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Consumer feces impact coral health in guild-specific ways

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Animal waste products are an important component of nutrient cycles and result in the trophic transmission of diverse microorganisms. There is growing recognition that the feces of consumers, such as predators, may impact resource species, their prey, *via* physical effects and/or microbial activity. We tested the effect of feces from distinct fish trophic groups on coral health and used heat-killed fecal controls to tease apart physical versus microbial effects of contact with fecal material. Fresh grazer/detritivore fish feces caused lesions more frequently on corals, and lesions were 4.2-fold larger than those from sterilized grazer/detritivore feces; in contrast, fresh corallivore feces did not cause more frequent or larger lesions than sterilized corallivore feces. Thus, microbial activity in grazer/detritivore feces, but not corallivore feces, was harmful to corals. Characterization of bacterial diversity in feces of 10 reef fish species, ranging from obligate corallivores to grazer/detritivores, indicated that our experimental findings may be broadly generalizable to consumer guild, since feces of some obligate corallivores contained ~2-fold higher relative abundances of coral mutualist bacteria (e.g., Endozoicomonadaceae), and lower abundances of the coral pathogen, *Vibrio coralliilyticus*, than feces of some grazer/detritivores. These findings recontextualize the ecological roles of consumers on coral reefs: although grazer/detritivores support coral reef health in various ways (e.g., promoting coral settlement and herbivory through the removal of detritus and sediments from the algal matrix), they also disperse coral pathogens. Corallivore predation can wound corals, yet their feces contain potentially beneficial coral-associated bacteria, supporting the hypothesized role of consumers, and corallivores in particular, in coral symbiont dispersal. Such consumer-mediated microbial dispersal as demonstrated here has broad implications for environmental management.

KEYWORDS

microbiome, lesion, *Vibrio*, corallivore, herbivore, grazer, detritivore, reef fish

Introduction

As consumers—including herbivores, predators and parasites—move through the environment and engage in consumer-resource interactions, they transmit microorganisms amongst the animals and plants with which they interact (Barron Pastor and Gordon, 2016; Burns et al., 2017; Ezzat et al., 2020; Vannette, 2020). This trophic transmission likely has important implications for microbiota assembly, as well as for resource species health and environmental acclimatization (Bosch and McFall-Ngai, 2011; McFall-Ngai et al., 2013; Grupstra et al., 2022; Fowler et al., 2023). Tropical coral reefs, for example, harbor diverse fishes that feed on benthic resource species, including stony corals and macroalgae (Hughes, 1994; Hughes et al., 2003; Bellwood et al., 2004). These fish defecate as they swim over the reef, generating a “persistent rain of feces”, containing high densities of live microbiota, that is deposited onto resident corals (Smriga et al., 2010). This frequent deposition of fish feces—along with the microbiota they contain—is likely to affect coral health through several potential mechanisms (Muller Parker, 1984; Ezzat et al., 2019; Ezzat et al., 2020; Umeki et al., 2020; Grupstra et al., 2021).

Corals are filter feeders that can take up nutrients from at least some fish waste products (Coma et al., 2001; Mills and Sebens, 2004; Allgeier et al., 2017); the extent and frequency that they do so from fish fecal material requires exploration. Yet, fish feces also contain particulate matter derived from reef sediments and silts (Krone et al., 2011); when sediments land on a coral colony, smothering and death of coral polyps can ensue, resulting in patches of mortality (lesions). Pathogenic or opportunistic bacteria in fish feces may also have negative effects on coral health (Aeby and Santavy, 2006; Nicolet et al., 2018; Ezzat et al., 2019; Ezzat et al., 2021; Renzi et al., 2022). For example, microbial opportunists in the feces of some grazer/detritivores can trigger lesion formation on corals (Ezzat et al., 2019; Ezzat et al., 2021). This is likely because detritus, turf-, and macroalgae, major food sources for these fishes, can contain diverse coral pathogens (Nugues et al., 2004; Dinsdale et al., 2008; Sweet et al., 2013; Franco et al., 2020) such as members of the genus *Vibrio*: *Vibrio coralliilyticus*, for example, can cause lesions or bleaching in corals (Ben-Haim et al., 2003; Vidal-Dupiol et al., 2011; Wilson et al., 2013).

Microbiota in fish feces may not always be detrimental to coral health, however. In fact, feces from corallivores might even support coral health through the delivery of probiotics—live, generally homologous microorganisms that can benefit the recipient animal—on some coral-dominated reefs (Grupstra et al., 2021; Grupstra et al., 2022). We infer this based on increased survival and growth of juvenile corals experimentally inoculated with cultured Symbiodiniaceae cells (Suzuki et al., 2013; McIlroy et al., 2016), as well as increased survival of adult corals under environmental stress that were provided with mixtures of beneficial bacteria or Symbiodiniaceae cells (Peixoto et al., 2017; