuals	36	9,819.57	272.77	—	—
COI	Habitat	2	408.88	204.44	12.45	<0.001
COI	Residuals	23	377.58	16.42	—	—

Appendix Table 1-A3b. Tukey HSD post-hoc comparisons, species richness. Pairwise habitat contrasts following the one-way ANOVA; differences on the original richness scale with 95% confidence intervals and family-wise adjusted p-values.

Marker	Comparison	Diff	Lower 95% CI	Upper 95% CI	p-adj
12S	Forereef-Backreef	-30.55	-46.18	-14.92	<0.0001
12S	Pelagic-Backreef	-67.38	-83.02	-51.75	<0.0001
12S	Pelagic-Forereef	-36.83	-53.31	-20.35	<0.0001
COI	Forereef-Backreef	-6.58	-10.82	-2.35	0.002
COI	Pelagic-Backreef	-10.92	-17.47	-4.37	0.001
COI	Pelagic-Forereef	-4.33	-10.94	2.28	0.249

Appendix Table 1-A4. Chao1 richness estimates and sampling completeness. Observed and Chao1-estimated species richness, with standard errors and resulting sampling completeness (observed / Chao1 $\times$ 100) by marker and habitat (non-rarefied, presence-absence).

Marker	Habitat	Observed species	Chao1 estimate	Chao1 SE	Completeness (%)	N samples
12S	Backreef	230	269.2	13.9	85.4	15
12S	Forereef	183	217.3	11.9	84.2	12
12S	Pelagic	51	84.9	16.4	60.1	12
COI	Backreef	56	79.8	12.4	70.1	12
COI	Forereef	32	50.4	11.7	63.5	11
COI	Pelagic	5	11.7	6.8	42.9	3

Appendix Table 1-A5a. Overall PERMANOVA, community composition by habitat (Jaccard dissimilarity, presence-absence, 999 permutations).

Test	Source	df	Sum Sq	R ²	F	p-value
Overall	Habitat general	2	2.991	0.212	4.84	0.001

Appendix Table 1-A5b. Pairwise PERMANOVA contrasts between habitats (Jaccard), with Bonferroni-adjusted *p*-values.

Comparison	df1	df2	Sum Sq	R ²	F	p-value	p-adj (Bonf.)
Backreef vs Forereef	1	25	1.108	0.144	4.22	0.001	0.003
Backreef vs Pelagic	1	25	2.100	0.216	6.87	0.001	0.003
Forereef vs Pelagic	1	22	1.252	0.135	3.42	0.001	0.003

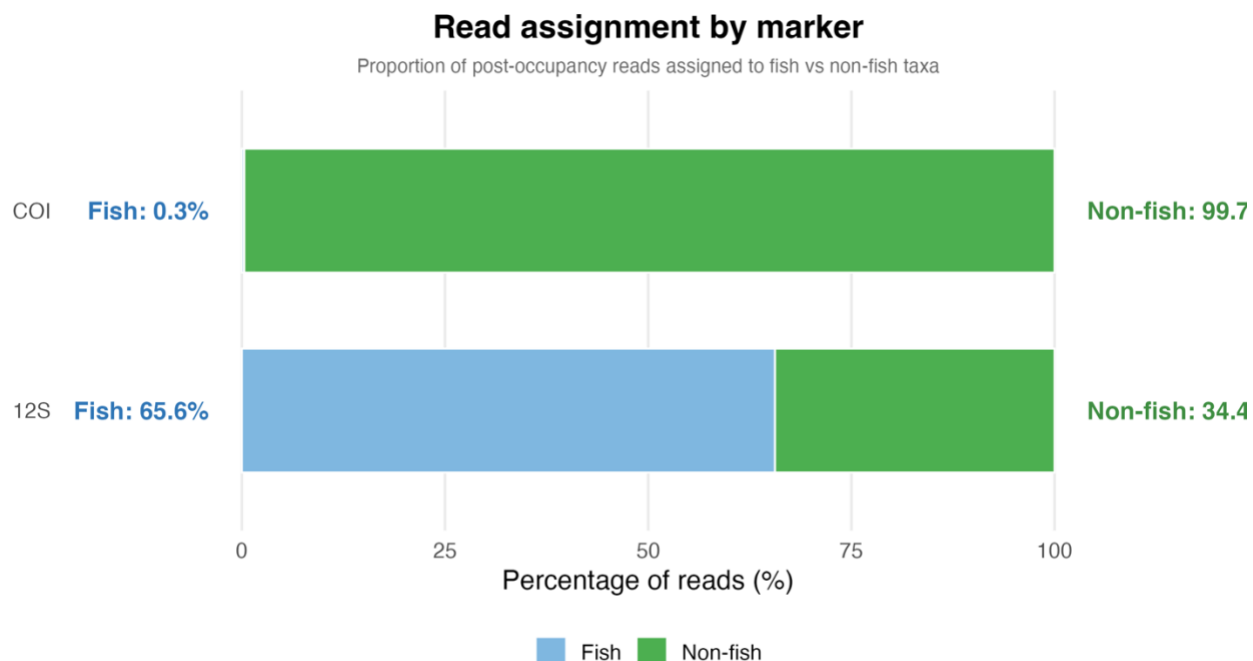
Appendix Table 1-A6. Indicator species analysis. Species with significant habitat associations identified by indicator species analysis (IndVal.g, $p < 0.05$; 999 permutations). 91 species are listed, grouped by their indicator habitat. No pelagic-only indicators were identified.

Species	Indicator habitat	IndVal statistic	p-value
Mulloidichthys flavolineatus	Backreef	0.866	0.001
Leptoscarus vaigiensis	Backreef	0.858	0.001
Thalassoma trilobatum	Backreef	0.853	0.001
Nannosalarias nativitatis	Backreef	0.851	0.001
Chrysiptera brownriggii	Backreef	0.851	0.001
Cirripectes variolosus	Backreef	0.829	0.001
Acanthurus guttatus	Backreef	0.814	0.001
Stegastes nigricans	Backreef	0.803	0.003
Stethojulis bandanensis	Backreef	0.781	0.001
Entomacrodus cymatobiotus	Backreef	0.775	0.001
Cirrhitus pinnulatus	Backreef	0.770	0.001
Acanthurus triostegus	Backreef	0.765	0.003
Thalassoma hardwicke	Backreef	0.751	0.002
Gomphosus varius	Backreef	0.740	0.001
Pomachromis fuscidorsalis	Backreef	0.730	0.001
Glyptoparus delicatulus	Backreef	0.730	0.001
Caracanthus maculatus	Backreef	0.730	0.001
Plectroglyphidodon leucozonus	Backreef	0.730	0.002
Apogonichthys ocellatus	Backreef	0.730	0.001
Siganus spinus	Backreef	0.730	0.002
Enneapterygius pyramis	Backreef	0.726	0.003
Plectroglyphidodon phoenixensis	Backreef	0.726	0.002

Species	Indicator habitat	IndVal statistic	p-value
Pseudogramma polyacantha	Backreef	0.696	0.003
Epinephelus merra	Backreef	0.685	0.006
Halichoeres margaritaceus	Backreef	0.685	0.006
Chromis vanderbilti	Backreef	0.685	0.003
Cirrhilabrus scottorum	Backreef	0.683	0.001
Rhinecanthus rectangulus	Backreef	0.683	0.002
Sargocentron microstoma	Backreef	0.679	0.007
Chaetodon ornatissimus	Backreef	0.637	0.019
Parapercis millepunctata	Backreef	0.632	0.005
Heniochus chrysostomus	Backreef	0.632	0.004
Enneapterygius hemimelas	Backreef	0.629	0.006
Callogobius sclateri	Backreef	0.629	0.006
Eviota afelei	Backreef	0.629	0.006
Pristiapogon kallopterus	Backreef	0.586	0.021
Hyporhamphus yuri	Backreef	0.586	0.041
Acanthurus lineatus	Backreef	0.577	0.010
Sebastapistes tinkhami	Backreef	0.577	0.009
Uropterygius alboguttatus	Backreef	0.577	0.006
Thalassoma purpureum	Backreef	0.577	0.006
Dascyllus trimaculatus	Backreef	0.575	0.019
Epinephelus hexagonatus	Backreef	0.575	0.009
Platybelone argalus platyura	Backreef	0.516	0.026
Siganus argenteus	Backreef	0.516	0.034
Gnathodentex aureolineatus	Backreef	0.516	0.035
Parupeneus cyclostomus	Backreef	0.516	0.042
Uropterygius micropterus	Backreef	0.516	0.030
Halichoeres marginatus	Backreef	0.516	0.025
Centropyge bispinosa	Forereef	0.769	0.001
Pterocaesio tile	Forereef	0.724	0.002
Acanthurus thompsoni	Forereef	0.645	0.001
Chromis iomelas	Forereef	0.645	0.001
Chromis xanthura	Forereef	0.599	0.011
Platax orbicularis	Forereef	0.599	0.010
Coris aygula	Forereef	0.598	0.012
Hologymnosus doliatus	Forereef	0.577	0.010
Monotaxis grandoculis	Forereef	0.562	0.024
Scarus forsteni	Forereef	0.527	0.036
Pseudocheilinus tetrataenia	Forereef	0.500	0.043

Species	Indicator habitat	IndVal statistic	p-value
<i>Chlorurus sordidus</i>	Backreef + Forereef	0.941	0.001
<i>Ctenochaetus striatus</i>	Backreef + Forereef	0.926	0.001
<i>Centropyge flavissima</i>	Backreef + Forereef	0.922	0.001
<i>Scarus psittacus</i>	Backreef + Forereef	0.901	0.001
<i>Balistapus undulatus</i>	Backreef + Forereef	0.901	0.001
<i>Neocirrhites armatus</i>	Backreef + Forereef	0.882	0.001
<i>Acanthurus nigrofusus</i>	Backreef + Forereef	0.880	0.001
<i>Cephalopholis argus</i>	Backreef + Forereef	0.816	0.001
<i>Naso lituratus</i>	Backreef + Forereef	0.815	0.003
<i>Cirripectes quagga</i>	Backreef + Forereef	0.793	0.002
<i>Pseudobalistes flavimarginatus</i>	Backreef + Forereef	0.793	0.002
<i>Gymnothorax buroensis</i>	Backreef + Forereef	0.793	0.015
<i>Myripristis amaena</i>	Backreef + Forereef	0.792	0.003
<i>Thalassoma quinquevittatum</i>	Backreef + Forereef	0.767	0.001
<i>Halichoeres hortulanus</i>	Backreef + Forereef	0.745	0.004
<i>Calotomus carolinus</i>	Backreef + Forereef	0.720	0.003
<i>Eviota</i> sp.	Backreef + Forereef	0.720	0.001
<i>Odonus niger</i>	Backreef + Forereef	0.694	0.009
<i>Myripristis violacea</i>	Backreef + Forereef	0.694	0.011
<i>Pseudocheilinus hexataenia</i>	Backreef + Forereef	0.692	0.027
<i>Chaetodon citrinellus</i>	Backreef + Forereef	0.667	0.019
<i>Scarus rubroviolaceus</i>	Backreef + Forereef	0.638	0.026
<i>Scarus niger</i>	Backreef + Forereef	0.638	0.018
<i>Myripristis berndti</i>	Backreef + Forereef	0.638	0.024
<i>Naso unicornis</i>	Backreef + Forereef	0.638	0.021
<i>Scarus oviceps</i>	Backreef + Forereef	0.638	0.016
<i>Coris gaimard</i>	Backreef + Forereef	0.638	0.024
<i>Caranx melampygus</i>	Backreef + Forereef	0.637	0.047
<i>Novaculichthys taeniourus</i>	Backreef + Forereef	0.609	0.048
<i>Tylosurus crocodilus</i>	Backreef + Forereef	0.609	0.041
<i>Thalassoma amblycephalum</i>	Backreef + Forereef	0.609	0.037

Appendix Figure



Appendix Figure 1-A1. Proportion of post-occupancy sequencing reads assigned to fish (Actinopteri and Chondrichthyes) versus non-fish taxa for the 12S and COI markers. The 12S marker assigned 65.6% of reads to fish, whereas COI assigned only 0.3% of reads to fish, with 99.7% amplifying non-target (predominantly non-fish metazoan) taxa. This non-specific amplification explains the markedly lower fish detection achieved by COI despite comparable sequencing depth.

Genus-specific host and spatial patterns of toxic benthic dinoflagellates on macroalgae in Mo'orea, French Polynesia

Abstract

Coral reefs worldwide are shifting from coral to macroalgal dominance, expanding the substrate available to epiphytic organisms including benthic toxic dinoflagellates (*Ostreopsis*, *Gambierdiscus*, *Prorocentrum*) that colonize macroalgae and produce potent biotoxins. Host macroalgal species differ in the dinoflagellate loads they support, but reported host preferences have been inconsistent, and how these communities respond as macroalgal assemblages shift remains poorly resolved. We counted toxic dinoflagellates on four macroalgal hosts (*Turbinaria ornata*, *Sargassum pacificum*, *Dictyota* spp., *Padina boryana*) at five sites in Mo'orea's north-shore lagoon during two sampling periods (September 2020 and July 2021; n = 600), and modeled cell densities per gram of host. Site mattered far more than host or sampling period, and it shaped both: how abundance differed among hosts, and how it changed between periods, depended on where we sampled. The three genera did not behave alike. *Gambierdiscus* was most abundant on *Turbinaria* and *Dictyota*, and this ranking held at every site. *Prorocentrum* was most abundant on the opposite hosts, *Padina* and *Dictyota*. *Ostreopsis*, which made up roughly 93% of all cells counted, showed little host preference. Per-gram rankings did not follow host weight: *Turbinaria*, the heaviest host, supported among the highest *Gambierdiscus* and *Ostreopsis* densities, while *Padina*, one of the lightest, supported the fewest. Host identity therefore shapes dinoflagellate abundance beyond simple differences in thallus mass. Total abundances were higher in July 2021, the drier of the two periods, but this came from *Ostreopsis* alone; *Gambierdiscus* and *Prorocentrum* were both more abundant in September 2020, when

rainfall was higher. As *Turbinaria ornata* continues to expand across Mo'orea's reefs, it adds substrate that supported among the highest *Gambierdiscus* densities we measured, consistently at every site. A growing standing stock of this host may therefore translate into greater lagoon-wide *Gambierdiscus* abundance and elevated ciguatera risk.

Introduction

Almost all maritime countries are affected by harmful algal blooms (HABs), many of which are caused by dinoflagellates (Hallegraeff 1993; Hallegraeff et al. 2021). During such blooms, algal toxins can bioaccumulate in primary consumers, leading to illness or even death in humans and other organisms that consume them (Shumway 1990; Van Dolah 2000). Toxic microalgae cause a variety of poisoning syndromes in humans, including diarrhetic, paralytic, amnesic, neurotoxic, and azaspiracid shellfish poisoning, among others (Van Dolah 2000; Wang 2008). Most of these syndromes are caused by planktonic species that bloom in the water column. A second group of harmful dinoflagellates instead lives on the benthos, attached to hard surfaces and to macroalgae.

On coral reefs, these benthic dinoflagellates occur largely as epiphytes on macroalgae an important but often overlooked part of the community. Dinoflagellates are extremely well-studied on coral reefs as coral endosymbionts that provide most coral nutrition and help corals adapt to changing ocean temperatures (Muller-Parker & D'Elia 1997; Graham & Wilcox 2000). However, free-living dinoflagellates are also an important and adaptable group of phytoplankton (Graham & Wilcox 2000), with many species thriving epiphytically on reef-associated macroalgae. Critically, many of these dinoflagellates produce toxins that can harm marine animals and humans (Van Dolah 2000; Duran-Riveroll et al., 2019). The most consequential of these for human health is ciguatera poisoning.

Ciguatera poisoning is caused by the ingestion of fish or invertebrates contaminated with ciguatoxins, lipid-soluble polyether toxins produced primarily by dinoflagellates in the genera *Gambierdiscus* and *Fukuyoa* (Lehane & Lewis 2000; Friedman et al. 2008; Chinain et al. 2021). These epiphytic dinoflagellates attach to macroalgae and other benthic substrates (Adachi & Fukuyo 1979; Parsons et al. 2011), and their toxins enter the food web when herbivorous fish and invertebrates graze on macroalgae. Because ciguatoxins are lipid-soluble, they bioaccumulate up the food web (Lehane & Lewis 2000; Friedman et al. 2008).

Affecting ~50,000 people annually, ciguatera poisoning is one of the most common seafood-borne illnesses worldwide (Lehane & Lewis 2000; Chinain et al. 2021; Skinner et al. 2011). Symptoms span gastrointestinal, cardiovascular, and neurological systems, typically arising within hours of consuming contaminated fish and occasionally persisting for years or, rarely, proving fatal (Friedman et al. 2008; Dalefield 2017). With increased commercial export of tropical fish, ciguatera is no longer confined to tropical regions but can occur wherever reef fish are consumed (Lehane & Lewis 2000; Chinain et al. 2021).

Other benthic dinoflagellates also produce toxins. Species in the genus *Prorocentrum* produce okadaic acid, a lipophilic toxin that causes diarrhetic shellfish poisoning (DSP) and has immunotoxic, mutagenic, and carcinogenic properties (Van Dolah 2000; Lee et al. 2016). Okadaic acid enters the food web both through filter-feeding bivalves that accumulate *Prorocentrum* cells and, as with *Gambierdiscus*, through herbivores that incidentally consume epiphytic *Prorocentrum* on macroalgae (Van Dolah 2000; Durán-Riveroll et al. 2019). Beyond the human health hazard, *Prorocentrum* blooms can cause substantial economic damage through shellfish fishery closures (Van Dolah 2000).

A third genus, *Ostreopsis*, causes both ecological harm and public health impacts. Like *Gambierdiscus* and some *Prorocentrum*, *Ostreopsis* is epiphytic on macroalgae, and its toxins enter the food web via herbivory (Totti et al. 2010; Rhodes 2011). The Mediterranean Sea has emerged as a major hotspot for *Ostreopsis* cf. *ovata* blooms, which have caused mass mortalities of benthic invertebrates and human health impacts including respiratory irritation, skin rashes, and ocular symptoms through direct seawater contact and inhalation of toxin-laden aerosols (Totti et al. 2010; Mangialajo et al. 2011; Tubaro et al. 2011). Of particular concern are palytoxin and related compounds produced by some *Ostreopsis* species, which rank among the most potent natural toxins known and can cause symptoms ranging from respiratory and gastrointestinal problems to fatal cardiac arrhythmias (Rhodes 2011; Tubaro et al. 2011; Patocka et al. 2015). The frequency and range of these events are projected to expand under future climate scenarios (Wells et al. 2015; Tester et al. 2020).

Evidence suggests that novel benthic habitat availability can drive outbreaks of ciguatera poisoning. Significant outbreaks followed World War II naval activities that destroyed live coral and created open substrate for colonization (Randall 1980; Ruff 1990). These cases share a mechanism: where coral dies, substrate opens and toxic dinoflagellates colonize it. Today that substrate is expanding for reasons unrelated to warfare.

Coral reefs are among the world's most diverse and economically valuable ecosystems (Roberts et al. 2002; Moberg & Folke 1999). Their structural complexity creates microhabitats that promote high diversity and abundance of marine organisms comparable to tropical rainforests (Connell 1978; Fisher et al. 2015) and provide critical ecosystem services such as coastline protection (Ferrario et al. 2014; Beck et al. 2018), pharmaceuticals (Cooper et al. 2014; Sang et

al. 2019), and recreation. Reefs also have tremendous economic value, generating nearly \$36 billion USD annually from ecotourism alone (Spalding et al. 2017), and are a major source of nutrition, providing a substantial fraction of animal protein for coastal populations worldwide (Eddy et al. 2021; Hicks et al. 2019).

In recent decades, however, anthropogenic stressors have transformed many reefs worldwide, often resulting in phase shifts from coral-dominated to communities dominated by larger, upright fleshy macroalgae (Done 1992; Fung et al. 2011). Macroalgae are typically nutrient limited in tropical waters (Mejia et al. 2012), but anthropogenic eutrophication can remove this limitation, allowing macroalgal biomass to accumulate rapidly on reefs (Fabricius et al. 2005; Teichberg et al. 2010). Moreover, although macroalgae are usually controlled by fish herbivory (Holbrook et al. 2016), overfishing reduces herbivory pressure, allowing macroalgae to occupy and hold space as coral cover declines rather than by directly displacing living coral (Bellwood et al. 2004; Holbrook et al. 2016). Eutrophication is exacerbated by global climate change, which increases thermal stress, lowers ocean pH, and intensifies storms and disease outbreaks (Hughes et al. 2003; Hoegh-Guldberg et al. 2007; Bellwood et al. 2004), resulting in further coral loss. Unfortunately, macroalgal-dominated reefs rarely transition back to coral-dominated states (Hoegh-Guldberg et al. 2007), resulting in the loss of the ecological goods and services that coral reefs provide to humans (Costanza et al. 1997).

Because of this global coral reef crisis, research on anthropogenic stressors has focused heavily on processes shaping the interaction between corals and macroalgae (Fung et al. 2011; McManus & Polsenberg 2004). However, eutrophication, sedimentation, and overfishing have wide-ranging impacts on reef environments beyond corals and macroalgae. For example,

eutrophication caused by terrestrial runoff can fuel harmful algal blooms caused by phytoplankton and create hypoxic “dead zones” (Hallegraeff 1993; Van Dolah 2000). Sedimentation can reduce fish species richness and alter feeding guilds (Moustaka et al. 2018), and overfishing not only depresses fish populations but also causes cascading social and economic impacts in coastal communities (Cesar et al. 2003). Toxic epiphytic dinoflagellates are another reef group affected by these stressors, and how they respond as macroalgal substrate expands remains poorly understood.

Climate change is reshaping global oceans by raising temperatures, lowering pH, and reducing dissolved oxygen (Hughes et al. 2003; Hoegh-Guldberg 1999; Wells et al. 2015). These changes are expanding the geographic range of HAB-forming species (Gobler et al. 2017; Wells et al. 2015), including poleward range expansions, and are increasing the duration, frequency, and intensity of HAB events (Van Dolah 2000; Wells et al. 2015). As a result, models predict that ciguatera fish poisoning will increase in some tropical regions and expand into higher latitudes under warming scenarios (Kibler et al. 2015). Combined with increased benthic substrate availability associated with macroalgal phase shifts on coral reefs, these trends indicate an urgent need to understand how toxic dinoflagellates respond to anthropogenic stressors and how such changes may affect public health.

Here, we quantify toxic dinoflagellate abundance on four macroalgal hosts (*Turbinaria ornata*, *Sargassum pacificum*, *Dictyota* spp., and *Padina boryana*) at five sites in the lagoons of Mo'orea, French Polynesia, sampled in two periods that differed in rainfall and storm activity. We address three questions: (1) Does abundance differ among macroalgal hosts, and do the three genera share the same host preferences? (2) Does abundance differ among sites, and are host patterns

consistent across them? (3) Does abundance differ between sampling periods, and does that difference hold across sites and hosts?

Study System

Mo'orea, French Polynesia, contains a diverse mosaic of lagoonal and reef habitats where repeated disturbances and nutrient inputs from developed watersheds have driven marked declines in coral cover and increases in fleshy macroalgae, particularly *Turbinaria ornata* (Stiger & Payri 1999; Bittick et al. 2019). Although its tough, leathery morphology makes it largely unpalatable to most herbivorous fishes (Sura et al 2021), *T. ornata* supports epiphytic communities that serve as trophic resources for juvenile reef-associated fishes (Fong et al. 2018). These epiphytic assemblages could include toxin-producing benthic dinoflagellates, particularly *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*, that are responsible for ciguatera poisoning, palytoxin-associated illnesses, and diarrhetic shellfish poisoning across the Pacific (Xu et al. 2014; Irola-Sansores et al. 2018).

The reefs of Mo'orea, French Polynesia have experienced several major disturbances that have promoted shifts from coral-dominated to macroalgal-dominated ecosystems. These include a massive outbreak of corallivorous sea stars (*Acanthaster planci*) from 2006 to 2009, a destructive cyclone in 2010, and increased agricultural activity that has introduced excess nutrients into nearshore waters (Adjeroud et al. 2005; Adam et al. 2011; Fong et al. 2020). As a result, live coral cover at some sites declined from approximately 46% in 2005 to less than 1% by 2010 (Adam et al. 2011). Together, these disturbances have contributed to phase shifts that favor macroalgae, increasing available substrate for potential colonization by toxic dinoflagellates.

The link between coral loss and ciguatera risk has regional precedent. Reef damage from atomic bomb tests at Bikini Atoll and nuclear weapons testing in French Polynesia preceded ciguatera outbreaks (Randall 1980; Ruff 1990), and work by Yasumoto and colleagues in the Gambier Islands subsequently linked *Gambierdiscus* with ciguatera poisoning and led to the description of *Gambierdiscus toxicus* (Adachi & Fukuyo 1979; Yasumoto et al. 1980, as cited in Ruff 1990). In some locations, the threat of ciguatera effectively turned reefs into de facto marine protected areas, as fish near heavily impacted sites became unsafe to eat (Ruff 1990; Skinner et al. 2011). Mo'orea's own coral loss over the past two decades represents the same kind of substrate release, driven by disturbance rather than by weapons testing.

Central to these dynamics are the macroalgae themselves, which provide the substrate on which toxic dinoflagellates live. In Mo'orea's lagoons, the fleshy macroalga *Turbinaria ornata* has expanded markedly since the 1980s and now dominates large portions of the backreef, alongside other common hosts including *Sargassum mangarevense*, *Dictyota bartayresiana*, and *Padina boryana* (Stiger & Payri 1999; Bittick et al. 2019). These macroalgae differ in morphology, surface complexity, and tissue density traits that can influence epiphytic attachment and shape the abundance and composition of the dinoflagellate communities they host (Irola-Sansores et al. 2018). Functional traits of several of these species have been quantified on Mo'orea's fringing reefs, revealing distinct trait strategies among species and substantial variation within species across sites (Cosca et al. 2024). Because dinoflagellate assemblages may therefore vary from one macroalgal host to another, the identity and relative abundance of macroalgae on a reef may directly influence the toxic potential of the system.

Understanding these dynamics is especially relevant in French Polynesia, which has historically experienced high rates of ciguatera poisoning (Chinain et al. 2021) and continues to undergo environmental change that may expand suitable habitat for toxin-producing dinoflagellates. These four hosts have been abundant components of Mo’orea’s lagoon benthos for at least two decades (Stiger & Payri 1999; Moorea Coral Reef LTER and Carpenter 2025), together providing a comparison for evaluating how host identity and environmental variation shape toxic epiphytic dinoflagellate communities.

Methods

To evaluate variation in toxic dinoflagellate abundance among sites, macroalgal hosts, and sampling periods. We collected macroalgal samples from five lagoonal locations along the northern coastline of Mo’orea, French Polynesia (Table 2-1, Figure 2-1). The five sampling sites spanned a west-to-east of the northern lagoon, beginning near $-17.492155, -149.851214$ and extending eastward through $-17.489219, -149.849047, -17.484365, -149.838024$, and $-17.487176, -149.825227$, terminating near $-17.482665, -149.813979$. These locations capture a representative section of the northern lagoon’s macroalgal-dominated habitats (Figure 2-1).

Table 2-1. Collection sites, numbered west to east along Mo’orea’s northern lagoon.

Site	Latitude	Longitude
Site 1	-17.492155	-149.851214
Site 2	-17.489219	-149.849047
Site 3*	-17.484365	-149.838024
Site 4	-17.487176	-149.825227
Site 5†	-17.482665	-149.813979

*Nearest site to MCR LTER 1 (≈ 1.0 km); †nearest site to MCR LTER 2 (≈ 1.3 km). All sites lie within 2.6 km of one or both LTER survey locations.

We sampled in two periods, 20 September–2 October 2020 and 1–12 July 2021 and refer to these as the wet and dry periods respectively. These labels denote rainfall and storm conditions observed during collection rather than climatological season: the wet season in the Society Islands runs approximately November through April, so both sampling periods fall within the regional dry season. For each period we screened both the two weeks preceding sampling and the sampling window itself for storm activity, so that any disturbance effect on epiphytic communities would be captured whether it preceded or occurred during collection. The September 2020 period coincided with elevated rainfall and with storm events on 21, 24, and 29 September, within the sampling window; the July 2021 period was drier, with no storm activity before or during sampling.

At each of the five sites, we sampled the four macroalgal species (*Turbinaria ornata*, *Sargassum pacificum*, *Padina boryana*, and *Dictyota* spp.). For each species at each site, we collected 15 replicate samples, where a single sample consisted of a sealable plastic bag filled with algal thalli of that species. This design yielded 75 replicate samples per species per sampling period (15 samples $\times$ 5 sites), 300 samples per period across all four species, and 600 samples in total across the two sampling periods. The design was fully crossed and balanced, with 15 replicates in every combination of site, host species, and sampling period.

To characterize environmental conditions during each sampling period, we obtained daily precipitation data from the University of California Gump meteorological station on the north shore of Mo'orea, French Polynesia (MCR LTER, knb-lter-mcr.9.50). We then calculated mean

daily rainfall for each sampling period and for the two-week window preceding each sampling event to capture potential lag effects of terrestrial nutrient runoff on nearshore dinoflagellate communities (Fong et al. 2020; Nifong et al. 2025).

Macroalgal cover analysis

To contextualize host-specific dinoflagellate loads against macroalgae host availability, we obtained long-term percent cover data for the four focal macroalgal species from the Moorea Coral Reef LTER benthic monitoring program (Moorea Coral Reef LTER and Carpenter 2025; knb-lter-mcr.8.38). These data provide the only continuous record of macroalgal cover change in Mo'orea's reef system over the past two decades. The MCR LTER program quantifies benthic community composition annually at six permanent sites around Mo'orea using fixed photoquadrat surveys; each of the six sites has three habitats – fringing, back, and fore reefs. Within each site habitat combination, five permanent 10-m transects are surveyed using ten 0.25 m² photoquadrats per transect (50 quadrats per site per year). We extracted data from the backreef habitat at LTER sites 1 and 2, the two LTER sites nearest our collection locations (Figure1): LTER 1 lies approximately 1 km from our Site 3 and LTER 2 approximately 1.3km from our Site 5, with every collection site within 2.6km of one or both. Data were pooled across the two sites (n = 100 quadrats per year) to describe host availability in the north-shore reef system surrounding our collection sites. We then calculated mean percent cover ($\pm$ SE) per year for *Turbinaria ornata*, *Sargassum pacificum*, *Dictyota* spp., and *Padina boryana* over the survey period 2006–2025. Data extraction and visualization were performed in R using the tidyverse and ggplot2 packages.

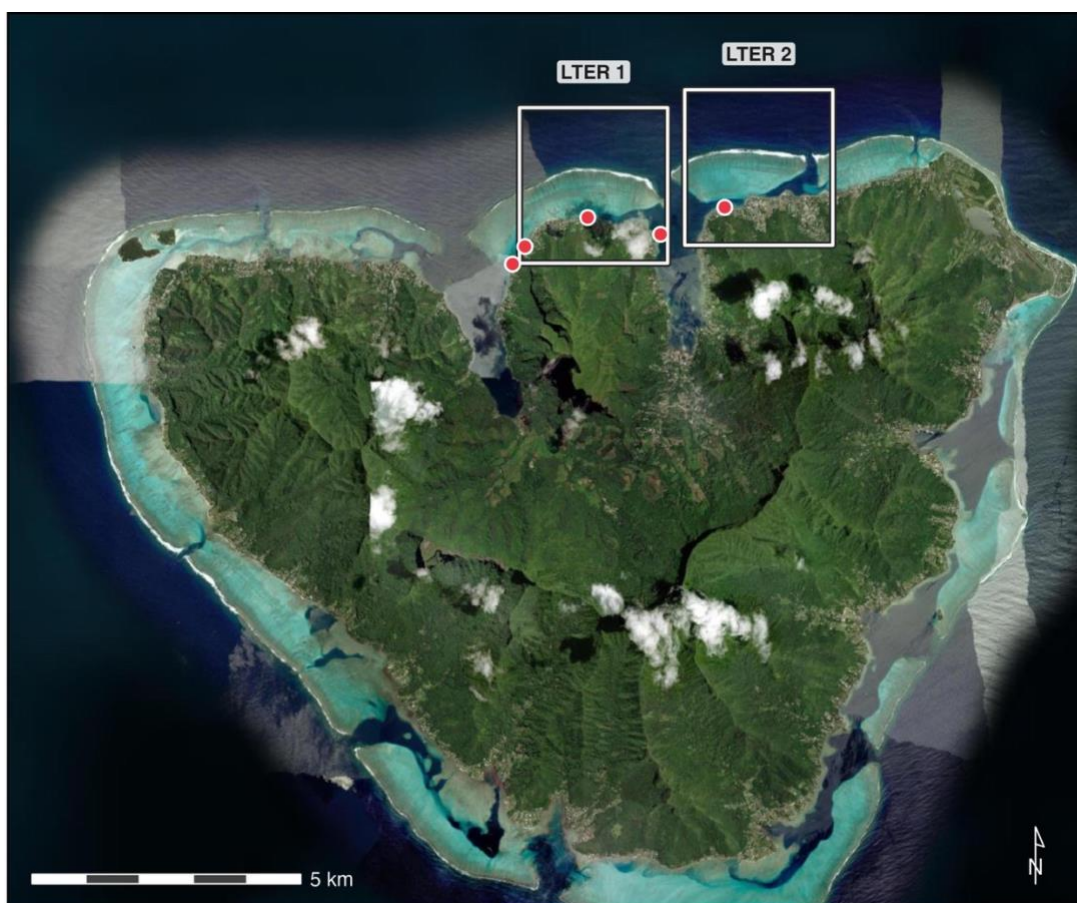


Figure 2-1. Map of Mo'orea, French Polynesia, showing the five macroalgal collection sites (red points), numbered 1 to 5 from west to east, and the MCR LTER survey areas (white boxes) from which macroalgal percent cover data were extracted. Boxes are centered on published LTER site coordinates and are illustrative; they do not represent formal survey boundaries, and backreef survey transects lie slightly inshore of the plotted positions.

Sample Collection

Macroalgal thalli were collected underwater using a modified version of the field protocol described by Xu and Richlen (2014). For each replicate, a 1 L Ziploc bag was gently placed over algal material while submerged, ensuring that half of the bag was filled with algae. The bag was

allowed to fill completely with seawater before the material was carefully cut at its base and the bag sealed underwater to minimize loss of epiphytic dinoflagellates. Bags were placed upright in a large cooler for transport to the laboratory to minimize disturbance prior to processing. A replicate therefore consisted of the algal material in a single bag, which in most cases comprised more than one thallus; for *Dictyota* spp. in particular, individual thalli could not be reliably distinguished in the field. Collections were made at depths of approximately 1–1.5 m in shallow lagoon and backreef habitat.

Sample Processing

In the laboratory, each bag was processed using the rinse–shake–sieve protocol to dislodge epiphytic dinoflagellates of Xu and Richlen (2014). Approximately half of the seawater in the bag was poured through a 200 μm sieve stacked above a 20 μm sieve, separating dinoflagellates from coarse debris. The filtered seawater (20 μm) was then added back to the bag, which was then shaken vigorously for ~10 seconds to detach epiphytic cells from the algal surface. The seawater was poured again through the sieve stack. This rinse–shake–sieve sequence was repeated five times per sample to dislodge the maximum number of epiphytic dinoflagellates. Next, macroalgal material was spun in a manual salad spinner until no further water was released, then weighed to obtain wet biomass, so that dinoflagellate abundances could be expressed as cells per gram of wet macroalgal weight.

Formalin Fixation

Following removal of the algal thalli, the remaining material on the 20 μm sieve was gently swirled and lightly rinsed with filtered seawater to concentrate the epiphytic cells. The retained

material was then backwashed directly into a 15 mL Falcon tube. Each tube contained a pre-measured amount of diluted formalin prepared from a 37% formaldehyde stock (Fisher Chemical, Formaldehyde Solution, 37% by weight with preservative, Certified ACS; catalog no. F79500). Filtered seawater was then added as needed so that each preserved sample reached a final volume of 15 mL with a final concentration of 4% formalin.

Microscopy and Enumeration

Dinoflagellate abundances for *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* were quantified at the University of California, Los Angeles (UCLA) using a 1 mL Sedgwick–Rafter counting chamber (Hausser Scientific™, Fisher Scientific catalog no. 02-671-56). Each preserved sample was gently inverted to homogenize the suspension before loading the chamber. The entire chamber area was then examined under a compound light microscope, ensuring complete enumeration of cells within the 1 mL volume.

Statistical Analyses

Our analyses addressed how three factors including host macroalga, the sampling site, and the sampling period shaped toxic dinoflagellate abundance. We additionally wanted to know whether their effects were independent of one another. Because every host species was sampled at every site in both periods, with 15 replicates in each combination ($n = 600$), we analyzed all three factors together in a single model rather than analyzing sampling periods separately. This allowed us to test not only whether each factor mattered on its own, but whether host preferences and seasonal differences were consistent across sites. All analyses were conducted in R version 4.5.1 (R Core Team 2025).

Because algal wet weight differed among host species, we modeled cell counts as raw integer counts with the natural logarithm of wet weight included as an offset. An offset is a predictor whose coefficient is fixed at one rather than estimated. Because these models use a log link, including the log of sample weight in this way is algebraically equivalent to modeling the log of cells per gram: expected counts scale directly with sample weight, so a sample twice as heavy is expected to carry twice as many cells, all else being equal. This expresses results on a per-gram basis while keeping the response as whole numbers, which a count model requires. Dividing counts by weight before fitting would instead produce fractional values that a negative binomial distribution cannot accommodate.

We first analyzed community-level structure using permutational multivariate analysis of variance (PERMANOVA; `adonis2` in the `vegan` package) on a Bray–Curtis dissimilarity matrix of genus-level abundances, with site, host species, sampling period, and all two- and three-way interactions as terms, and 9,999 permutations. Because the design was balanced, the order in which terms were entered did not affect how variation was partitioned among them. A significant PERMANOVA result can mean that groups differ in their average community composition, that they differ in how variable their samples are, or both. We therefore tested each factor separately for differences in variability using `betadisper` with 9,999 permutations.

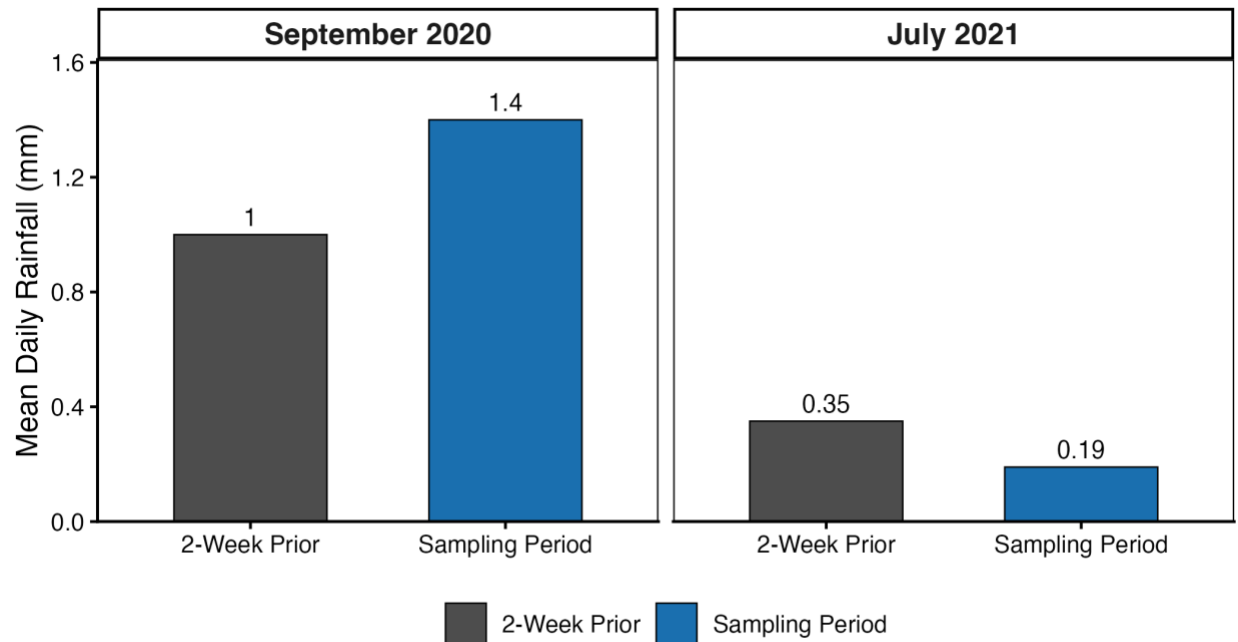
We then analyzed each genus using negative binomial generalized linear models (`glm.nb` in the `MASS` package), chosen over Poisson models because all three genera were strongly overdispersed. For each genus we fitted two two-factor models, each including an interaction: site $\times$ sampling period, and site $\times$ host species. These models include two factors each; the three-way interaction among site, host, and period is examined only in the PERMANOVA. We tested each

term by comparing the fit of the model containing it against the fit of the same model with that term removed. The interaction was tested against a model with both factors, but no interaction and each factor was tested against a model containing only the other. Where a term was significant, we used the emmeans package to estimate mean abundance for each group and to compare groups pairwise, adjusting p-values for multiple comparisons with the Bonferroni correction. We evaluated statistical significance at $\alpha = 0.05$. Analyzing each genus separately assumes that the three genera do not directly affect one another abundance. We could not test this with our data, since we did not measure competition or facilitation among them. The PERMANOVA, which treats the three genera together as a community, provides a complementary view.

Figures were produced using ggplot2, with multi-panel layouts assembled using patchwork.

RESULTS

Precipitation differed markedly between sampling periods (Figure 2-2). Mean daily rainfall was approximately seven times higher during the September 2020 sampling period (1.40 mm) than during July 2021 (0.19 mm). Similarly, mean daily rainfall in the two weeks preceding September 2020 sampling (1.00 mm) was nearly three times that recorded prior to July 2021 sampling (0.35 mm), suggesting that nearshore nutrient inputs from terrestrial runoff were substantially greater during the September 2020 sampling period (Fong et al. 2020; Nifong et al. 2025).



Source: MCR LTER knb-lter-mcr.9.50

Figure 2-2. Mean daily rainfall (mm) at the GUMP meteorological station, Mo'orea, French Polynesia, during the two sampling periods. Bars show mean daily rainfall during the 2-week pre-sampling window (gray) and the sampling period itself (blue) for September 2020 (left panel) and July 2021 (right panel). Data source: MCR LTER (knb-lter-mcr.9.50)

Community Structure

Community composition differed among sites, among host macroalgae, and between sampling periods, and all four interaction terms were also significant (Table 2-2; all $p = 0.0001$, the lowest value attainable with 9,999 permutations). Site explained 32.2% of variation in composition, compared with 3.4% for host and 3.9% for sampling period.

Two interaction terms explained more variation than the main effects they involve: site $\times$ period (7.8%) exceeded period alone (3.9%), and site $\times$ host (5.4%) exceeded host alone (3.4%). The

three-way interaction explained 3.1%. Residual variation among the 15 replicates within each site–host–period combination accounted for 42.9%.

Multivariate dispersion was homogeneous among host species ($p = 0.26$) and between sampling periods ($p = 0.64$), indicating that those differences reflect shifts in average composition.

Dispersion differed among sites ($p = 0.001$), so the site effect reflects both differences in average composition and differences in how variable samples were within a site.

Table 2-2. Permutational multivariate analysis of variance (PERMANOVA) of toxic dinoflagellate community composition, based on Bray–Curtis dissimilarities of genus-level abundances ($n = 600$, 9,999 permutations, sequential terms).

Term	df	R ²	F	p	Dispersion p
Site	4	0.322	105.07	0.0001	0.001
Host macroalga	3	0.034	14.60	0.0001	0.26
Sampling period	1	0.039	50.60	0.0001	0.64
Site × Host	12	0.054	5.88	0.0001	—
Site × Period	4	0.078	25.33	0.0001	—
Host × Period	3	0.013	5.79	0.0001	—
Site × Host × Period	12	0.031	3.40	0.0001	—
Residual	560	0.429	—	—	—
Total	599	1.000	—	—	—

All p-values reached 0.0001, the lowest value attainable with 9,999 permutations; R² therefore distinguishes the relative importance of terms. Dispersion p-values are from betadisper (999 permutations) and are reported for main effects only.

Host Macroalgae

Host species influenced abundance in all three genera, but the three genera did not share the same preferences (Figure 2-3; Appendix Table 2-A1). *Gambierdiscus* abundance differed among hosts (LR = 50.3, df = 3, $p < 0.001$). This was the only genus for which the site $\times$ host interaction was not significant (LR = 20.0, df = 12, $p = 0.067$). Densities were highest on *Dictyota* spp. (0.043 cells g^{-1}) and *Turbinaria ornata* (0.041), which did not differ from one another ($p = 1.0$), intermediate on *Sargassum pacificum* (0.025), and lowest on *Padina boryana* (0.015). Each tier differed from the next (*Turbinaria* vs *Sargassum*, $p = 0.003$; *Sargassum* vs *Padina*, $p = 0.011$).

Ostreopsis showed the smallest host effect of the three genera (LR = 16.4, df = 3, $p < 0.001$) and the largest site $\times$ host interaction (LR = 82.9, df = 12, $p < 0.001$). Densities on *Dictyota* (10.5 cells g^{-1}), *Sargassum* (10.3), and *Turbinaria* (9.3) did not differ from one another (all $p = 1.0$), while *Padina* supported roughly half as many (5.9; all $p < 0.001$). *Prorocentrum* showed the largest host effect (LR = 210.8, df = 3, $p < 0.001$). Densities were highest on *Padina* (1.82 cells g^{-1}) and *Dictyota* (1.41), which differed from each other ($p = 0.007$), and two to three times lower on *Turbinaria* (0.64) and *Sargassum* (0.61), which did not differ ($p = 1.0$; all cross-group comparisons $p < 0.001$). Mean sample wet weight differed among hosts: *Turbinaria* 103.3 g, *Sargassum* 82.4 g, *Padina* 53.0 g, and *Dictyota* 43.8 g. Per-gram rankings did not follow this order for any genus.

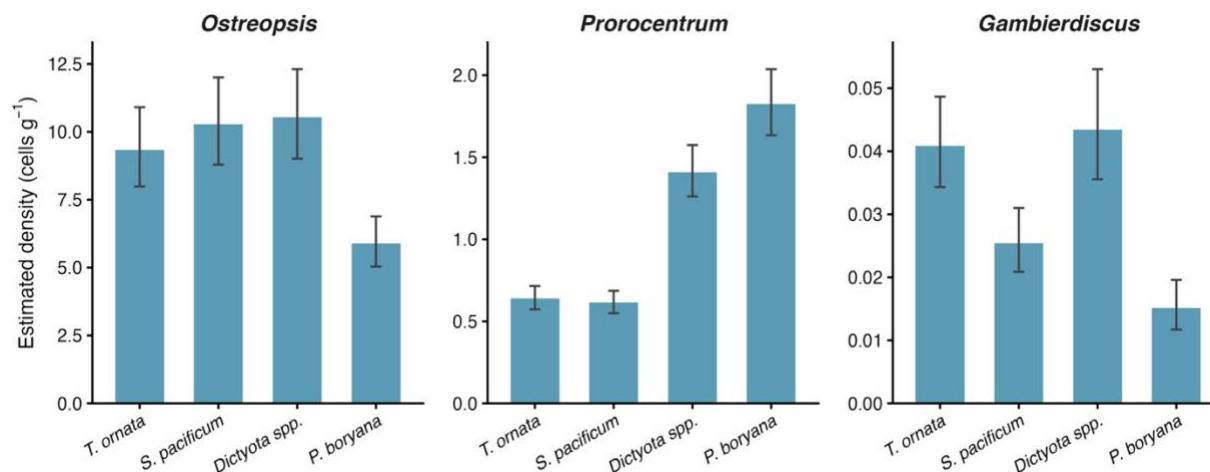


Figure 2-3. Model-estimated dinoflagellate density on each host macroalga. Bars show estimated marginal means from negative binomial models fitted separately to *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*, with 95% confidence intervals, averaged across sites and sampling periods. Densities are expressed per gram of wet algal tissue. Note that the y-axis scale differs among panels. Underlying estimates are given in Appendix Table 2-A1.

Site

Site influenced abundance in all three genera, but the sites supporting the highest densities differed by genus (Figure 2-4). *Gambierdiscus* was most abundant at Site 1 (0.083 cells g⁻¹), where densities were 2.4 times those at Site 2 (0.034) and 5.4 times those at Site 4 (0.016), the lowest (all comparisons with Site 1, $p < 0.001$). Sites 3, 4, and 5 did not differ from one another (all $p > 0.14$).

Ostreopsis spanned a 31-fold range across sites. Densities were 26.7 cells g⁻¹ at Site 2, 24.3 at Site 1, and 18.6 at Site 5, which did not differ from one another (all $p > 0.33$), 5.1 at Site 3, and 0.86 at Site 4 (all $p < 0.001$). *Prorocentrum* spanned a two-fold range, from 1.27 cells g⁻¹ at Site 2 to 0.66 at Site 4. Site 4 differed from every other site (all $p < 0.001$); no other pair differed after

correction except Sites 2 and 3 ($p = 0.040$). Site 4 supported the lowest densities of all three genera. The range across sites was 31-fold for *Ostreopsis*, 5.4-fold for *Gambierdiscus*, and two-fold for *Prorocentrum*.

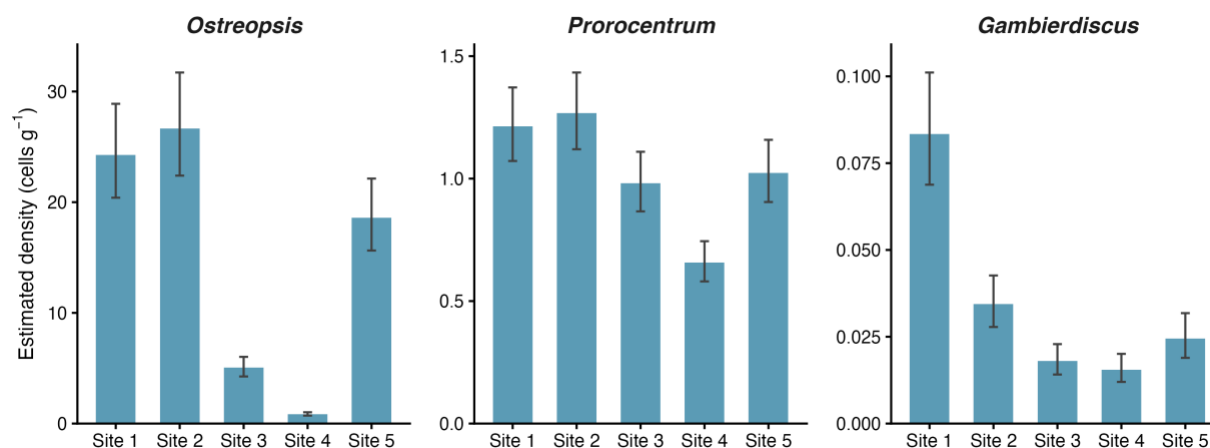


Figure 2-4. Model-estimated dinoflagellate density at each collection site. Bars show estimated marginal means from negative binomial models fitted separately to each genus, with 95% confidence intervals, averaged across host species and sampling periods. Densities are expressed per gram of wet algal tissue. Sites are numbered west to east (Table 2-1). Note that the y-axis scale differs among panels.

Sampling Period

Abundances differed between the two sampling periods for all three genera, but not in the same direction. *Ostreopsis* was 2.1 times more abundant in July 2021 than in September 2020 ($p < 0.001$), whereas *Gambierdiscus* was 1.7 times more abundant in September ($p < 0.001$) and *Prorocentrum* 1.9 times more abundant ($p < 0.001$). Because *Ostreopsis* accounted for roughly 93% of all cells counted, total abundance followed *Ostreopsis* and was higher in July 2021 (Figure 2-5). These differences depended on site (Table 2-3; Appendix Table 2-A2). For

Ostreopsis, the July increase was significant at only two of five sites. Site 1, where July densities were 11 times those of September, and Site 3 (3.6 times) and absent at the other three (all $p > 0.26$). For *Gambierdiscus*, the September increase was significant at Sites 2, 3, and 4 (ratios of 2.0, 2.8, and 5.1) and reversed direction, though not significantly, at Sites 1 and 5. *Prorocentrum* was higher in September at all five sites, significantly so at four (ratios 1.6 to 2.9).

Table 2-3. Ratio of estimated dinoflagellate density in September 2020 to July 2021, by site and genus. Values above 1 indicate higher densities in September; values below 1 indicate higher densities in July.

Site	<i>Gambierdiscus</i>	<i>Ostreopsis</i>	<i>Prorocentrum</i>
Site 1	0.71	0.089*	1.30
Site 2	1.97*	1.15	2.25*
Site 3	2.82*	0.27*	2.87*
Site 4	5.06*	0.72	1.80*
Site 5	0.65	1.15	1.58*
Overall	1.67*	0.47*	1.89*

Ratios are from negative binomial models of cell counts with log sample wet weight as an offset.

Asterisks and bold indicate contrasts significant at $\alpha = 0.05$ after Bonferroni correction across the five sites within each genus; the Overall row is the contrast averaged across sites.

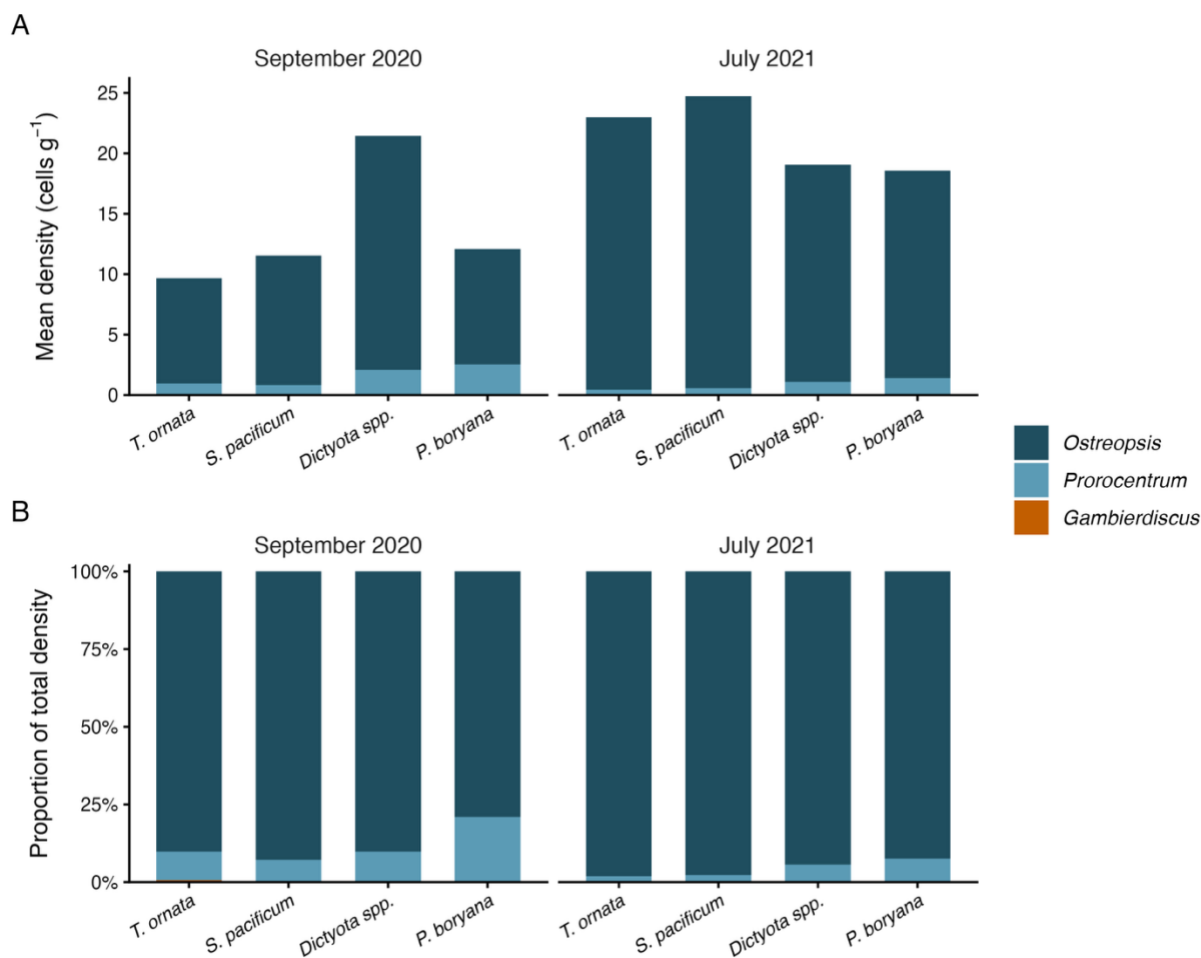


Figure 2-5. Contribution of each dinoflagellate genus to total cell density across host macroalgae and sampling periods. (A) Mean density (cells per gram of wet algal tissue) of each genus on each host, stacked. (B) The same data expressed as the proportion of total density. $n = 150$ per host per sampling period. *Gambierdiscus* is present on every host in both periods but at densities too low to be visible at this scale.

Macroalgal cover context (MCR LTER data)

Long-term photoquadrat data from the Moorea Coral Reef LTER program show that all four host macroalgae have been documented at the north-shore backreef sites in every year of the survey

since 2006 (Figure 2-6; Moorea Coral Reef LTER and Carpenter 2025). In the surveys conducted in April 2020 and April 2021, the years bracketing our sampling, mean percent cover ($\pm$ SE) of the four host taxa across the pooled north-shore quadrats was: *Dictyota* spp. $12.0\% \pm 1.6$ (2020) and $12.4\% \pm 1.9$ (2021); *Turbinaria ornata* $8.3\% \pm 1.2$ (2020) and $8.0\% \pm 1.3$ (2021); *Sargassum pacificum* $3.4\% \pm 0.8$ (2020) and $3.8\% \pm 1.0$ (2021); and *Padina boryana* $1.2\% \pm 0.3$ (2020) and $1.9\% \pm 0.5$ (2021). In both LTER survey years, *Dictyota* spp. and *Turbinaria ornata* were the two most abundant fleshy macroalgae in the north-shore backreef, together accounting for over 80% of the cover represented by these four taxa.

The broader 2006–2025 LTER time series places our sampling within a longer-term cover record (Figure 2-6). *Turbinaria ornata* increased from less than 2% cover in 2008–2009 to a stable post-2013 plateau of approximately 8–11%, where it remained throughout our sampling. *Dictyota* spp. increased from less than 1% in 2006–2007 to peak values of 12–16% during 2014–2018, then settled at approximately 12% during the 2020–2021 sampling window. *Sargassum pacificum* increased gradually after 2012, peaked at approximately 5% in 2018, and was near 3–4% during the sampling period. *Padina boryana* remained a minor component throughout the time series, with cover consistently below 2%. None of the four taxa showed pronounced expansions or declines during the sampling window relative to the surrounding years.

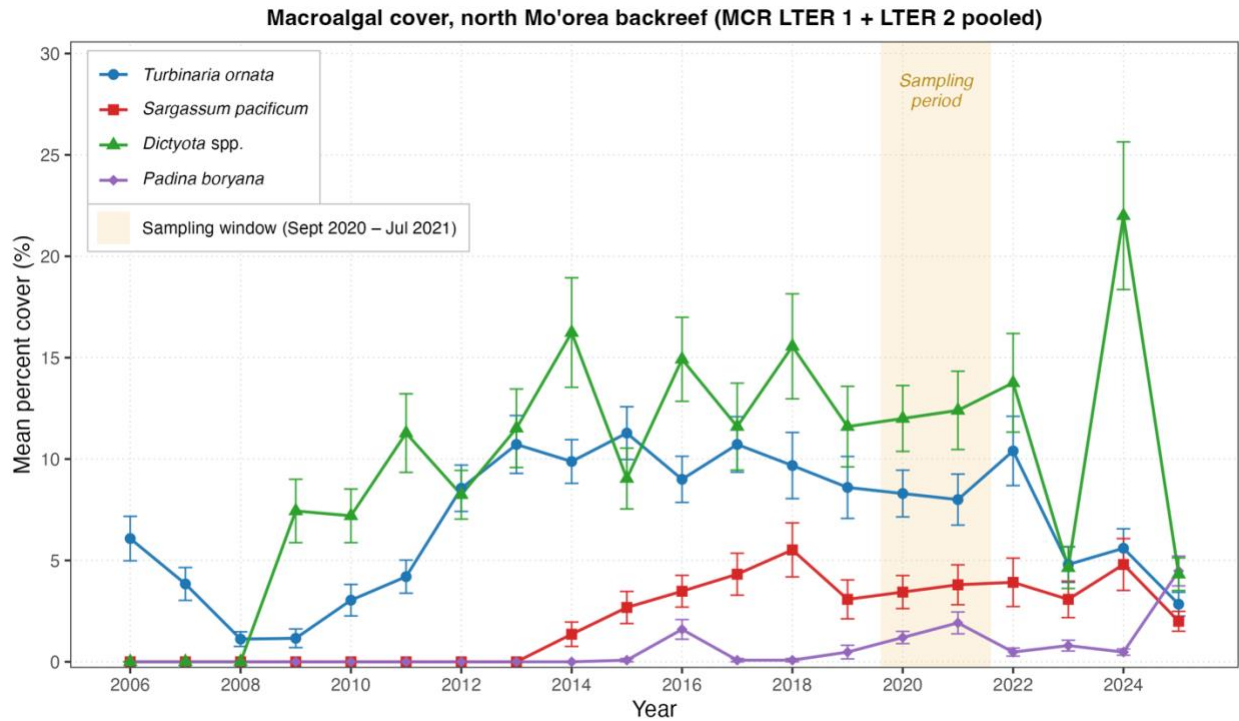


Figure 2-6. Long-term percent cover (mean $\pm$ SE) of the four host macroalgae at north-shore Mo'orea backreef habitats, 2006–2025. Data are pooled across MCR LTER sites 1 and 2 backreef photoquadrat surveys ($n = 100$ quadrats per year). Shaded band indicates the sampling window for the present study (September 2020 – July 2021). Source: Moorea Coral Reef LTER and Carpenter 2025 (knb-lter-mcr.8.38).

Discussion

Toxic Dinoflagellate Abundance and Algal Hosts

Toxic epiphytic dinoflagellates (*Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*) underpin the production of ciguatoxins, palytoxins, and related marine biotoxins that pose substantial public-health risks across tropical reef systems (Parsons et al. 2012; Tester et al. 2020). In the South Pacific, *Gambierdiscus* has been linked to high regional incidences of ciguatera poisoning, yet

the specific macroalgal hosts that sustain these dinoflagellate communities, and the conditions under which they proliferate, remain incompletely characterized (Chinain et al. 2010; Rains & Parsons 2015). Prior work indicates that host macroalgae differ in the dinoflagellate loads they support, though the degree of host specificity varies: some studies report clear preferences, with dinoflagellates attaching readily to certain macroalgae while avoiding others (Parsons et al. 2011; Rains & Parsons 2015), whereas others find weak or inconsistent host associations (Parsons & Preskitt 2007). Increasingly, this variation has been attributed less to taxonomic host identity than to host traits particularly thallus structure and surface area to biomass ratio, which govern the amount of colonizable, sheltered surface available to epiphytic cells (Tester et al. 2022; Accoroni et al. 2023). Across five sites and two sampling periods on Mo'orea's north-shore lagoon, this study shows that host macroalgal species influences toxic dinoflagellate abundance, but that the three genera do not share the same host preferences. *Gambierdiscus* reached its highest densities on *Turbinaria ornata* and *Dictyota* spp., while *Prorocentrum* was most abundant on the opposite hosts, *Padina boryana* and *Dictyota* spp., and *Ostreopsis* differed among hosts only in supporting fewer cells on *Padina Boryana*. Host identity therefore shapes dinoflagellate abundance, but in genus-specific directions rather than through a single ranking of favorable and unfavorable substrates.

Per-gram rankings did not follow host mass. *Turbinaria ornata*, the heaviest host sampled, supported among the highest *Gambierdiscus* and *Ostreopsis* densities, while *Padina boryana*, one of the lightest, supported the fewest of both. Differences among hosts therefore reflect more than differences in thallus mass, though wet weight remains an imperfect proxy for the colonizable surface actually available to epiphytic cells. Lobel et al. (1988) demonstrated that *Gambierdiscus* abundances differed by host when expressed as cells per gram wet weight, but

the relationship reversed when normalized to surface area, highlighting how the choice of normalization unit can shape ecological interpretations. Parsons & Preskitt (2007) similarly noted that morphologically distinct hosts with differing structural densities present an inherent challenge for wet-weight-based comparisons. While surface-area measurements were not feasible given the large sample sizes in this study ($n = 600$ samples collected by a single researcher), incorporating them in future work would provide a more accurate picture of dinoflagellate colonization across structurally diverse hosts and strengthen cross-study comparisons. Functional traits of several of these species have since been quantified on Mo'orea's fringing reefs (Cosca et al. 2024), offering a route to relating dinoflagellate loads to measured host traits rather than to mass alone.

The one host pattern that did hold everywhere belonged to *Gambierdiscus*. Its ranking among hosts did not vary significantly across the five sites, whereas host effects for *Ostreopsis* and *Prorocentrum* both depended strongly on site. For the genus responsible for ciguatera, then, host preference appears to be a property of the host itself rather than of local conditions. *Ostreopsis* was the numerically dominant genus across all four hosts during both sampling periods, consistent with reports from other tropical and subtropical reef systems where *Ostreopsis* outnumbers *Gambierdiscus* and *Prorocentrum* by orders of magnitude (Parsons & Preskitt 2007; Irola-Sansores et al. 2018). When the host-specific dinoflagellate patterns observed here are placed against long-term cover data from the Moorea Coral Reef LTER program, the relative abundance of host macroalgae alone does not account for the dinoflagellate distribution, indicating that host identity, morphology, and seasonal dynamics jointly shape the structure of toxic epiphytic dinoflagellate communities. As macroalgal coverage continues to expand on coral reefs in Moorea and worldwide, these findings have direct implications for assessing

ciguatera risk and for the design of monitoring programs targeting benthic harmful algal blooms in Indo-Pacific reef ecosystems.

The *Gambierdiscus* abundances documented here contribute to a limited body of recent work on this genus in French Polynesia. Despite four decades of ciguatera research in the region, most contemporary *Gambierdiscus* surveys have concentrated on Tahiti and the more distant Marquesas, Australes, and Tuamotu archipelagos rather than Mo'orea, leaving a spatial gap in the regional record (Chinain et al. 2023). Because French Polynesia is a global *Gambierdiscus* hotspot of co-occurring species with differing toxicities with *G. polynesiensis* the principal source of Pacific ciguatoxins (Chinain et al. 2010, 2021) our genus-level counts likely represent a mixed-species assemblage whose true ciguatera risk depends on the relative contribution of *G. polynesiensis* versus less toxic congeners. The host-specific differences we observed are also consistent with the marked spatial heterogeneity reported across French Polynesian islands and habitats (Chinain et al. 2023). Our own results show comparable heterogeneity across a few kilometres of Mo'orea's north shore, from Opunohu Bay area to Maharepa, where site accounted for approximately eight times more variation in community composition than either host or sampling period. This reinforces the value of host-resolved sampling and extends host-specific *Gambierdiscus* estimates to Mo'orea's north-shore lagoons as a baseline for future molecular, toxin-resolved monitoring.

Seasonal Variation in Toxic Dinoflagellate Communities

Abundances differed between sampling periods for all three genera, but not in the same direction. Total cell counts were higher in the drier July 2021 period, and *Ostreopsis* drove that difference, it was roughly twice as abundant in July as in September. It accounted for

approximately 93% of all cells counted, it determined the pooled pattern. *Gambierdiscus* made up 0.2% of cells and *Prorocentrum* 6.8%, so a metric dominated by *Ostreopsis* carries almost no information about the genus responsible for ciguatera. Both other genera moved the opposite way. *Gambierdiscus* was 1.7 times more abundant in September 2020, and *Prorocentrum* 1.9 times more abundant. A summary based on total cell counts would therefore have reported a dry period increase for a community in which two of three genera were more abundant in the wetter period.

These differences also depended on where we sampled. For *Ostreopsis*, the July increase was significant at only two of five sites and absent at the other three, while for *Gambierdiscus* the September increase was significant at three sites and reversed, though not significantly, at the remaining two. Only *Prorocentrum* moved consistently in the same direction at every site.

The September 2020 sampling window included storm activity on three days and higher daily rainfall than July 2021, so the two periods differed in both physical disturbance and terrestrial input. These act in opposite directions on epiphytic cells. Strong water motion dislodges dinoflagellates from macroalgal surfaces; Fernández-Zabala et al. (2022) reported lower *Gambierdiscus* abundances in shallow, high-flow environments, and Selina et al. (2014) found reduced *Ostreopsis* abundances under strong wave action. Runoff-driven nutrient input, by contrast, is expected to promote growth (Fong et al. 2020; Gobler et al. 2017). The genus-specific directions we observed are consistent with these mechanisms operating unequally across genera, with *Ostreopsis* more sensitive to physical disturbance and *Gambierdiscus* and *Prorocentrum* responding more to conditions associated with the wetter period.

We cannot separate these mechanisms with two sampling periods, one in each year. Season, year, and storm exposure differ together, and any of them could underlie the differences reported here.

Phase Shifts, *Turbinaria* Expansion and Ciguatera Risk

Interpreting these host specific patterns at the habitat scale requires accounting for how much reef area each host occupies, not only how many cells it supports per thallus. Habitat level dinoflagellate burden is effectively the product of per thallus cell load and areal cover, so host contributes disproportionately to the reef wide epiphytic substrate only when it is both heavily colonized and spatially dominant. Although our measurements quantify cell loads per gram of host tissue rather than per unit reef area, the high lagoon cover of *Turbinaria ornata* and *Dictyota* spp. (Figure 2-6), combined with the fact that these were also the two highest-ranked hosts for *Gambierdiscus*, suggests that they contribute disproportionately to the reef-wide substrate available for epiphytic colonization by the ciguatera-causing genus. Conversely, *Padina boryana*, despite occasionally supporting high per-gram densities, occupies very limited reef area and is therefore unlikely to be a major contributor to habitat-level dinoflagellate biomass. Direct integration of host-specific cell counts with concurrent reef-wide cover surveys would be required to formally evaluate habitat-level dinoflagellate burdens, but the cover patterns documented here point to *Turbinaria ornata* and *Dictyota* spp. as the macroalgal hosts most likely to dominate epiphytic dinoflagellate substrate availability in the north-shore lagoon under current reef conditions.

The macroalgae *T. ornata* ranks among the highest hosts for *Gambierdiscus* is particularly noteworthy given its dramatic expansion across Mo'orea's back-reef and lagoon habitats since

the 1980s (Stiger & Payri 1999; Bittick et al. 2019). As *T. ornata* continues to colonize reef areas previously occupied by coral, its tough leathery thallus provides persistent, stable substrate for epiphytic dinoflagellate colonization, a dynamic that has direct implications for benthic HAB risk in phase-shifted reef systems. Of additional concern is that *T. ornata* and *S. pacificum* can detach and form floating rafts that drift on surface currents. Early work by Bomber et al. (1988) demonstrated that *Gambierdiscus* cells remain viable on drift algae and can be transported across considerable distances, helping explain the circumtropical distribution of ciguatera-producing dinoflagellates. Because French Polynesia sits within the broader Pacific current system, rafting on detached *T. ornata* and *S. pacificum* thalli could similarly facilitate the dispersal of toxic dinoflagellates to unimpacted reef areas, potentially expanding the geographic footprint of ciguatera risk. Taken together with the elevated *Gambierdiscus* densities observed on this hosts, reef-wide increases in *T. ornata* cover could translate to heightened ciguatera risks for communities that rely on reef fish and invertebrates for food (Xu et al. 2014; Parsons et al. 2012). Future monitoring in Mo'orea and similar phase-shift-impacted systems should therefore prioritize *T. ornata* as a sentinel host, since its continued proliferation and dispersal potential may serve as both an indicator and a driver of elevated benthic HAB dynamics.

Understanding the relationship between host macroalgae and toxic dinoflagellates is more than just an ecological question. Over the multi-decade LTER record, both *T. ornata* and *Dictyota* spp. have expanded substantially on Mo'orea's reefs (Figure 2-6). As these fleshy macroalgae replace coral, phase shifts from coral- to macroalgal-dominated reefs could increase the entry of ciguatoxins into reef food webs as herbivorous fishes graze colonized thalli. Notably, the same shift would not raise all benthic HAB risks equally: *Prorocentrum* reached its highest densities on *Padina* the host with the lowest and most stable reef cover of the four.

Conclusion

This study shows that toxic epiphytic dinoflagellate communities on Mo'orea's north-shore lagoon are shaped by site, host identity, and sampling period, but that the three genera do not respond alike to any of them. Site was by far the strongest influence, accounting for roughly eight times more variation in community composition than host or period. *Gambierdiscus* reached its highest densities on *Turbinaria ornata* and *Dictyota* spp., a ranking that held at every site; *Prorocentrum* favoured the opposite hosts; and *Ostreopsis*, which made up around 93% of all cells counted, differed among hosts only in supporting fewer cells on *Padina*. Because *Ostreopsis* dominates numerically, summaries based on total cell counts describe *Ostreopsis* rather than the community. At the habitat scale, long-term cover data indicate that *T. ornata* and *Dictyota* spp., owing to their high reef cover, contribute disproportionately to the substrate available for *Gambierdiscus* colonization.

Several priorities emerge for future work in Mo'orea and similar reef systems. Incorporating surface area or other functional trait measurements alongside biomass-based abundance estimates would resolve much of the ambiguity introduced by host morphology and would enable more direct comparisons across studies and host species. Functional traits have now been quantified for several of these species on Mo'orea's reefs (Cosca et al. 2024), providing a starting point alongside biomass-based abundance estimates would resolve much of the ambiguity introduced by host morphology and would enable more direct comparisons across studies and host species. Species-level molecular characterization of *Gambierdiscus* in Mo'orea, particularly the relative contribution of the highly toxic *G. polynesiensis* versus less toxic congeners, is an important next step for translating cell-count data into meaningful ciguatera risk assessments. Extending sampling to additional seasonal windows, broader spatial coverage around the island,

and integrating concurrent dinoflagellate cell counts with site-specific macroalgal cover surveys would strengthen the link between host availability and dinoflagellate dynamics.

From a public-health and reef-management perspective, these results have direct implications for assessing ciguatera risk and for the design of monitoring programs targeting benthic harmful algal blooms in Indo-Pacific reef ecosystems. The elevated *Gambierdiscus* densities observed on *Turbinaria ornata*, a host that has expanded substantially across French Polynesian reefs over the past four decades and that retains the capacity to detach and form drifting rafts, indicate that continued *Turbinaria* proliferation in phase-shifted reef systems may translate to elevated ciguatera risks for communities dependent on reef fisheries.

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Appendix

Appendix Table 2-A1. Model-estimated dinoflagellate density (cells per gram of wet algal tissue) on each host macroalga, by genus. Estimates are marginal means from negative binomial models, averaged across sites and sampling periods.

Genus	Host	Estimate	SE	95% CI
<i>Gambierdiscus</i>	<i>Turbinaria ornata</i>	0.0409	0.00364	0.0343 – 0.0487

Genus	Host	Estimate	SE	95% CI
<i>Ostreopsis</i>	<i>Sargassum pacificum</i>	0.0254	0.00257	0.0209 – 0.0310
	<i>Dictyota spp.</i>	0.0434	0.00443	0.0355 – 0.0530
	<i>Padina boryana</i>	0.0151	0.00199	0.0117 – 0.0196
	<i>Turbinaria ornata</i>	9.33	0.742	7.99 – 10.91
	<i>Sargassum pacificum</i>	10.27	0.816	8.79 – 12.00
	<i>Dictyota spp.</i>	10.53	0.838	9.01 – 12.31
<i>Prorocentrum</i>	<i>Padina boryana</i>	5.89	0.469	5.04 – 6.89
	<i>Turbinaria ornata</i>	0.640	0.0361	0.573 – 0.715
	<i>Sargassum pacificum</i>	0.614	0.0348	0.549 – 0.686
	<i>Dictyota spp.</i>	1.409	0.0797	1.261 – 1.574
	<i>Padina boryana</i>	1.824	0.1030	1.634 – 2.037

These are the estimates plotted in Figure 2-3.

Appendix Table 2-A2. Model-estimated dinoflagellate density (cells per gram of wet algal tissue) by genus, site, and sampling period, averaged across host species.

Genus	Site	September 2020	July 2021
<i>Gambierdiscus</i>	Site 1	0.0691	0.0969
	Site 2	0.0480	0.0244
	Site 3	0.0294	0.0104
	Site 4	0.0330	0.0065
	Site 5	0.0193	0.0297
<i>Ostreopsis</i>	Site 1	4.19	46.98
	Site 2	28.55	24.92
	Site 3	2.36	8.59
	Site 4	0.73	1.01
	Site 5	20.00	17.32
<i>Prorocentrum</i>	Site 1	1.37	1.05
	Site 2	1.76	0.78
	Site 3	1.42	0.50
	Site 4	0.86	0.48
	Site 5	1.25	0.79

The ratios reported in Table 2-3 are derived from these estimates. Values are rounded; recompute from the model output for exact ratios.

Toxic dinoflagellates on macroalgae and their diet-structured transfer to fishes in French Polynesia

Abstract

Worldwide, coral reefs are shifting from coral to macroalgal-dominated states. These macroalgae provide substrate for benthic harmful algal bloom (BHAB) dinoflagellates, whose ciguatoxins, palytoxins, and related biotoxins accumulate in food webs, posing public-health risks. Ciguatera ecology conventionally assumes these toxins enter the food web when fishes graze the dinoflagellate-bearing substrate and accumulate through the food web to high trophic levels. However, that pathway has rarely been tested directly in part because species within toxic dinoflagellate genus can differ in toxicity by orders of magnitude, and field surveys resolve them only to genus. Here, we apply D1–D2 LSU metabarcoding to both ends of this pathway in French Polynesia: Results showed that *Ostreopsis* (78.5% of BHAB reads) dominated the toxic dinoflagellate community on macroalgae, rather than the ciguatera-causing *Gambierdiscus* (7.0%); nearly all *Ostreopsis* reads belonged to two poorly characterized species, *O. rhodesae* and *O. lenticularis*, whose documented hazards are to reef organisms rather than to human consumers. Community structure was shaped principally by site (marginal $R^2 = 0.32$) and host macroalga ($R^2 = 0.12$), consistent with fine-scale patchiness and host-specific colonization. Feeding mode imposed a sharp filter on exposure with detritivores carrying the heaviest and most diverse hindgut loads, with toxic dinoflagellates representing 4.58% of sequence reads. In contrast, herbivore loads were only 1.56%, and corallivores were 400-fold less at 0.011%, roughly 400-fold lower than detritivores. These differences held across the three islands sampled and remained significant after accounting for body size, sequences depth and phylogeny. Hindgut assemblages were nearly completely nested within substrate assemblages

with 23 of 24 gut species also found on macroalgae. One notable exception is that of ten *Gambierdiscus* species detected on macroalgae, only *G. pacificus* was recovered from any fish, and in only a single individual. Toxic dinoflagellates therefore do not appear to transfer to consumers as an undifferentiated whole, and the genus of greatest public-health concern (*Gambierdiscus*) is least likely to be observed in the gut of the fishes that carry its toxins, pointing to an indirect trophic pathway and to limits on what cell-based surveys can reveal about ciguatera risk.

Introduction

Coral reefs worldwide are shifting from coral- to macroalgal-dominated states (Done 1992; Fung et al. 2011). In addition to the loss of coral, macroalgal expansion provides substrate for epiphytic dinoflagellates, including toxin-producing species of *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum*, whose ciguatoxins, palytoxins, and okadaic acid pose well-documented risks to reef food webs and human health (Van Dolah 2000; Wang 2008). These risks include ciguatera poisoning, which carries significant health and socioeconomic impacts on affected communities (Rongo and van Woesik 2012, Kouakou and Poder 2019). As such, understanding the distribution of toxic dinoflagellates on these novel macroalgal communities, and the processes by which they enter the food web, and onto dinner tables, is paramount.

Ciguatera poisoning is among the most widespread non-bacterial seafood-borne illnesses worldwide (Lehane & Lewis 2000; Skinner et al. 2011; Chinain et al. 2021). Ciguatera poisoning is caused by ciguatoxins, chemicals produced primarily by species of *Gambierdiscus* and the related genus *Fukuyoa* (Friedman et al. 2008; Chinain et al. 2021). These species, and other toxic dinoflagellates such as *Ostreopsis* and *Prorocentrum*, attach epiphytically to macroalgae and other benthic substrates (Adachi & Fukuyo 1979; Parsons et al. 2011), and enter the food web

when consumed by primary consumers, particularly fishes (Cruz-Rivera & Villareal 2006; Holmes & Lewis 2025). These toxins then bioaccumulate through the food web, with piscivorous fishes typically having the highest toxin loads (Lehane & Lewis 2000), representing the greatest danger to human consumers of fish (Friedman et al. 2008).

Although there are many species of toxic dinoflagellates, within any given genus toxicity can vary by orders of magnitude. For example, *G. polynesiensis* is among the most toxic species known, whereas *G. pacificus* produces little or no ciguatoxin (Chinain et al. 2010a; Dolah et al. 2020; Chinain et al. 2023). Despite toxicity differences, morphology alone cannot reliably distinguish among species, as the genus *Gambierdiscus* comprises numerous morphologically similar and cryptic species (Litaker et al. 2009; Litaker et al. 2010). Similar challenges arise in *Ostreopsis* and *Prorocentrum*, where morphological plasticity, cryptic diversity, and overlapping traits impede reliable species-level identification (Rhodes 2011; Durán-Riveroll et al. 2019). As such, field methods focused on tracking human health risk from toxic dinoflagellates rely primarily on genus level identification, comparing the abundance of *Gambierdiscus* to that of the less toxic *Ostreopsis* and *Prorocentrum* (Litaker et al. 2010; Parsons et al. 2012). However, such genus-level abundance patterns provide only partial insight into toxin risk and ecological dynamics.

Ciguatera risk is more closely tied to the presence of highly toxic species such as *Gambierdiscus polynesiensis* and to shifts in species composition than to total dinoflagellate abundance alone, in part because toxin content per cell can differ markedly among co-occurring congeners (Litaker et al. 2010; Liefer et al. 2021; Chinain et al. 2023). Because toxic and non-toxic species frequently co-occur and are morphologically indistinguishable, traditional microscopy approaches of the study of toxic dinoflagellate communities cannot reveal the transitions in community structure

that underpin changes in toxin production, ecological interactions, or public-health risk (Parsons et al. 2012; Chinain et al. 2021). As such, fully understanding the dynamics of toxic dinoflagellate communities and the risk that they pose to human health require new approaches. High-throughput DNA metabarcoding provides a powerful approach to overcoming the limitations of traditional microscopy-based monitoring of toxic dinoflagellates. By amplifying and sequencing ribosomal markers such as the nuclear rDNA, particularly D1/D2 large-subunit (LSU) and V4 small-subunit (SSU), metabarcoding enables the simultaneous identification of many species within sampled dinoflagellate communities (Smith et al. 2017; Kassim et al. 2025), including cryptic and morphologically similar taxa (Hariganeya et al. 2013; Kassim et al. 2025). For the benthic harmful genera *Gambierdiscus* and *Ostreopsis* in particular, the LSU rDNA marker resolves species that are difficult or impossible to separate morphologically and provides finer taxonomic resolution than SSU (Kassim et al. 2025). This level of taxonomic resolution allows for a more comprehensive understanding of benthic dinoflagellate community structure. Characterizing the toxic dinoflagellate community, however, addresses only the first step in the pathway from environmental presence to human health risk. Benthic dinoflagellate toxins become a public-health concern only when they enter marine food webs, and the principal route of entry is the incidental ingestion of epiphytic dinoflagellates as fishes and invertebrates graze on macroalgae and other benthic substrates (Lehane & Lewis 2000; Holmes & Lewis 2025). Because ciguatoxins are lipid-soluble, they bioaccumulate and biomagnify as higher-trophic-level organisms consume contaminated prey, ultimately reaching humans through the consumption of reef-associated fish (Friedman et al. 2008; Chinain et al. 2021). Reef fishes, however, are not uniform in how they access the benthos. For example, herbivores crop fleshy and turf algae, detritivores ingest surface biofilm and sediment-associated material, and

corallivores feed directly on coral tissue. Each guild therefore contacting a different physical microhabitat and, potentially, a different exposures to toxic dinoflagellate communities (Bellwood et al. 2004; Parravicini et al. 2020). Because *Gambierdiscus* and related genera are ingested incidentally during feeding, the composition of toxic dinoflagellates recovered from a fish's digestive tract offers a direct, integrative record of recent dietary exposure and, by extension, of the trophic pathways through which benthic algal toxins enter reef food webs (Casey et al. 2019; Coker et al. 2023). Yet, the transfer of toxic dinoflagellates from substrate to consumer remains poorly resolved, particularly at the species level.

Although DNA metabarcoding has been applied separately to benthic dinoflagellate communities on reef substrates (Kassim et al. 2025) and to the gut contents of reef fishes (Casey et al. 2019; Coker et al. 2023), these approaches have not been paired within a single system. As such, it remains unknown whether the toxic dinoflagellate species most abundant on macroalgal substrates are the same species that accumulate in reef fishes, or whether feeding guild systematically structures this exposure.

Here, we pair DNA metabarcoding of macroalgal-associated toxic dinoflagellate communities with those recovered from the hindguts of reef fishes from three islands of French Polynesia, spanning multiple feeding guilds. Zerecero et al (2026; Chapter 2) documented strong host-specific variation in the abundance of *Gambierdiscus*, *Ostreopsis*, and *Prorocentrum* across four macroalgal hosts on the fringing reefs of Mo'orea. Here we apply next-generation DNA sequencing and metabarcoding to these samples to test whether macroalgal taxa differ in the species of toxic dinoflagellate species they support. We then apply the same approach to the toxic dinoflagellate communities recovered from the hindguts of reef fishes across three islands of French Polynesia, testing the prediction that feeding guild structures dietary exposure. These

complementary approaches allow for a direct test of whether the toxic dinoflagellate species most prominent on macroalgal substrates are those that transfer into reef fish consumers, or whether substrate-to-consumer transfer is selective. Together, these aims address a central question for reef toxin ecology: how the species composition of benthic harmful dinoflagellate communities is shaped across substrates, consumers, and space, and what that means for the trophic pathways underlying ciguatera and other seafood-borne toxin risks.

Methods

Toxic dinoflagellate metabarcoding of macroalgal substrates

Sample collection and subsampling

Sampling was conducted across three islands of French Polynesia, Mo'orea, Tetiaroa, and Mangareva. Mo'orea in particular has incurred repeated crown-of-thorns sea star outbreaks, cyclones, coral bleaching and nutrient enrichment (Adjeroud et al. 2009; Adam et al. 2011; Fong et al. 2020). As a result, fleshy macroalgae, particularly *Turbinaria ornata*, have become a dominant feature of these ecosystems, alongside other common lagoon macroalgae including *Sargassum mangarevense*, *Dictyota bartayresiana*, and *Padina boryana*, (Stiger & Payri 1999; Adam et al. 2011; Moorea Coral Reef LTER and Carpenter 2025). This phase shift to macroalgal dominated reefs provides extensive substrate for epiphytic dinoflagellate colonization, and an ideal location to understand the impacts of phase shifts on toxic dinoflagellate communities and the potential associated health risks.

Macroalgal samples were collected on Mo'orea and processed as described in (Zerecero et al. 2026; Chapter 2). Briefly, intact thalli of the four host macroalgae (*Turbinaria ornata*, *Sargassum mangarevense*, *Padina boryana*, *Dictyota bartayresiana*) were bagged underwater,

and epiphytic dinoflagellates were dislodged using a repeated rinse–shake–sieve protocol over stacked 200 μm and 20 μm sieves. All collection and sample processing through preservation were conducted at the University of California, Berkeley Richard B. Gump South Pacific Research Station on Mo‘orea, French Polynesia. From the 20 μm sieve fraction retaining the target dinoflagellates, and after the retained material was thoroughly resuspended, a 1 mL subsample was drawn and pipetted into a 2 mL screw-cap tube, then preserved with an equal volume of RNAlater and stored at $-20\text{ }^{\circ}\text{C}$. The remainder of each sample was fixed in formalin for the microscopy (see Zerecero et al. 2026; Chapter 2). Preserved subsamples were subsequently transported to the University of California, Los Angeles (UCLA) for molecular processing.

In total, we selected 120 samples from Zerecero et al. (2026; Chapter 2) for DNA metabarcoding, comprising five biological replicates for each of the four host species in both sampling periods (September–October 2020 and July 2021) across four of the five sites: Site 2 (17.4892° S , $149.8490^{\circ}\text{ W}$), Site 3 (17.4844° S , $149.8380^{\circ}\text{ W}$), Site 4 (17.4872° S , $149.8252^{\circ}\text{ W}$), and Site 5 (17.4827° S , $149.8140^{\circ}\text{ W}$). Samples were selected to represent the full range of host, site, and seasonal combinations sampled for microscopy. Three samples failed to yield sufficient sequence data and were removed during quality filtering, giving a final dataset of 117 samples. Importantly, the two sampling periods differed markedly in rainfall and storm activity. In the two weeks preceding the first collection day, mean daily rainfall was nearly three times higher in the September 2020 sampling period than in the July 2021 (1.00 versus 0.35 mm), and rainfall during the sampling periods themselves differed roughly seven-fold (1.40 versus 0.19mm) (Moorea Coral Reef LTER and Carpenter 2025; Zerecero et al. 2026, Chapter 2). The 2020 period also coincided with storm events on 21, 24 and 29 of September, whereas the July

2021 period was without storm activity. We therefore define these as the wet and dry periods respectively, following Zerecero et al. (2026; Chapter 2). Throughout, wet and dry refer to the rainfall and disturbance regime observed during each collection rather than to fixed calendar months.

DNA extraction

Prior to extraction, each subsample was centrifuged at $4,500 \times g$ for 15 min to pellet dinoflagellate cells. We then removed and retained the supernatant and resuspended the pellet in 250 μL of supernatant to ensure consistent volumes for DNA extraction. Because a portion of fine sediment naturally passes through the sieve with the sample, we extracted DNA using the Qiagen DNeasy PowerSoil Kit (Qiagen, cat. no. 12888-100), whose bead-beating step is well suited to lysing dinoflagellate cells and maximizing DNA recovery. We added the full 250 μL resuspended pellet directly to the PowerBead tube in place of the 0.25 g of soil specified in the manufacturer protocol. Cells were then lysed by vortex bead-beating, and DNA extracted following the manufacturer protocol, eluting the extracted DNA into 50 μL of Solution C6 rather than the 100 μL specified by the manufacturer, to increase DNA concentration.

PCR amplification

DNA metabarcoding targeted the D1–D2 domains of the large-subunit (LSU) rDNA gene using the dinoflagellate-specific primers D1R (5'-ACCCGCTGAATTTAAGCATA-3') and 305R (5'-TTTAAYTCTCTTTYCAAAGTCC-3'), which amplify a ~365 bp product (Smith et al. 2017). As in Zerecero et al. (2026; Chapter 1), both primers incorporated Illumina Nextera adapters to enable two-step indexing following the workflow of Curd et al. (2019). We conducted PCR reactions in 25 μL volumes using the Qiagen Multiplex 2 $\times$ Master Mix, consisting of 12.5 μL of master mix, 0.5 μL each of the forward and reverse primers (2 μM), 10.5 μL of ddH₂O, and 1 μL

of DNA template. Thermocycling began with an initial 15 min denaturing step at 95 °C followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 10 s, and extension at 72 °C for 45 s, concluding with a final extension at 72 °C for 5 min. As in Zerecero et al. (2026; Chapter 1), we amplified each sample in triplicate to account for PCR stochasticity and minimize false positives (Alberdi et al. 2018). All PCRs included a negative control in which molecular-grade water replaced the DNA template, alongside a positive PCR control consisting of genomic DNA from cultured *Gambierdiscus caribaeus* strain provided by Dr. Richlen (Woods Hole Oceanographic Institution). We confirmed all PCR amplifications and product size through electrophoresis on a 2% agarose gel stained with SybrGreen in TBE.

Library preparation and sequencing

To create sequencing libraries, we pooled 5 µL of each of the three replicate PCRs per sample and then purified by gel extraction using the QIAquick Gel Extraction Kit (Qiagen). We then electrophoresed pooled amplicons on a 3% low-melt agarose gel (3 g low-melt agarose per 100 mL TBE, prepared with molecular-grade water) at 90 V for 90 min, and excised the target band. We then recovered the cleaned, pooled PCR products following the manufacturer protocol, incorporating the following modifications reported to improve recovery: we 1) added one gel volume of isopropanol to the tube prior to binding, 2) performed the DNA-binding centrifugation step at a reduced speed of 3,000 rpm (with all subsequent steps at 13,000 rpm), and 3) eluted DNA in 30 µL of Buffer EB applied directly to the membrane and incubated for 1 min before centrifugation. We then prepared indexed libraries in a secondary PCR using unique dual-indexed Illumina Nextera XT adapters. Each 25 µL indexing reaction contained 12.5 µL of KAPA HiFi HotStart master mix, 2.5 µL each of the i5 and i7 indices, 5 µL of primary PCR product, and 2.5 µL of molecular-grade water. Indexing thermocycling conditions were 95 °C for

3 min; 12 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min. We verified success of indexing reactions through electrophoresis on a 2% agarose gel, and then quantified indexed libraries using a Qubit 4 Fluorometer with the dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific), and then pooled all indexed reactions normalized to equimolar concentrations. We purified the final sequencing library using a second and final gel extraction, described above. We sequenced this final library pool on an Illumina MiSeq using a 600-cycle MiSeq Reagent Kit v3 (2 × 300 bp paired-end) with 30% PhiX at the University of California, Davis.

Bioinformatic processing

We processed raw sequencing reads using the Anacapa Toolkit (Curd et al. 2019), which performs primer trimming, quality filtering, and amplicon sequence variant (ASV) inference using DADA2 (Callahan et al. 2016), followed by taxonomic assignment using the Bowtie2 BLCA classifier with a 60% bootstrap confidence cutoff. We employed quality filtering at a Phred score of Q25, with a minimum merge length of 345 bp, and assigned taxonomy against the D1-D2 LSU reference database of Smith et al. (2017), provided by the authors and formatted for use within the Anacapa Toolkit. We identified and removed any potential contaminants using the DECONTAM package (Davis et al. 2018) based on frequency and prevalence profiles, and also removed singleton ASVs prior to downstream analyses. We employed a threshold for taxon detection in a sample at a minimum of 3 reads.

Toxic dinoflagellate metabarcoding of reef-fish hindguts

Sample origin and selection

To test whether primary consumers ingest toxic dinoflagellate communities in proportion to community composition on benthic substrates, we examined the species composition of dinoflagellates from reef-fish hindgut samples from a companion study by Degregori et al. (2024) focused on fish gut microbiomes. Briefly, adult fishes representing different feeding guilds were collected by spearfishing across three South Pacific islands (Mo‘orea , Tetiaroa, and Mangareva) and transported on ice for dissection. Intestines were removed using sterile technique, and hindgut digesta was squeezed into sterile 2 mL tubes using sterile forceps and stored at -80°C . Total genomic DNA was extracted from the digesta using the Qiagen DNeasy PowerSoil Kit following the manufacturer protocol (Degregori et al. 2024). All collection and animal-handling protocols were approved by the University of California, Los Angeles Animal Research Committee (ARC-2017-045).

From these DNA extractions, we selected 57 for toxic-dinoflagellate metabarcoding based on sample availability and traceability, using only extractions with sufficient DNA remaining and an unambiguous match to their corresponding sample metadata. The samples included 8 fish species spanning feeding guilds as defined by Degregori et al. (2024) (detritivore, $n = 19$, corallivore, $n = 18$, herbivore, $n = 17$, carnivore, $n = 3$) and three islands (Mangareva, $n = 20$; Tetiaroa, $n = 19$; Mo‘orea, $n = 18$) .

DNA Sequencing and Bioinformatic processing

PCR amplification, library preparation and bioinformatic processing followed the same protocols described for the macroalgal substrate samples, above. However, sequencing libraries were purified by a second gel extraction and sequenced on an Element Biosciences AVITI platform using a 600-cycle kit (2 x 300 bp paired-end) with PhiX added, at the University of California, Davis DNA Technologies Core.

Statistical analysis

All statistical analyses were performed in R version 4.5.1 (2025-06-13) (R Core Team), with community data handled using the phyloseq package (McMurdie & Holmes 2013). In all analyses, read counts were converted to proportions of total dinoflagellate reads (total-sum scaling, TSS) to account for variation in sequencing depth; rarefaction was not applied, as it discards data and reduces statistical power (McMurdie & Holmes 2014).

Macroalgal substrate communities

We tested for seasonal differences in the relative abundance of each BHAB genus using Wilcoxon rank-sum tests with Benjamini–Hochberg (BH) correction. We then compared community composition using Bray–Curtis dissimilarities of TSS-normalized proportions, visualized by non-metric multidimensional scaling (NMDS) and by a distance-based ordination constrained on season (CAP; capscale, vegan). Next, we tested for differences in composition among sites, host macroalgae, and seasons using a permutational multivariate analysis of variance (PERMANOVA; 999 permutations) using marginal (Type III) sums of squares (adonis2, vegan) and then tested for homogeneity of multivariate dispersion for each factor using betadisper. We identified species associations with season with an indicator species analysis (IndVal.g; De Cáceres & Legendre 2009; 499 permutations, BH-adjusted). To assess whether the seasonal signal persisted after accounting for sequencing effort, we related BHAB proportion to total dinoflagellate reads by Spearman rank correlation, conducting a rank-based analysis of covariance with the $\log_{10}$ of total dinoflagellate reads as a covariate. As a targeted check on the confounding between season and sequencing depth, we repeated the seasonal comparison on depth-standardized data (rarefied to 2,000 dinoflagellate reads, 100 iterations) and reran the

PERMANOVA on one rarefied dataset; rarefaction was used only for this check, not for primary inference (McMurdie & Holmes 2014).

Reef-fish hindgut communities

For each fish, BHAB load was expressed as the proportion of dinoflagellate reads assigned to the six focal BHAB genera, and BHAB richness as the number of BHAB species detected at ≥ 3 reads in that fish. Species counts include provisionally named taxa (e.g., *Prorocentrum* sp., *Amphidinium* sp. D2-CMSTAC021) resolved as distinct by the D1–D2 marker but lacking formal species names. We include aggregate load at the genus to provide direct comparison to cell counts of Zerecero et al. (2026; Chapter 2) and with traditional field monitoring focused on genera. The species level analyses that follow address a separate question: which taxa are transferred, which microscopy cannot resolve. Samples returning no dinoflagellate reads were assigned a BHAB proportion of zero and retained in all univariate analyses of load, and in detection frequency summaries where absence is informative. Because Bray–Curtis dissimilarity is undefined for samples containing no BHAB reads, ordination and PERMANOVA were restricted to samples with at least one BHAB read (104 of 117 macroalgal samples; 46 of 57 fish). At the species level, we tested for differences in BHAB proportion and richness among detritivores, herbivores, and corallivores using a Kruskal–Wallis rank-sum test followed by Dunn's post-hoc tests (BH-adjusted) for pairwise comparisons (FSA package), excluding carnivores due to low sample size. We then assessed consistency of guild effects across islands using a Kruskal–Wallis tests performed separately within each island. Next, we assessed relationships between BHAB proportion, and both fish body size and total dinoflagellate reads using a Spearman rank correlation, testing for differences between the two detritivore families via Wilcoxon rank-sum test. To determine whether the guild effect persisted after accounting for

potential confounds, we performed a rank-based analyses of covariance with the $\log_{10}$ of total dinoflagellate reads, and with both total reads and fish body size, included as covariates. Lastly, to test whether guilds differed in community composition rather than only in load, we computed Bray–Curtis dissimilarities on TSS-normalized BHAB proportions and tested effects of guild and island by PERMANOVA (marginal terms, 9,999 permutations), with multivariate dispersion assessed by betadisper, excluding samples with no BHAB reads as Bray–Curtis is undefined for empty samples. Ordination used principal coordinates analysis rather than NMDS, which failed to converge on the sparse hindgut data.

Results

Toxic dinoflagellate communities on macroalgal substrates

Overall abundance and genus composition

Sequencing of D1–D2 LSU across 117 macroalgal samples recovered 7,749,624 dinoflagellate reads assigned to 212 dinoflagellate ASVs. Of these, 53 ASVs belonged to the 6 focal genera of toxic dinoflagellate, totaling 3,083,014 reads and 52 species, only 37 of which met the ≥ 3 reads per sample detection threshold. Sequencing depth varied widely, averaging 63,294 per sample (SD 116,011; median 34,126, IQR 3,778–69,052; range 0–1,009,807), with BHAB reads averaging 26,351 (median 5,191, IQR 799–27,431; range 0–378,251). BHAB reads accounted for 39.8% of total dinoflagellate reads. BHAB sequence reads were dominated by *Ostreopsis* (78.5%) followed by *Prorocentrum* (12.6%), *Gambierdiscus* (7.0%), *Amphidinium* (1.3%), and *Coolia* (0.5%); *Fukuyoa* was not detected in any sample (Figure 3-1; Appendix Table 3-A1). Within *Ostreopsis*, two species accounted for >99.9% of all reads; *O. rhodesae* (82.8% of *Ostreopsis* reads) and *O. lenticularis* (17.2%). Five additional *Ostreopsis* species contributed 33 reads combined.

The fraction of toxic members of dinoflagellate communities varied among host macroalgae. Averaged across seasons, BHAB proportion was highest on *Sargassum* (42.5% of dinoflagellate reads) and *Padina* (34.8%), and lower on *Turbinaria* (26.6%) and *Dictyota* (24.7%). Order differed based on detection frequency (the percentage of samples in which one or more BHAB taxa were recovered) with 97% of *Dictyota* samples containing BHAB, followed by *Sargassum* (90%), *Padina* (86%), and *Turbinaria* (83%) (Appendix Table 3-A1).

Analysis of seasonal variation showed that BHAB proportion was roughly twice as high in the dry season (43.6% of dinoflagellate reads; a mean of ~48,900 BHAB reads per sample) as in the wet season (21.1%; ~4,900 reads per sample) (Figure 3-1; Appendix Table 3-A1). This difference held at the genus level for four of the five detected genera, with *Gambierdiscus*, *Ostreopsis*, *Prorocentrum*, and *Amphidinium* all being significantly more abundant in the dry season (Wilcoxon, all BH-adjusted $p < 0.05$), whereas *Coolia* showed no seasonal difference ($p = 0.52$) (Table 3-1). However, seasonal differences were not consistent across sites. In the two of four sites sampled in both periods, BHAB proportion did not differ between seasons (median 27.3% dry versus 26.4% wet, $n = 73$; Wilcoxon $p = 0.66$), indicating that the pooled seasonal contrast partly reflects sites that were unevenly represented across the two collection periods. Because samples were not rarefied, we further assessed whether the enrichment could reflect uneven sequencing effort. BHAB proportion covaried with total dinoflagellate reads per sample (Spearman $\rho = 0.45$, $p = 2.8 \times 10^{-7}$), which were themselves roughly 14-fold higher in dry-season samples (median 56,848 versus 3,979 reads; Kruskal–Wallis $p = 4.9 \times 10^{-12}$). With this term included as a covariate in a rank-based ANCOVA, the seasonal effect attenuated but remained significant ($F_{1,114} = 4.47$, $p = 0.037$). Standardizing sequencing depth did not remove the seasonal difference: the contrast was significant in all 100 rarefactions (median $p = 0.0024$)

and the rarefied PERMANOVA recovered a comparable seasonal term ($R^2 = 0.046$, $p = 2 \times 10^{-4}$; Appendix Table 3-A7). The attenuation seen in the ANCOVA reflects the tight confounding of depth with period rather than an absence of seasonal signal. Four macroalgal samples and three fish returned no dinoflagellate reads; excluding them did not alter the seasonal difference in BHAB proportion ($p = 2.5 \times 10^{-5}$ versus 3.7×10^{-6} when included).

Table 3-1. Seasonal difference in genus-level BHAB proportion (Wilcoxon rank-sum, BH-adjusted).

Genus	Dry median (%)	Wet median (%)	Dry mean (%)	Wet mean (%)	Adjusted p (BH)	Significance
<i>Gambierdiscus</i>	2.03	0.00	3.44	0.75	3.96e-09	significant
<i>Ostreopsis</i>	24.83	6.62	34.68	15.44	1.78e-05	significant
<i>Prorocentrum</i>	3.45	1.67	4.82	4.02	5.31e-03	significant
<i>Amphidinium</i>	0.33	0.08	0.53	0.46	1.40e-02	significant
<i>Coolia</i>	0.06	0.01	0.17	0.47	5.23e-01	n.s.

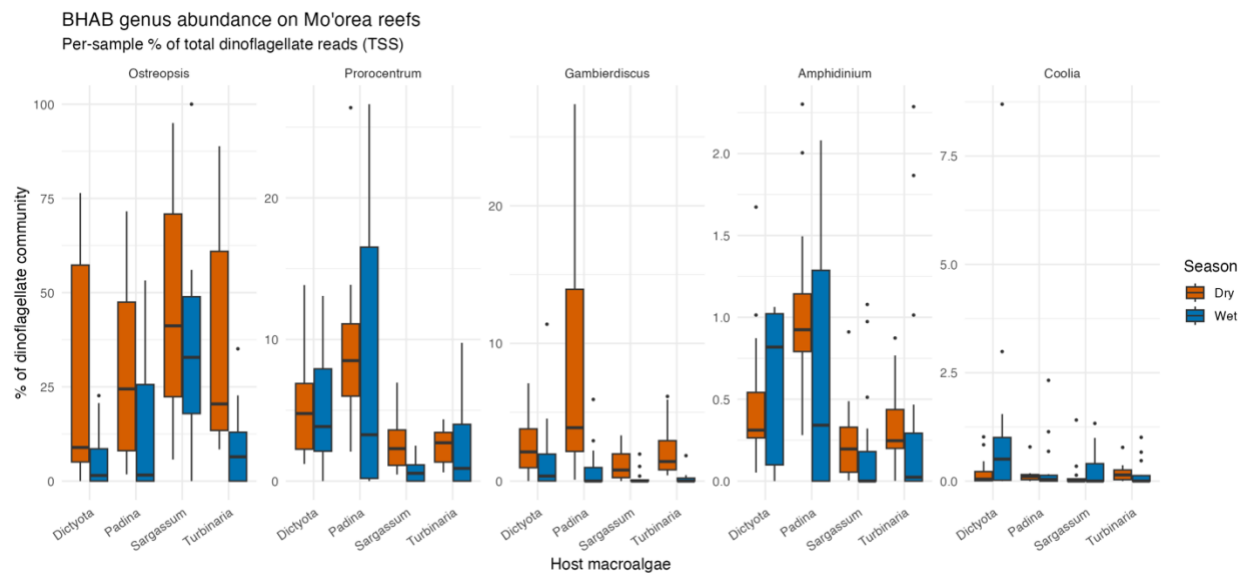


Figure 3-1. Relative abundance of the five detected toxic dinoflagellate (BHAB) genera across four macroalgal hosts in the dry and wet seasons. Values are per-sample percentages of total dinoflagellate reads (TSS normalization).

Species-level Detection and Host Sharing

At the species level, the four host macroalgae supported largely the same core assemblage of toxic dinoflagellate species. Of the 37 BHAB species detected across hosts, 24 were present on all four macroalgal species, and richness was comparable among hosts, ranging from 29 to 32 species (Figure 3-2). Only four species were unique to a single host, all of them *Gambierdiscus*: three on *Padina* and one on *Turbinaria*, with none unique to *Dictyota* or *Sargassum* (Figure 3-2).

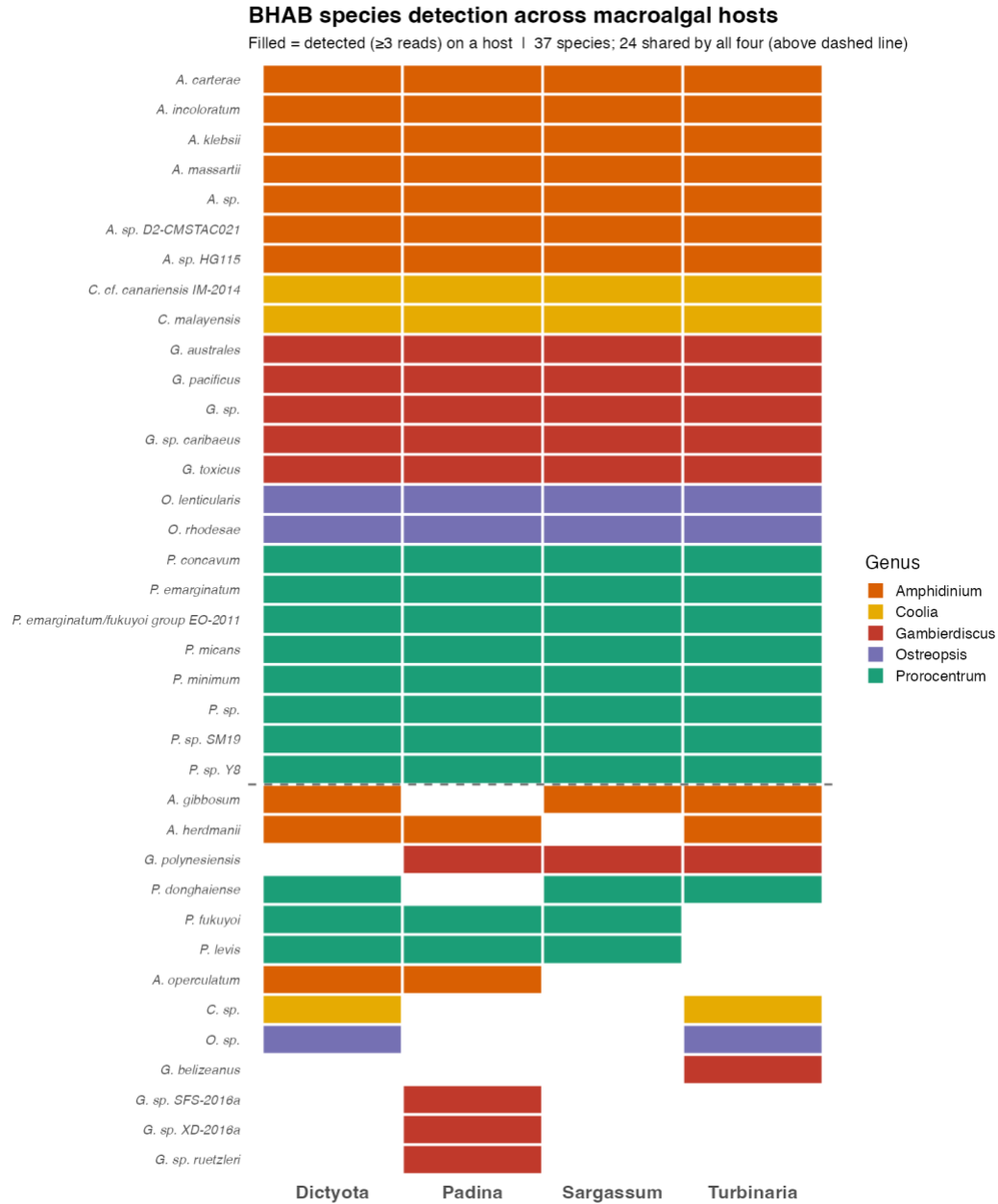


Figure 3-2. Detection of each BHAB species across the four macroalgal hosts (filled = detected at ≥ 3 reads on that host), colored by genus. Species above the dashed line ($n = 24$) were detected on all four hosts; those below were host-restricted to varying degrees. The near-complete core

block shows that hosts shared most species, differing mainly in a small set of rare, mostly *Gambierdiscus* taxa.

Detection frequency, the percentage of samples in which a taxon was detected (≥ 3 reads), was highest for *Prorocentrum* sp., at 81% of samples, followed by *Ostreopsis lenticularis* (79%), *Ostreopsis rhodesae* (64%), *Amphidinium* sp. D2-CMSTAC021 (63%), and *Gambierdiscus pacificus* (59%) (Figure 3-3; Appendix Table 3-A2). Abundance and prevalence were therefore decoupled: *Ostreopsis* dominated read abundance (78.5% of BHAB reads), but *Prorocentrum* sp. was the most frequently detected taxon. Detection frequency tracked the seasonal abundance pattern closely, with the species with the highest detection frequency being detected far more often in the dry season than the wet season. For example, *Gambierdiscus pacificus* was detected in 91% of dry-season samples but only 28% of wet-season samples, and *O. rhodesae* in 86% versus 43% (Figure 3-3).

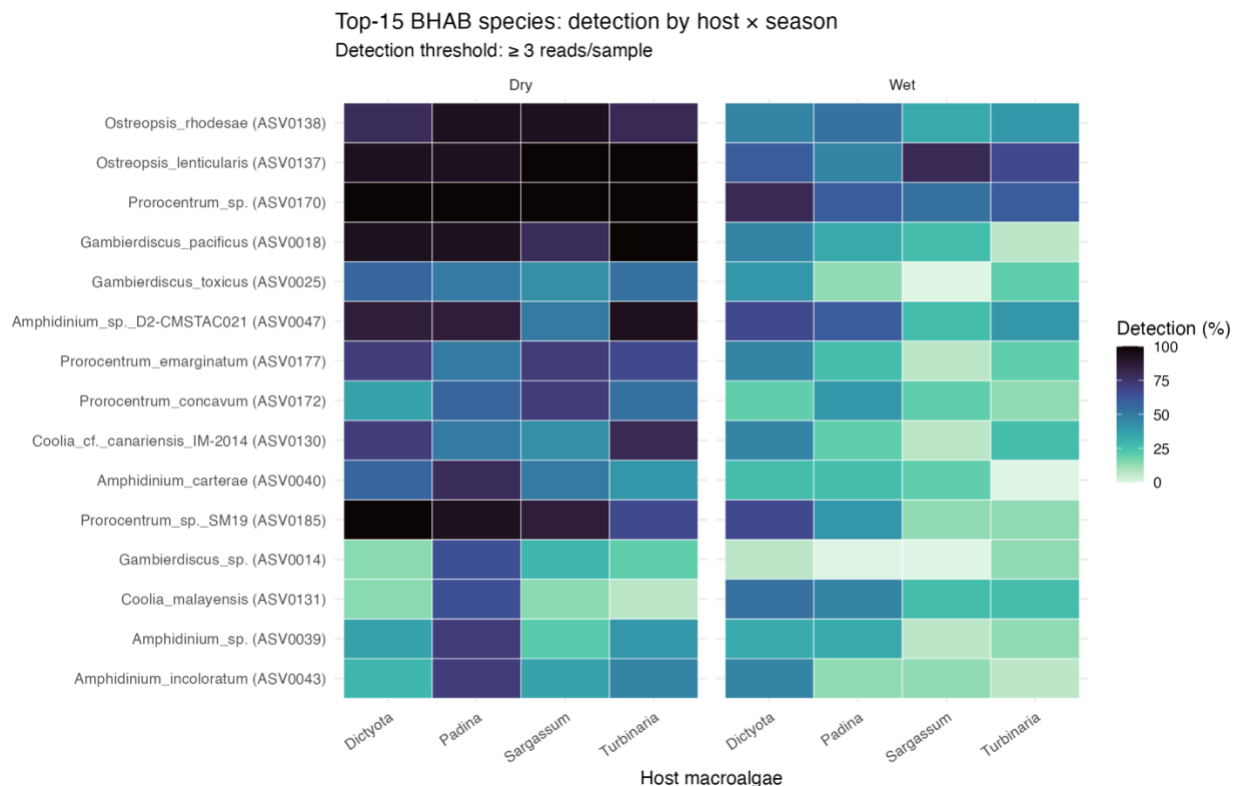


Figure 3-3. Detection frequency of the 15 most abundant BHAB species across the four macroalgal hosts in the dry and wet seasons (detection defined as ≥ 3 reads per sample). Darker cells indicate a higher proportion of samples with the species present. Detection was consistently higher in the dry season across most species.

Species Composition Within the Dominant Genera

Because each genus contains species that differ in toxicity, we examined species-level composition within the dominant genera (Figure 3-4). Reads of *Ostreopsis*, the most abundant genus, included just two species: *O. rhodesae* (82.8% of *Ostreopsis* reads) and *O. lenticularis* (17.2%), with *O. rhodesae* being the dominant species in the dry season (87.4%), whereas *O. lenticularis* was dominant in the wet season (74.1%). *O. lenticularis* was also the more frequently detected of the two (79% vs. 64% of samples).

For *Prorocentrum*, a single, taxonomically unresolved lineage (*Prorocentrum* sp.) comprised 91.0% of reads and was the single most frequently detected BHAB species in the dataset (81% of samples) where as *P. emarginatum* (3.1%), *P. concavum* (2.9%), and *P. sp.* SM19 (2.2%), were comparatively rare; composition did not shift seasonally. A similar pattern held for the minor genus *Amphidinium*, which was dominated by an unresolved lineage (*Amphidinium* sp. D2-CMSTAC021, 46.9%).

The highly toxic *G. pacificus* represented 83.6% of *Gambierdiscus* sequence reads followed by *G. toxicus* at 10.5%. Sequences of *G. pacificus* dominated the dry season (88.8% of reads), and *G. toxicus* in the wet season (53.7% of reads). In contrast, *G. polynesiensis*, regarded as the principal ciguatoxin producer in the Pacific, was rare throughout, accounting for just 126 of 215,879 *Gambierdiscus* reads (<0.1%) and was detected in only 4% of samples.

Species composition within the dominant BHAB genera, by season

Proportion of each genus's reads; species <0.1% in both seasons pooled as "Other"

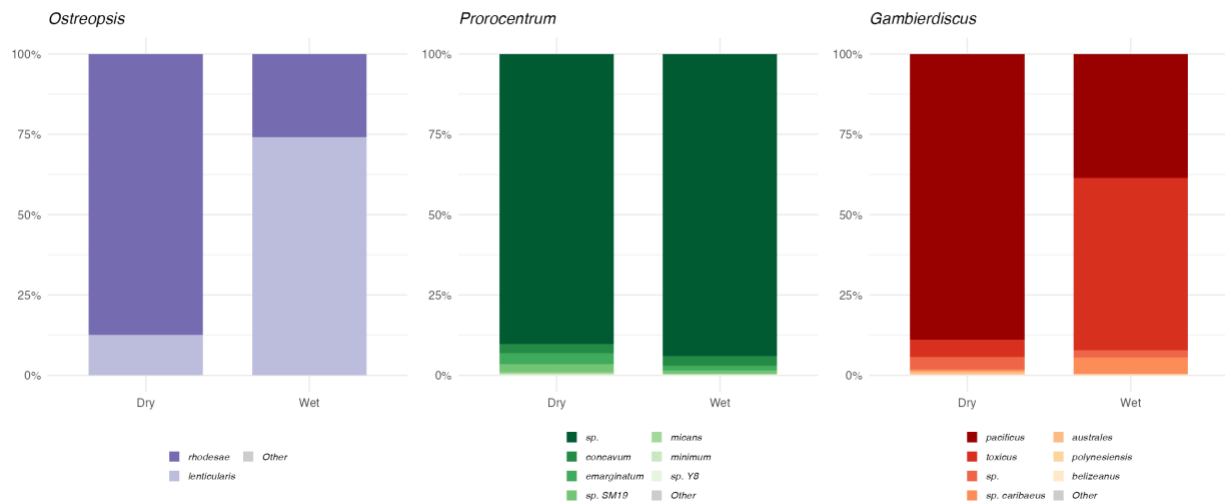


Figure 3-4. Species composition within the three dominant BHAB genera (*Ostreopsis*, *Prorocentrum*, *Gambierdiscus*) in the dry and wet seasons, shown as the proportion of each genus's reads assigned to each species (species <0.1% pooled as "Other"). *Ostreopsis* and *Gambierdiscus* both showed seasonal reversals in composition, whereas *Prorocentrum* was dominated by a single unresolved lineage in both seasons.

Community structure: site, host and seasonal effects

Toxic dinoflagellate community composition varied along three significant axes (Table 3-2, Figure 3-5). Spatial variation among sites was the dominant signal, explaining roughly a third of compositional variation independently of all other terms (marginal $R^2 = 0.32$, $p = 0.001$). Host macroalga and season explained smaller but significant shares (host $R^2 = 0.12$; season $R^2 = 0.04$; both $p = 0.001$). The host effect was invariant to how the model was specified its marginal and sequential estimates were identical ($R^2 = 0.12$), indicating that host-associated community differences are independent of both where and when a sample was collected. The seasonal effect was significant but modest once spatial structure was accounted for: its marginal contribution ($R^2 = 0.04$) was roughly a third of its value when site was omitted ($R^2 = 0.11$), the difference

reflecting variance that season shares with site under a design in which the two collection periods fell in different years.

Table 3-2. PERMANOVA on Bray–Curtis dissimilarities of TSS-normalized BHAB proportions (marginal / Type III R^2). Dispersion p-values from betadisper.

Term	Df	R^2	F	p (PERMANOVA)	Dispersion p
Site	3	0.322	22.88	0.001	0.001
Host macroalga	3	0.116	8.22	0.001	0.012
Season	1	0.036	7.71	0.001	0.383
Residual	96	0.450	—	—	—
Total	103	1.000	—	—	—

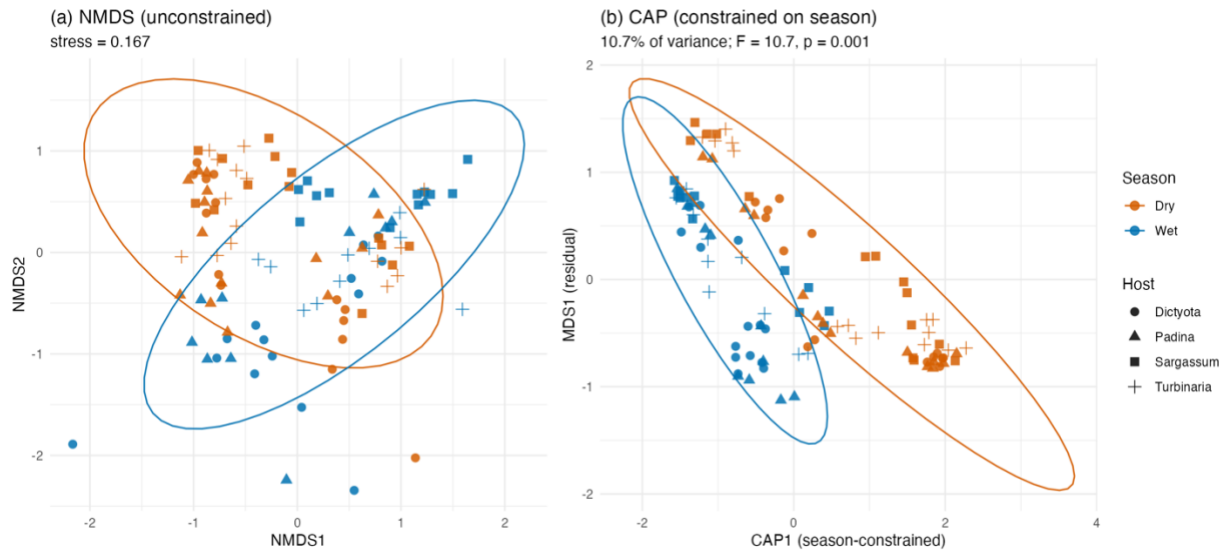


Figure 3-5. Community structure of the substrate BHAB assemblage across seasons, based on Bray–Curtis dissimilarities of TSS-normalized read proportions. (a) Unconstrained ordination (NMDS, stress = 0.167), in which dry- and wet-season samples overlap broadly. (b) Constrained ordination (CAP) conditioned on season, which separates the seasons along the constrained axis

(CAP1) while retaining substantial overlap (10.7% of variance, $p = 0.001$). Points are individual samples, colored by season and shaped by host; ellipses show 95% confidence regions.

Tests of multivariate dispersion indicated that seasonal effects reflected a genuine shift in community composition rather than differences in within-group variability (betadisper, $p = 0.38$). Dispersion was heterogeneous among hosts ($p = 0.012$), indicating that group variances differed. Indicator species analysis (IndVal.g) identified 21 BHAB species significantly associated with season (all BH-adjusted $p < 0.05$). All 21 were dry-season indicators, and none characterized the wet season (Appendix Table 3-A6). The strongest dry-season indicators included *Prorocentrum* sp. SM19 (IndVal = 0.94), *Gambierdiscus pacificus* (0.94), and *Ostreopsis rhodesae* (0.92), the same toxic taxa that dominated overall abundance.

Toxic dinoflagellate communities in reef-fish hindguts

BHAB load across feeding guilds

Across 57 reef fish, hindgut dinoflagellate communities comprised 159 taxa, of which 25 species, accounting for 1,054,278 reads, belonged to the six focal BHAB genera; 24 of these met the ≥ 3 -read detection threshold in at least one fish. Dinoflagellate reads averaged 443,001 per fish (SD 389,303; median 380,987, IQR 65,305–812,901; range 0–1,240,091) and BHAB reads averaged 18,496 (median 942, IQR 47–9,899; range 0–198,597). BHAB taxa accounted for 4.2% of dinoflagellate reads with non-toxic dinoflagellates accounting for the remaining 95.8%.

Libraries were not rarefied.

Total dinoflagellate reads did not differ among feeding guilds (Kruskal–Wallis $p = 0.097$).

However, BHAB load, expressed as the proportion of a sample's dinoflagellate reads assigned to BHAB genera, differed strongly among the three feeding guilds (Kruskal–Wallis $\chi^2 = 26.4$, $df =$

2, $p = 1.9 \times 10^{-6}$; Figure 3-6). Detritivores carried the heaviest loads (median 4.58% of dinoflagellate reads, IQR 0.88–11.30%, $n = 19$), followed by herbivores (median 1.56%, IQR 0.24–2.43%, $n = 17$), although the two guilds did not differ significantly from one another (Dunn $p = 0.27$). Corallivores carried significantly lower toxic dinoflagellate by two to three orders of magnitude (median 0.011%, IQR 0–0.068%, $n = 18$), differing from both detritivores ($p = 2.5 \times 10^{-6}$) and herbivores ($p = 3.1 \times 10^{-4}$). Despite differences in total load, this pattern did not translate to detection frequency, with BHAB reads recovered from 100% of herbivores, 89% of detritivores, 67% of corallivores, and none of the three carnivores.

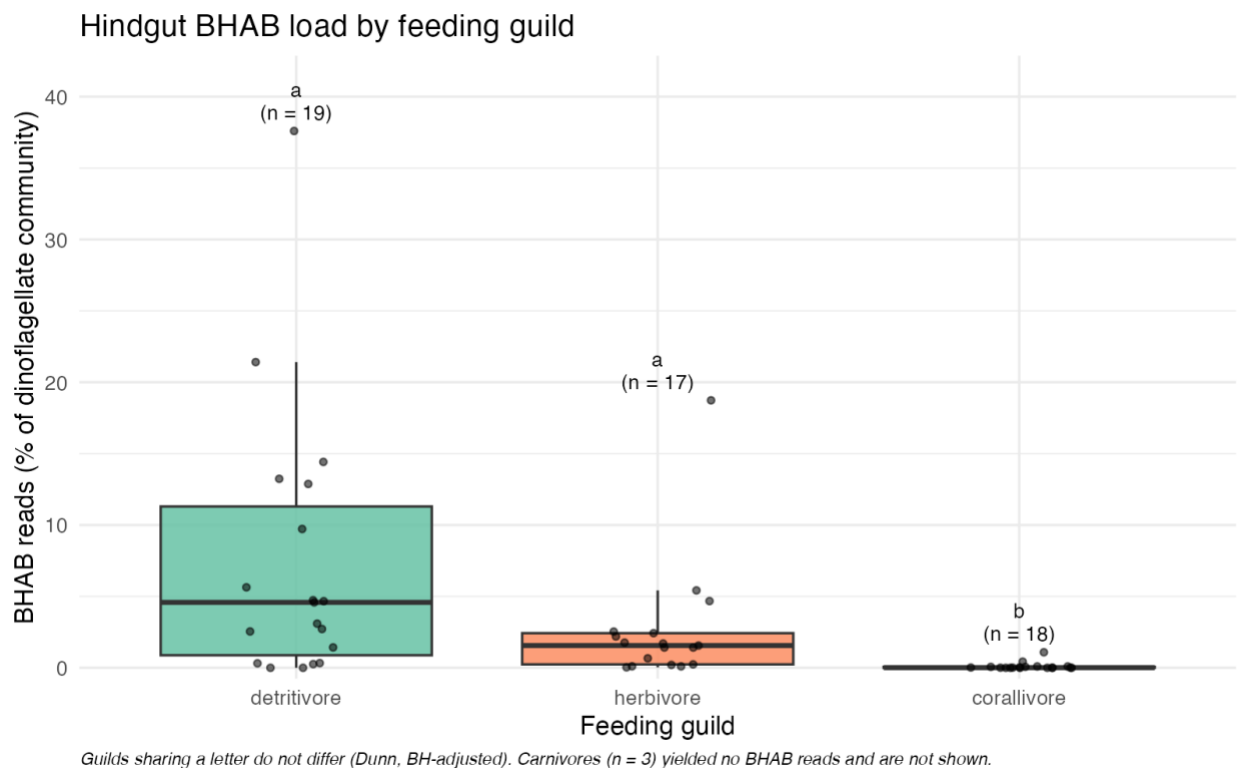


Figure 3-6. Hindgut BHAB load across reef-fish feeding guilds in 54 fishes, expressed as the percentage of each fish's dinoflagellate reads assigned to the six focal BHAB genera (TSS-normalized). Guilds are ordered by decreasing median load. Boxes show the median and interquartile range, whiskers extend to $1.5 \times$ the interquartile range, and points are individual fish,

jittered horizontally. Detritivores and herbivores did not differ from one another ($p = 0.27$), and both carried significantly higher loads than corallivores ($p = 2.5 \times 10^{-6}$ and $p = 3.1 \times 10^{-4}$, respectively). Carnivores ($n = 3$) yielded no BHAB reads and were excluded from both the figure and all statistical tests.

Consistency Across Islands

Effect of fish feeding guild was consistent across the three islands, a significant effect of feeding guild at Mangareva ($n = 20$, $p = 0.001$), Tetiaroa ($n = 17$, $p = 0.017$), and Mo'orea ($n = 17$, $p = 0.035$), and all three remained significant after correcting for the three island-level tests (BH-adjusted $p = 0.004$, 0.026 , and 0.035 , respectively). The rank order of guilds was identical at every island: detritivores carried the heaviest median load, herbivores an intermediate load, and corallivores the lightest (Figure 3-7). Median detritivore load ranged from 2.07% of dinoflagellate reads at Mo'orea to 5.64% at Mangareva, while median corallivore load did not exceed 0.07% at any island. These sites span roughly 1,700 km across two archipelagos: the Society Islands (Mo'orea, Tetiaroa) and the Gambier Islands (Mangareva). They were sampled in March, June, and October 2018, respectively.

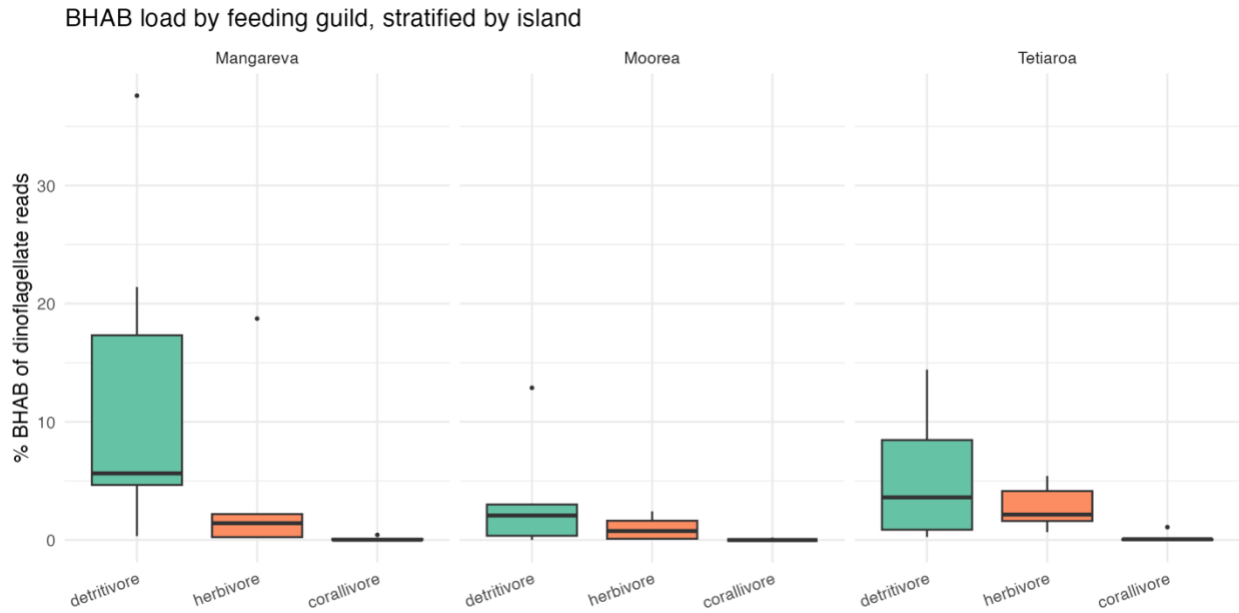


Figure 3-7. Hindgut BHAB load by feeding guild, stratified by island. Each panel shows fishes collected at one island: Mangareva ($n = 20$), Mo'orea ($n = 17$), and Tetiaroa ($n = 17$). Boxes show the median and interquartile range, and whiskers extend to $1.5\times$ the interquartile range. Feeding guild had a significant effect on BHAB load at every island (Kruskal–Wallis $p = 0.001$, 0.035 , and 0.017 , respectively; all significant after Benjamini–Hochberg correction across the three tests), and the rank order of guilds was identical throughout. Islands were sampled in June, March, and October 2018, respectively, so island and sampling month are fully confounded. Carnivores ($n = 3$) are omitted.

In addition to BHAB load, feeding guild also structured hindgut community composition. PERMANOVA on Bray–Curtis dissimilarities of TSS-normalized BHAB proportions recovered a significant effect of guild (marginal $R^2 = 0.128$, $F_{2,41} = 3.35$, $p = 0.0001$) and a weaker effect of island ($R^2 = 0.079$, $F_{2,41} = 2.06$, $p = 0.0033$). Multivariate dispersion did not differ among guilds (betadisper $p = 0.91$). The first two principal coordinate axes captured 35.6% of variance (Figure 3-8).

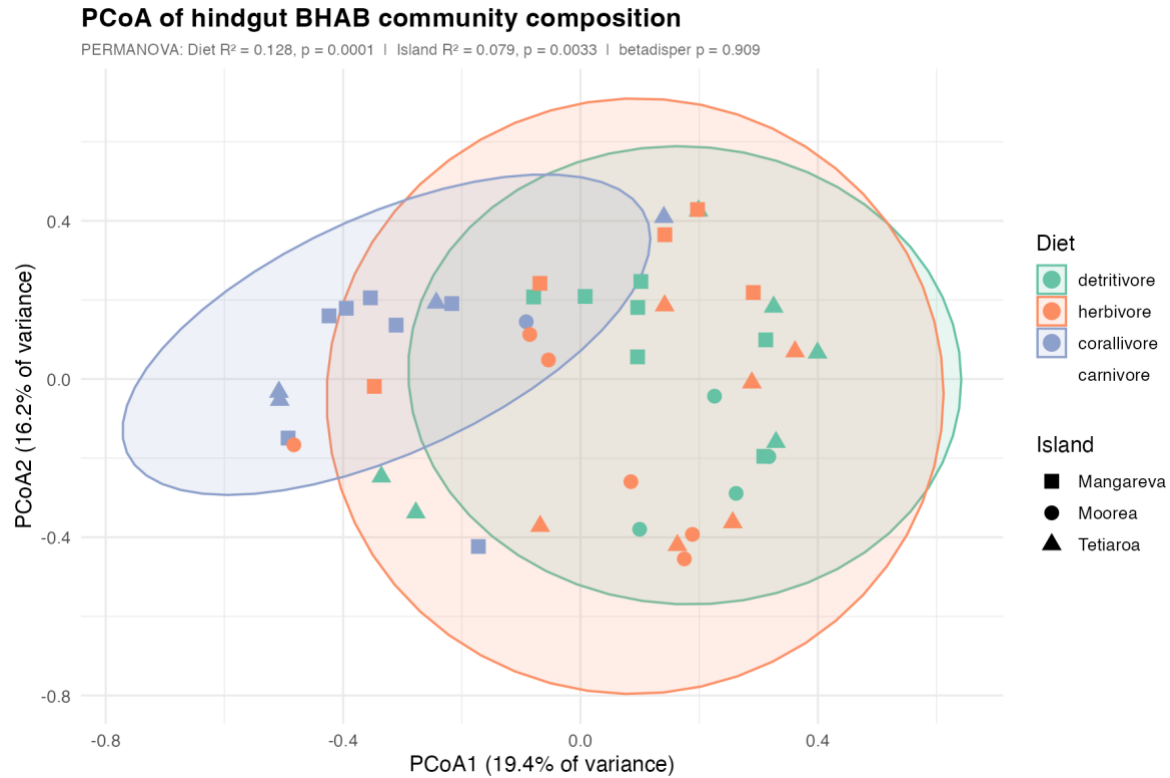


Figure 3-8. Principal coordinates analysis of hindgut BHAB community composition, based on Bray–Curtis dissimilarities of TSS-normalized proportions. Points are individual fish, colored by feeding guild and shaped by island; ellipses show 95% confidence regions for guilds with at least four fish. Based on 46 fish; 11 with no BHAB reads are not shown, including all three carnivores.

Robustness across families, body size, and sequencing effort

Detritivores included two families, each represented by a single species: Labridae (*Chlorurus spilurus*, $n = 13$) and Acanthuridae (*Ctenochaetus striatus*, $n = 6$). BHAB load did not differ between these species (Wilcoxon $W = 31.5$, $p = 0.54$; Figure 3-9), and both species were detected at all three islands. Herbivores comprised two acanthurids (*Naso lituratus*, $n=9$; *Zebrasoma scopas*, $n=8$) and corallivores three chaetodontids (*Chaetodon reticulatus*, $n=10$; *C. ornatissimus*, $n=6$; *C. auriga*, $n=2$). Applying the same test within each guild, corallivore species

did not differ (Kruskal–Wallis $\chi^2 = 3.73$, $df = 2$, $p = 0.15$), and the difference between the two herbivore species was nominally significant but did not survive correction across the three within-guild tests (Wilcoxon $W = 60$, $p = 0.021$; BH-adjusted $p = 0.062$). The only comparison spanning two families the detritivore contrast showed no difference, whereas the suggestive difference occurred between two same family acanthurids.

Because fish were also not rarefied, we tested whether the guild effect could reflect sequencing effort or body size, using the same rank-based ANCOVA approach applied to the substrate data. BHAB load increased with fish body size (Spearman $\rho = 0.40$, $p = 0.002$) and covaried with total dinoflagellate reads ($\rho = 0.38$, $p = 0.004$), but total reads did not differ among guilds (Kruskal–Wallis $p = 0.097$). Feeding guild remained significant with total reads as a covariate ($F_{2,50} = 35.0$, $p = 3.1 \times 10^{-10}$), and with both total reads and total length included ($F_{2,49} = 19.8$, $p = 5.0 \times 10^{-7}$).

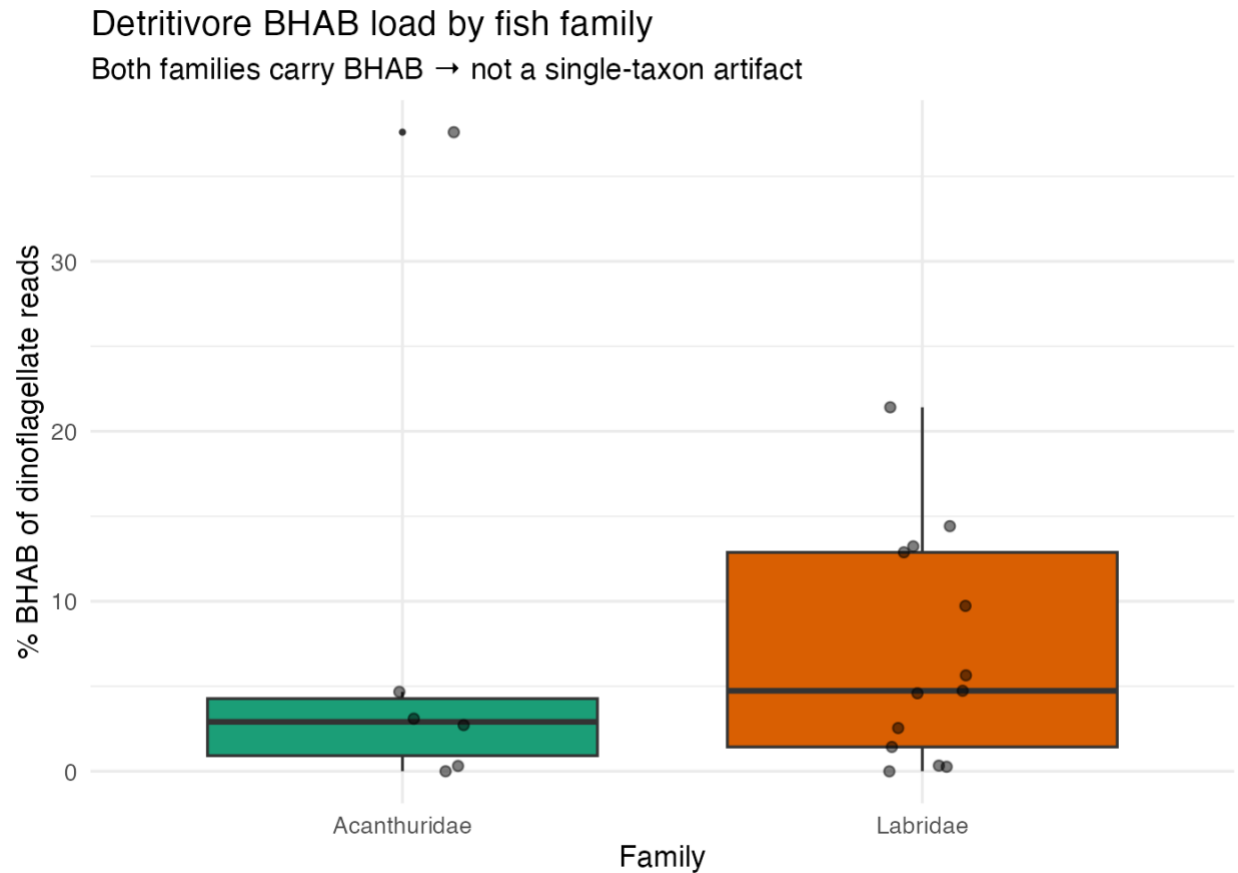


Figure 3-9. BHAB load in detritivorous fishes, by family. Each detritivore family was represented by a single species: Labridae by the excavating parrotfish *Chlorurus spilurus* (n = 13) and Acanthuridae by the brushing bristletooth *Ctenochaetus striatus* (n = 6). Loads did not differ (Wilcoxon W = 31.5, p = 0.54). Boxes show the median and interquartile range; points are individual fish, jittered horizontally.

Diversity and Guild Sharing

BHAB taxon richness differed among the three feeding guilds (Kruskal–Wallis $\chi^2 = 18.3$, df = 2, p = 1.1×10^{-4} ; Figure 3-10). Detritivores had the greatest richness (median 5, IQR 3–10, range 0–15), followed by herbivores (median 3, IQR 1–4, range 1–11) and corallivores (median 1, IQR 0–2, range 0–3). No BHAB taxa were recovered from any carnivore, including two individuals

sequenced to 6,680 and 27,659 dinoflagellate reads.. Guild remained a significant predictor of richness with total dinoflagellate reads included as a covariate ($F_{2,50} = 18.4$, $p = 1.0 \times 10^{-6}$).

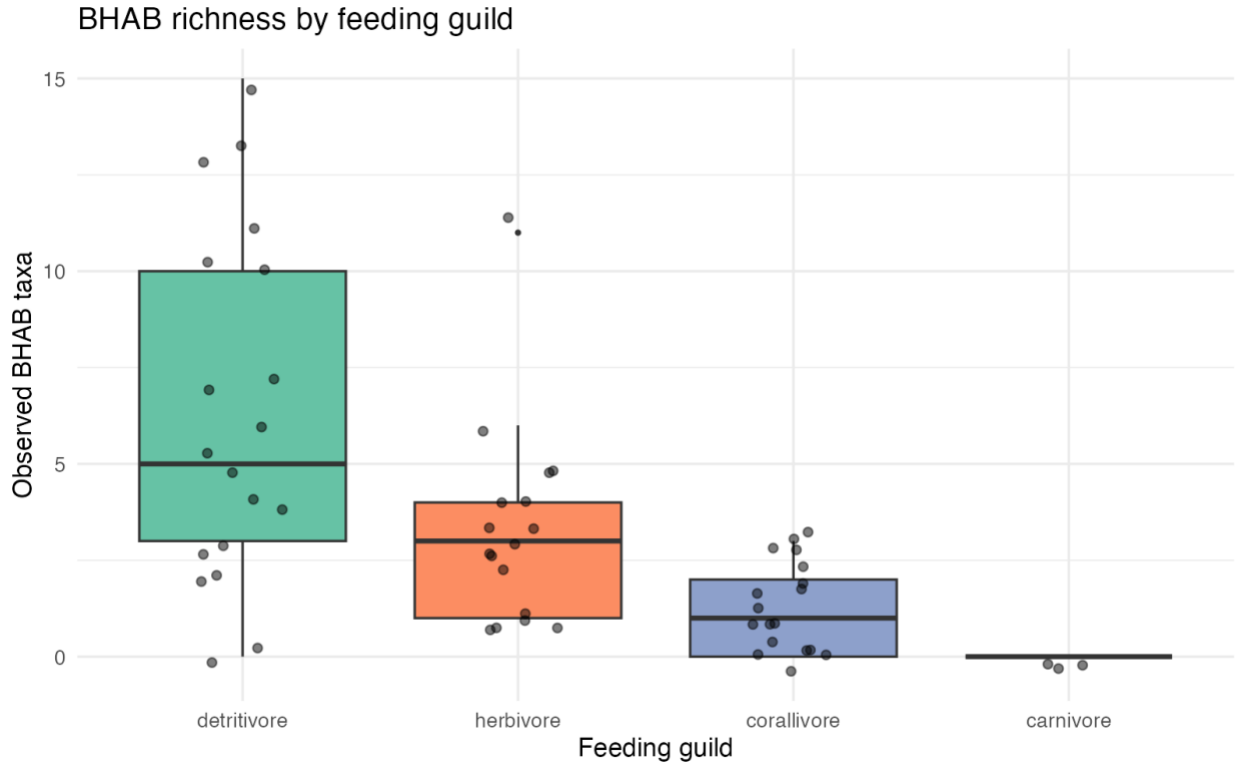


Figure 3-10. Observed BHAB taxon richness across reef-fish feeding guilds, ordered by decreasing median richness. Boxes show the median and interquartile range, whiskers extend to $1.5\times$ the interquartile range, and points are individual fish. Richness differed among the three well-replicated guilds (Kruskal–Wallis $\chi^2 = 18.3$, $df = 2$, $p = 1.1 \times 10^{-4}$) and remained significant after controlling for total dinoflagellate reads ($p = 1.0 \times 10^{-6}$). Carnivores ($n = 3$) yielded no BHAB taxa and are shown for reference only.

Twenty-four BHAB species were detected across the three guilds (≥ 3 reads in at least one fish), of which six occurred in all three: *Coolia malayensis*, *Ostreopsis lenticularis*, *Ostreopsis* sp., *Prorocentrum emarginatum*, *P. minimum*, and *P. sp.* Detritivores carried 21 species, eight of which were found in no other guild; herbivores carried 16, three of them unique; corallivores

carried six, none of which were unique. The corallivore assemblage was a strict subset of both grazer assemblages (Figure 3-11). *Gambierdiscus pacificus* was detected in detritivores only, in a single *Chlorurus spilurus* from Mangareva (5,212 reads); it was the only *Gambierdiscus* species recovered from any fish and no *Gambierdiscus* reads were found in herbivores, corallivores, or carnivores. The other nine *Gambierdiscus* species, including *G. toxicus*, despite being detected on macroalgae were never recovered in hindgut samples.

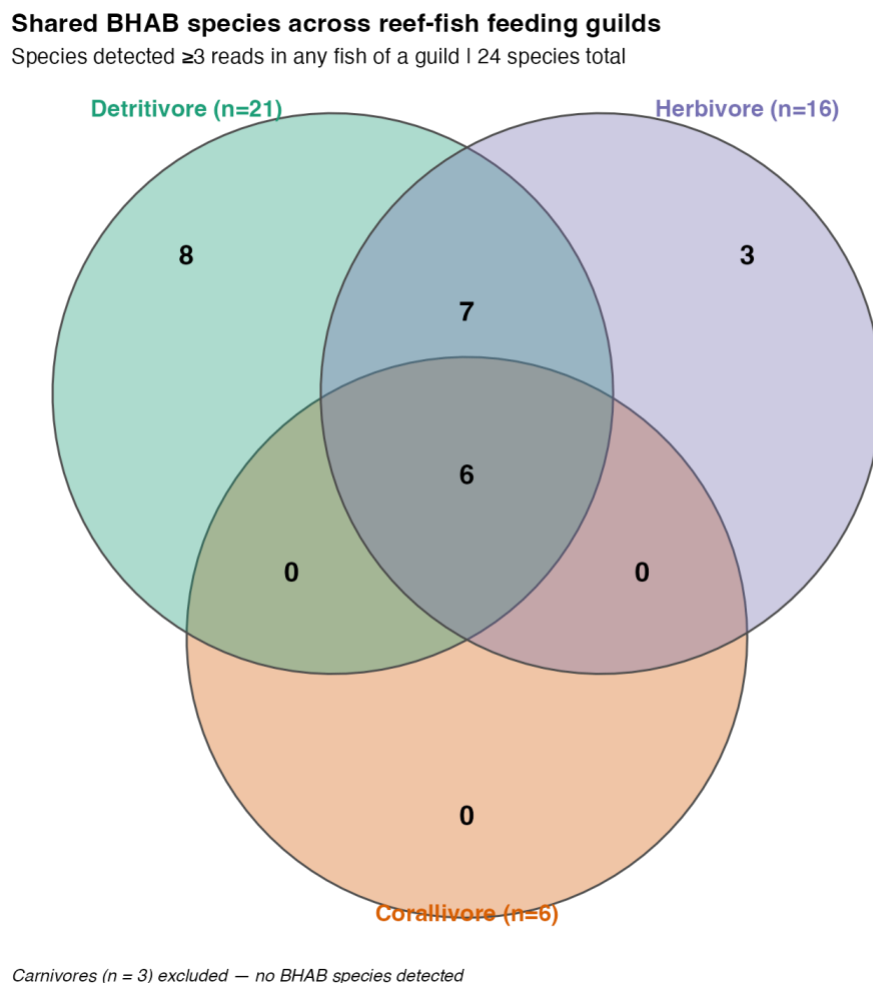


Figure 3-11. BHAB species shared among reef-fish feeding guilds. A species was scored as present in a guild if it reached ≥ 3 reads in at least one fish of that guild. Twenty-four species

were detected in total. Six occurred in all three guilds; detritivores carried eight species found in no other guild and herbivores three, while corallivores carried no unique species; their entire assemblage was a strict subset of both grazer assemblages. Carnivores ($n = 3$) are omitted, no BHAB species having been detected in any of the three.

Correspondence between substrate and consumer assemblages

Macroalgal and hindgut samples were collected independently: macroalgae from four sites on Mo'orea, and fishes from Mo'orea, Tetiaroa, and Mangareva in 2018. The two components were not matched in time, and only Mo'orea was sampled for both.

Applying the detection threshold used throughout (≥ 3 reads in at least one sample), results showed 37 BHAB species on macroalgal substrates and 24 in reef-fish hindguts, of which 23 occurred in both. A single species, *Prorocentrum elegans*, was recovered from fish hindguts but not from any macroalgal sample. Fourteen species were detected on macroalgae but in no fish hindguts.

Restricting the comparison to Mo'orea, the one island sampled for both components, all 13 BHAB species detected in Mo'orea fishes were also detected on Mo'orea macroalgae, with none being unique to hindguts. *Prorocentrum elegans* occurred in fishes from Mangareva or Tetiaroa, where macroalgal substrates were not sampled.

Nine of the 14 substrate-only species were *Gambierdiscus*. Of the ten *Gambierdiscus* species identified from macroalgal substrates, only *G. pacificus* was recovered from a hindgut, from a single *Chlorurus spilurus* at Mangareva (5,212 reads); the other nine, including *G. toxicus*, were detected on macroalgae but never in fish. Across all 57 fish, only one individual carried any

Gambierdiscus. The remaining genera were shared between substrate and gut almost completely: *Ostreopsis* (3 of 3 species), *Coolia* (3 of 3), *Prorocentrum* (9 of 11), and *Amphidinium* (7 of 10).

Discussion

DNA metabarcoding of toxic dinoflagellates on macroalgal-dominated reefs of Mo'orea, French Polynesia showed substantial variation in dinoflagellate communities across both space and time (wet vs dry season). Although spatial (Richlen & Lobel 2011; Parsons et al. 2012) and temporal (Chinain et al. 2010b) variation in toxic dinoflagellates has been reported in previous studies, our study was able to identify these communities to the level of species, rather than genus, a critical difference given that, even within the most toxic genera, levels of toxicity can vary greatly (Chinain et al. 2010a). As such, metabarcoding approaches allow for a more accurate determination of species composition and associated health risk to potentially affected human populations.

Not only did metabarcoding shed novel insights into changes in toxic dinoflagellate communities associated with expanding macroalgal populations, but it also demonstrated that detritivores have the highest ingestion of toxic dinoflagellates, relative to herbivores and corallivores a pattern consistent across all three islands and robust to body size, sequencing depth, and phylogeny. Feeding guild structured not only how much fishes ingested, but also which taxa they ingested. Most BHAB species were shared between substrate and hindgut, confirming the view that fishes draw broadly from the pool present on the surrounding benthos (DiBattista et al. 2023). The lone exception was *Gambierdiscus*, where of ten species recovered from macroalgal substrates, only one, *G. pacificus*, was recovered from any fish, and only from a single individual. This result suggests that the ingestion of toxic dinoflagellates is not completely random and provides novel insights into the introduction of ciguatoxins into the food chain (Lehane & Lewis 2000; Holmes

& Lewis 2025). Combined these results indicate that metabarcoding of algae and primary consumer fishes could provide important insights shaping our understanding of ciguatera and other forms of food poisoning associated with toxic dinoflagellates.

Composition of Toxic dinoflagellate communities on macroalgal substrates

A key limitation of studies of the ecology of toxic dinoflagellates, including Zerecero et al. (2026; Chapter 2) is that morphological identification frequently does not resolve the species-level diversity. Lack of species level identification is problematic because many genera contain toxic and non-toxic species that are indistinguishable by light microscopy (Litaker et al. 2009). This limitation is particularly consequential for *Gambierdiscus*, in which ciguatoxin production varies by orders of magnitude among congeners (Litaker et al. 2010; Dolah et al. 2020).

By identifying BHABs to species, this study demonstrated the presence of a core community of BHABs living epiphytically on macroalgae, with few species being unique to any given macroalgal species. Moreover, species level identification provided other important insights. For example, *Ostreopsis*, dominated all BHAB reads, with the ciguatera-causing genus *Gambierdiscus* comprising only 7.0%. Although counter intuitive for a ciguatera-endemic region (Chinain et al. 2023) this result is consistent with a growing recognition that, as a widespread member of tropical epibenthic dinoflagellate communities (Rhodes 2011; Parsons et al. 2012), *Ostreopsis* can numerically dominate dinoflagellate assemblages on Pacific reefs (Zerecero et al. 2026; Chapter 2). Importantly, results showed that more than 99.9% all *Ostreopsis* reads belonged to just two species, *O. rhodesae* (82.8% of *Ostreopsis* reads) and *O. lenticularis* (17.2%), the toxicological significance of which is not straightforward. Although several *Ostreopsis* produce potent palytoxin analogues associated with respiratory irritation, dermatitis, and marine-organism mortality (Ramos & Vasconcelos 2010), the two species dominating these

reefs are not among them: *O. lenticularis* produces ostreotoxins of considerably lower potency whose classification as palytoxin analogues remains unresolved (Ramos & Vasconcelos 2010). In contrast the most abundant species in our study, *O. rhodesae*, is only recently described and is not known to produce palytoxin or its analogues, although its extracts are cytotoxic to fish gill cell lines (Verma et al. 2016). The dominance of these two taxa, and their seasonal shifts (see below), identifies a substantial but poorly characterized component of the reef toxic-dinoflagellate burden, one whose principal hazard may lie less in human palytoxin poisoning than in direct toxicity to reef organisms, and which a *Gambierdiscus*-focused survey would overlook entirely. The low overall abundance of *Gambierdiscus* sequence reads, however, does not diminish its epidemiological importance. Because ciguatoxins bioaccumulate and biomagnify through reef food webs (Friedman et al. 2008; Chinain et al. 2021), even a numerically minor substrate presence can translate into significant risk at higher trophic levels.

Drivers of Variation in Toxic Dinoflagellates Communities

The structure of dinoflagellate assemblages on macroalgae was shaped by three distinct sources of variation. The dominant driver was spatial position among sites, which explained roughly a third of community variation, a degree of small-scale heterogeneity that underscores how patchily these epibenthic communities are distributed even within a single reef system, where microhabitat factors such as water motion and depth strongly structure abundance (Richlen & Lobel 2011). Host macroalga explained a smaller but robust share and the effect of host was remained even after accounting for other factors, specifically spatial variation and sampling season, indicating that different species of algae host different dinoflagellate communities. Such host structuring is consistent with the substratum preferences documented among benthic dinoflagellates, in which macroalgal architecture, surface chemistry, and epiphyte load influence

which taxa colonize a given host (Parsons & Preskitt 2007; Rains & Parsons 2015). This inference warrants some caution, however: multivariate dispersion differed among hosts (betadisper $p = 0.012$), so the host signal may partly reflect differences in community variability rather than fully distinct assemblages.

Species composition turned over between periods within the two most toxicologically relevant genera. In *Ostreopsis*, *O. rhodesae* accounted for 87.4% of reads in the dry period, whereas *O. lenticularis* accounted for 74.1% in the wet. In *Gambierdiscus*, *G. pacificus* dominated the dry period (88.8%) while *G. toxicus* rose to 53.7% in the wet. Because these are proportions computed within a genus and within a sample, they are insensitive to the difference in sequencing depth between periods, and the absence of a dispersion difference (betadisper $p = 0.38$) indicates a genuine compositional shift rather than a change in variability. This turnover is invisible to genus-level microscopy, which would record both genera as present in both periods at broadly similar relative abundance.

Total BHAB load was also higher in the drier July period, consistent with the cell counts of Zerecero et al. (2026; Chapter 2) and with storm-associated wave disturbance suppressing epibenthic assemblages (Richlen & Lobel 2011). The metabarcoding evidence for this abundance difference is the weaker of the two results, however, and the microscopy in Chapter 2 provides the stronger support, as cell counts are not subject to the sequencing-depth asymmetry described below.

Three limitations constrain interpretation of the effect of seasonality. First, season explained roughly an order of magnitude less variance than site, and its apparent effect shrank once spatial structure was accounted for. Sites were not evenly represented across the two collection periods, and when the comparison was restricted to the two sites sampled in both, no seasonal difference

was detectable so part of what looks seasonal is spatial. Second, sequencing depth was roughly fourteen-fold higher in dry-period samples and covaried with BHAB proportion, and every indicator taxon was associated with the dry period, a pattern depth asymmetry alone could produce. Standardizing depth by rarefaction did not remove the seasonal difference, so depth asymmetry contributes to but does not generate the pattern; the residual caveat is that rarefaction discards the shallowest samples, all of them from the wet period. Third, each period was sampled in a single year, so period, interannual, and storm-related variation cannot be separated. Distinguishing them will require sampling replicated across multiple years and disturbance regimes.

The toxic fraction of dinoflagellate communities also varied among species of macroalgae. BHAB taxa made the largest proportion of dinoflagellate reads on *Sargassum* (42.5%) and *Padina* (34.8%), with smaller proportions on *Turbinaria* (26.6%) and *Dictyota* (24.7%). This ranking differs from the absolute cell abundances reported in (Zerecero et al. 2026; Chapter 2), where toxic loads peaked on *Turbinaria*. While seemingly contradictory, these results are complementary, as the metabarcoding proportion reflects the relative dominance of BHAB taxa within the dinoflagellate community on a host, whereas microscopy captures total cell density per unit of algal tissue. A host may therefore support a high absolute abundance of toxic cells while those cells represent a smaller fraction of an especially rich overall dinoflagellate assemblage, or vice versa. The most epidemiologically informative pattern, however, emerged not at the level of total BHAB proportion but within *Gambierdiscus* where the community was dominated by *G. pacificus* followed by *G. toxicus*, both ciguatoxin-associated species within a genus whose toxicity varies by orders of magnitude among congeners (Litaker et al. 2010; Chinain et al. 2023). Both were detected across all four host macroalgae, establishing a broad

reservoir of ciguatera-relevant cells available to grazing consumers regardless of which macroalga they feed upon, suggesting that phase shifts from coral to algal dominate reefs could increase the introduction of ciguatoxins into tropical coastal foodwebs.

Toxic dinoflagellate communities in reef fish hindguts

If macroalgal substrates constitute the environmental reservoir of toxic dinoflagellates, the reef fishes that graze them determine which of those toxins enter the food web. Across the 57 fish sampled, hindgut BHAB load was structured strongly and significantly by feeding guild, with detritivores carrying the heaviest toxic dinoflagellate burden, followed by herbivores, while corallivores carried almost none. Although carnivore samples were not sufficient for statistical analyses, all three samples had undetectable levels. This gradient in toxic dinoflagellate load aligns directly with how each guild contacts the benthos: detritivores ingest surface biofilm, sediment, and detrital material (Parravicini et al. 2020), precisely the microhabitat where epibenthic dinoflagellates settle and accumulate (Parsons et al. 2011; Rains and Parsons 2015). In contrast, herbivores crop fleshy and turf algae. Although these algae clearly harbor substantial toxic dinoflagellate communities (Zerecero et al 2026: Chapter 2), by consuming the algae, herbivores are incidentally ingesting toxic dinoflagellates, rather than scraping the surfaces of the benthos or macroalgae where toxic dinoflagellates accumulate (Cruz-Rivera & Villareal 2006; Lehane & Lewis 2000), so it is expected that the dinoflagellates would be a smaller portion of their diets. The near-absence of BHABs in corallivores and carnivores is expected. Corallivores consume live coral, where there are likely to be few toxic dinoflagellates. Similarly, piscivorous fishes acquire toxins through their prey rather than by direct substrate contact, and any ciguatoxin they carry would be present as biomagnified tissue-bound compound rather than intact dinoflagellate cells detectable by metabarcoding (Lehane & Lewis 2000; Chinain et al.

2021). Consistent with this, eleven of the 57 fish yielded no BHAB reads at all, and they were not randomly distributed across guilds: all three carnivores and six of eighteen corallivores were empty, compared with two of nineteen detritivores and none of the herbivores. That these fishes had to be excluded from the compositional analysis is itself informative for guilds with little direct benthic contact, BHAB ingestion appears to be incidental rather than routine. As such, feeding mode imposes a clear filter on which fishes encounter toxic dinoflagellates at the base of the exposure pathway.

The same guild structure was evident in taxon richness: detritivores harbored significantly more BHAB species per fish, followed by herbivores and corallivores, echoing differences observed in load. Detritivores thus not only carried heavier toxic loads but encountered a wider diversity of toxic taxa, consistent with the broad, indiscriminate ingestion of surface and sediment material that characterizes their feeding. Critically, this pattern was consistent across the three islands sampled. Combined, these data suggest that detritivores are likely the major pathway for the introduction of ciguatoxins and other dinoflagellate toxins into the food webs.

Further support for feeding guild as a predictor of toxic dinoflagellate exposure comes from examination of the effects of body size and sequencing depth. Although dinoflagellate load increased significantly as a function of both body size and sequencing depth, feeding guild remained a strong predictor of load after body size and sequencing effort were included as covariates. Additionally, total reads did not differ significantly among guilds. Since feeding guilds are not evenly distributed across fish families, phylogeny could also impact BHAB load. However, the excavating parrotfish *Chlorurus spilurus* (Labridae) and the brushing bristletooth *Ctenochaetus striatus* (Acanthuridae), despite belonging to different families and employing distinct feeding mechanisms, had statistically indistinguishable BHAB loads. Within-guild

species differences were likewise absent among corallivores, and only marginal among herbivores, where *Naso lituratus* and *Zebrasoma scopas* differed nominally but not after correction. Feeding guild therefore remains the dominant structure, though species-level differences in foraging within a guild may contribute a plausible reading given that *N. lituratus* browses fleshy macroalgae while *Z. scopas* crops turf.

This trophic structuring echoes decades of ciguatera ecology. Early gut-content surveys of *Ctenochaetus* surgeonfish reported near-ubiquitous *Gambierdiscus* (Campbell et al. 1987), and recent bioaccumulation modeling predicts that detritivorous *C. striatus* should accumulate more toxin than co-occurring herbivorous parrotfishes, owing to its larger grazing area and longer gut retention time (Holmes & Lewis 2025). Our metabarcoding recovers this same gradient directly across three islands and two archipelagos. These patterns establish that feeding mode governs how much toxic dinoflagellate material a fish ingests, and how many toxic taxa it encounters.

Correspondence between substrate and consumer assemblages

Ciguatera ecology has long assumed that the toxic dinoflagellates accumulating in fish are drawn broadly from the pool present on the surrounding benthos (Cruz-Rivera & Villareal 2006; Lehane & Lewis 2000). Of 37 BHAB species detected on macroalgal substrates and 24 in reef-fish hindguts, 23 were common to both. Only a single species, *Prorocentrum elegans*, was found in hindguts but never on the sampled substrates. Restricting this analysis to Mo'orea, where both fish and algae were sampled, all 13 BHAB species recovered from fishes were also present on macroalgae, with no gut-exclusive taxa. As such, the toxic dinoflagellates found in reef fishes are, almost without exception, a subset of those on the benthos they graze, a pattern expected if the substrate community is the source of consumer exposure. In this respect, the present study

extends gut-content metabarcoding established for reef-fish food webs in this same system by Casey et al. (2019) to the specific question of toxic-dinoflagellate transfer.

Not only was there a near-complete overlap in the toxic dinoflagellate communities broadly, but there was also strong correspondence at the generic level with most BHAB genera moving from the benthos to fish with high fidelity: *Ostreopsis* (3 of 3 species), *Coolia* (3 of 3), *Prorocentrum* (9 of 11), and *Amphidinium* (7 of 10), indicating that substrate presence largely translates into consumer presence. However, there was one striking exception—the genus that matters most for ciguatera. Of the ten *Gambierdiscus* species detected on macroalgal substrates, only one, *G. pacificus*, was recovered from any fish, a single individual of *Chlorurus spilurus* (a detritivorous parrotfish) at Mangareva. In total, 9 of the 14 BHABs found algae, but never in fish, were *Gambierdiscus*, and only one of 57 fish had *Gambierdiscus* in their digestive system. This pattern is a sharp departure *Ostreopsis* and *Prorocentrum*, where there was nearly complete overlap between algal BHAB communities and fish hindgut contents. This result indicates that toxic dinoflagellate communities do not transfer to consumers as an undifferentiated whole.

Several explanations could account for this pattern. Given that *Gambierdiscus* typically occurs at low absolute cell densities relative to other genera (Zerecero et al. 2026; Chapter 2; Richlen & Lobel 2011), herbivorous and detritivorous fishes may ingest it infrequently. This baseline scarcity, however, can be punctuated by episodic *Gambierdiscus* blooms, during which cell densities can reach nearly 5,000 cells g⁻¹ (Chinain et al. 1999). As such, even in areas impacted by ciguatera, probability of observing *Gambierdiscus* in fish guts during baseline conditions is likely extremely low. The decoupling implied here is that *Gambierdiscus* abundance need not predict what reaches consumers, a result that is consistent with recent work from the Cook Islands, where ciguatoxin levels in the sentinel detritivore *Ctenochaetus striatus* tracked the

presence of the rare, highly toxic *G. polynesiensis* rather than overall *Gambierdiscus* abundance (Smith et al. 2026).

A second explanation for not observing *Gambierdiscus* in fish guts is trophic pathway itself. In the classical model of ciguatera transfer, *Gambierdiscus* cells are consumed not primarily by fish that ultimately become toxic, but rather by small intermediate grazers, including herbivorous invertebrates and micrograzers, which accumulate ciguatoxins and are, in turn, consumed by larger fishes (Holmes et al. 2021). Under this model, ciguatoxin can biomagnify in piscivorous fishes without its prey ever directly ingesting an intact *Gambierdiscus*. This pathway is consistent with two features of the present data: the near-absence of *Gambierdiscus* in carnivores, which would acquire toxin through prey rather than by grazing, and the single detection in a detritivorous parrotfish, a direct grazer positioned to ingest cells before they enter the invertebrate-mediated chain.

A third explanation is methodological, and it reinforces the second. Because metabarcoding detects intact cells and their DNA rather than the ciguatoxins the cells produce, and because those toxins are retained and biomagnified in fish tissue long after the source cells are digested (Lehane & Lewis 2000; Holmes et al. 2021), a fish may carry a substantial ciguatoxin body burden while yielding little or no *Gambierdiscus* DNA at the moment of sampling. This limitation is not unique to the present study: recent work coupling *Gambierdiscus* metabarcoding with LC-MS/MS confirmation notes that molecular detection alone has been unable to link *Gambierdiscus* presence to realized ciguatoxin transfer in fish, precisely because the toxin and the cells that produce it are decoupled in trophic space and time (Smith et al. 2026). Together, the trophic and methodological explanations imply the same caution: cell-based surveys of

consumers will systematically underestimate ciguatoxin exposure, because the toxin outlasts and outreaches the cells that produce it.

A fourth and final explanation is that fish may actively avoid ingestion of *Gambierdiscus*. A recent study by Li et al (2022) indicates that toxins associated with *Gambierdiscus* alters fish behavior, potentially making them more susceptible to predation. As such, there would be strong selection pressure to avoid consuming *Gambierdiscus*. These explanations are not mutually exclusive, and the present data cannot fully distinguish among them. What they establish jointly is that the presence of a toxigenic genus on the benthos does not translate straightforwardly into its detectable presence in consumers, a decoupling with direct consequences for how ciguatera risk should be monitored.

Conclusion

With a global increase in phase shifts from coral to algal dominated reefs, it is essential to understand how this increase in substrate may impact the risk of ciguatera and other fish poisonings associated with toxic dinoflagellates. Despite conducting this study in a region where ciguatera is an important public health concern (Chinain et al. 2010b, 2019) dinoflagellate communities on macroalgae were not dominated not by the ciguatera-causing *Gambierdiscus*, but rather by two species of *Ostreopsis*, whose greatest hazard is to reef organisms, not human consumers. Results also showed that feeding ecology, more than location, determined which fishes encounter toxic dinoflagellates, and that despite being the genus of greatest public-health concern, *Gambierdiscus* was rarely detected in fish guts. All these important insights resulted from the application of metabarcoding approaches that allowed the identification of BHABs to the level of species. However, our results also show that molecular studies cannot, on their own, quantify the ciguatoxin risk those communities pose. Robust ciguatera monitoring will require

pairing the species-level community resolution demonstrated here with the direct toxin-detection approaches already central to ciguatera research (Smith et al. 2026), and with sampling designed to capture episodic *Gambierdiscus* blooms. Our data clarify why this pairing matters—molecular surveys and toxin assays measure different things, and the *Gambierdiscus* bottleneck marks exactly where a cell-based census and a toxin-based methods would diverge.

Even so, our metabarcoding approach delivers what genus-level and microscopy-based surveys cannot: a species-resolved census of the entire toxic-dinoflagellate assemblage across both substrate and consumer. Given the lack of *Gambierdiscus* in fish guts relative to algal surfaces, application of the same metabarcoding approach to macroalgal-associated invertebrate communities would help reveal whether these grazers harbor the *Gambierdiscus* diversity that is present on the substrate yet missing from fish and thereby closing the loop between benthic dinoflagellate production and realized ciguatera risk. Such a study would complete the exposure pathway traced only partway here: from toxic dinoflagellates on macroalgae, through the invertebrate grazers that consume them, to the fishes and other marine organisms in which their toxins accumulate and ultimately to our dinner tables.

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Appendix

Appendix Table 3-A1. BHAB relative abundance and detection by host and season.

(a) By host macroalga

Host macroalga	No. of samples	Mean BHAB (% of dinoflagellate reads)	Mean BHAB reads per sample	Samples with BHAB detected (%)
<i>Sargassum</i>	29	42.5	27,443	90
<i>Padina</i>	29	34.8	22,777	86
<i>Turbinaria</i>	30	26.6	26,100	83
<i>Dictyota</i>	29	24.7	29,090	97

(b) By season

Season	No. of samples	Mean BHAB (% of dinoflagellate reads)	Mean BHAB reads per sample	Samples with BHAB detected (%)
Dry	57	43.6	48,892	100
Wet	60	21.1	4,936	78

Appendix Table 3-A2. All BHAB species detected on macroalgal substrates (≥ 3 reads/sample; $n = 37$). Asterisks (*) mark the 15 most abundant species.

Species	Overall detection (%)	Dry detection (%)	Wet detection (%)
<i>Prorocentrum_sp.*</i>	81	100	63
<i>Ostreopsis_lenticularis*</i>	79	96	63

Species	Overall detection (%)	Dry detection (%)	Wet detection (%)
<i>Ostreopsis_rhodesae</i> *	64	86	43
<i>Amphidinium_sp._D2-CMSTAC021</i> *	63	79	48
<i>Gambierdiscus_pacificus</i> *	59	91	28
<i>Prorocentrum_sp._SM19</i> *	59	86	33
<i>Prorocentrum_emarginatum</i> *	44	65	25
<i>Prorocentrum_minimum</i>	44	61	27
<i>Coolia_cf._canariensis_IM-2014</i> *	43	61	25
<i>Prorocentrum_concavum</i> *	38	54	23
<i>Amphidinium_carterae</i> *	37	56	18
<i>Gambierdiscus_toxicus</i> *	34	51	18
<i>Amphidinium_incoloratum</i> *	32	46	20
<i>Amphidinium_sp._*</i>	32	42	22
<i>Coolia_malayensis</i> *	32	25	38
<i>Amphidinium_klebsii</i>	21	30	13
<i>Amphidinium_sp._HG115</i>	20	30	10
<i>Gambierdiscus_sp._*</i>	18	32	5
<i>Gambierdiscus_sp._caribaeus</i>	13	23	3
<i>Prorocentrum_emarginatum/fukuyoi_group_EO-2011</i>	13	19	7
<i>Amphidinium_gibbosum</i>	10	14	7
<i>Gambierdiscus_australes</i>	10	19	2
<i>Prorocentrum_micans</i>	9	18	2
<i>Amphidinium_massartii</i>	9	9	8
<i>Prorocentrum_sp._Y8</i>	9	16	2
<i>Amphidinium_herdmanii</i>	5	7	3
<i>Gambierdiscus_polynesiensis</i>	4	2	7
<i>Prorocentrum_donghaiense</i>	4	5	3
<i>Prorocentrum_fukuyoi</i>	4	7	2
<i>Prorocentrum_levis</i>	4	9	0
<i>Gambierdiscus_sp._ruetzleri</i>	3	7	0
<i>Coolia_sp._</i>	3	4	2
<i>Amphidinium_operculatum</i>	2	4	0
<i>Ostreopsis_sp._</i>	2	4	0
<i>Gambierdiscus_belizeanus</i>	1	2	0
<i>Gambierdiscus_sp._SFS-2016a</i>	1	2	0

Species	Overall detection (%)	Dry detection (%)	Wet detection (%)
<i>Gambierdiscus_sp._XD-2016a</i>	1	2	0

Appendix Table 3-A3. *Ostreopsis* species composition.

Species	Reads	% of genus reads	Dry-season %	Wet-season %
<i>Ostreopsis_rhodesae</i>	2,003,657	82.82	87.4	25.9
<i>Ostreopsis_lenticularis</i>	415,707	17.18	12.6	74.1
<i>Ostreopsis_sp.</i>	19	0.00	0.0	0.0
<i>Ostreopsis_cf._siamensis_MGP-2015</i>	7	0.00	0.0	0.0
<i>Ostreopsis_spCAWD 203</i>	5	0.00	0.0	0.0
<i>Ostreopsis_cf._ovata</i>	1	0.00	0.0	0.0
<i>Ostreopsis_cf._siamensis_Dn20EHU</i>	1	0.00	0.0	0.0

Appendix Table 3-A4. *Prorocentrum* species composition.

Species	Reads	% of genus reads	Dry-season %	Wet-season %
<i>Prorocentrum_sp.</i>	354,508	90.96	90.2	93.9
<i>Prorocentrum_emarginatum</i>	11,936	3.06	3.4	1.5
<i>Prorocentrum_concavum</i>	11,191	2.87	2.8	3.0
<i>Prorocentrum_sp._SM19</i>	8,622	2.21	2.5	0.9
<i>Prorocentrum_micans</i>	1,614	0.41	0.5	0.2
<i>Prorocentrum_minimum</i>	929	0.24	0.2	0.2
<i>Prorocentrum_sp._Y8</i>	568	0.15	0.2	0.1
<i>Prorocentrum_levis</i>	137	0.04	0.0	0.0
<i>Prorocentrum_emarginatum/fukuyoi_group_EO-2011</i>	125	0.03	0.0	0.0
<i>Prorocentrum_dong</i>	58	0.01	0.0	0.0

Species	Reads	% of genus reads	Dry-season %	Wet-season %
<i>haiense</i>				
<i>Prorocentrum_fukuyoi</i>	33	0.01	0.0	0.0
<i>Prorocentrum_dentatum</i>	5	0.00	0.0	0.0
<i>Prorocentrum_elegans</i>	4	0.00	0.0	0.0
<i>Prorocentrum_bimaculatum</i>	2	0.00	0.0	0.0
<i>Prorocentrum_consutum</i>	1	0.00	0.0	0.0
<i>Prorocentrum_rhathymum</i>	1	0.00	0.0	0.0

Appendix Table 3-A5. Gambierdiscus species composition and reference-database status.

Species	Reads	% of genus reads	Dry-season %	Wet-season %
<i>Gambierdiscus_pacificus</i>	180,426	83.58	88.8	38.5
<i>Gambierdiscus_toxicus</i>	22,684	10.51	5.5	53.7
<i>Gambierdiscus_sp.</i>	7,995	3.70	3.9	2.2
<i>Gambierdiscus_sp._caribaeus</i>	2,296	1.06	0.6	5.1
<i>Gambierdiscus_austriales</i>	1,601	0.74	0.8	0.1
<i>Gambierdiscus_belizeanus</i>	640	0.30	0.3	0.0
<i>Gambierdiscus_polyneisensis</i>	126	0.06	0.0	0.4
<i>Gambierdiscus_sp._ruetzleri</i>	73	0.03	0.0	0.0
<i>Gambierdiscus_sp._XD-2016a</i>	17	0.01	0.0	0.0
<i>Gambierdiscus_sp._SFS-2016a</i>	16	0.01	0.0	0.0
<i>Gambierdiscus_cfyasumotoi</i>	3	0.00	0.0	0.0
<i>Gambierdiscus_sp._carpenteri</i>	1	0.00	0.0	0.0
<i>Gambierdiscus_yasumotoi</i>	1	0.00	0.0	0.0

Appendix Table 3-A6. Dry-season indicator species (IndVal.g, BH-adjusted $p < 0.05$).

Species	Indicator season	IndVal	Adjusted p (BH)
<i>Prorocentrum_sp._SM19</i>	Dry	0.943	0.007
<i>Gambierdiscus_pacificus</i>	Dry	0.942	0.007
<i>Ostreopsis_rhodesae</i>	Dry	0.917	0.007
<i>Prorocentrum_sp.</i>	Dry	0.898	0.007
<i>Prorocentrum_emarginatum</i>	Dry	0.817	0.007
<i>Amphidinium_sp._D2-CMSTAC021</i>	Dry	0.815	0.007
<i>Ostreopsis_lenticularis</i>	Dry	0.815	0.016
<i>Prorocentrum_minimum</i>	Dry	0.790	0.007
<i>Amphidinium_carterae</i>	Dry	0.784	0.007
<i>Coolia_cf._canariensis_IM-2014</i>	Dry	0.757	0.007
<i>Prorocentrum_concavum</i>	Dry	0.692	0.007
<i>Amphidinium_incoloratum</i>	Dry	0.673	0.007
<i>Prorocentrum_micans</i>	Dry	0.632	0.007
<i>Prorocentrum_emarginatum/fukuyoi_group_EO-2011</i>	Dry	0.609	0.007
<i>Gambierdiscus_sp.</i>	Dry	0.603	0.007
<i>Prorocentrum_sp._Y8</i>	Dry	0.588	0.012
<i>Amphidinium_sp._HG115</i>	Dry	0.580	0.007
<i>Prorocentrum_fukuyoi</i>	Dry	0.466	0.007
<i>Gambierdiscus_austerales</i>	Dry	0.457	0.012
<i>Ostreopsis_sp.</i>	Dry	0.408	0.016
<i>Amphidinium_operculatum</i>	Dry	0.373	0.030

Appendix Table 3-A7. BHAB species detected on macroalgal substrates, in reef-fish hindguts, or both (≥ 3 reads/sample).

Genus	Species	Category
<i>Amphidinium</i>	<i>Amphidinium_carterae</i>	Both

Genus	Species	Category
<i>Amphidinium</i>	<i>Amphidinium_incoloratum</i>	Both
<i>Amphidinium</i>	<i>Amphidinium_klebsii</i>	Both
<i>Amphidinium</i>	<i>Amphidinium_massartii</i>	Both
<i>Amphidinium</i>	<i>Amphidinium_sp.</i>	Both
<i>Amphidinium</i>	<i>Amphidinium_sp._D2-CMSTAC021</i>	Both
<i>Amphidinium</i>	<i>Amphidinium_sp._HG115</i>	Both
<i>Coolia</i>	<i>Coolia_cf._canariensis_IM-2014</i>	Both
<i>Coolia</i>	<i>Coolia_malayensis</i>	Both
<i>Coolia</i>	<i>Coolia_sp.</i>	Both
<i>Gambierdiscus</i>	<i>Gambierdiscus_pacificus</i>	Both
<i>Ostreopsis</i>	<i>Ostreopsis_lenticularis</i>	Both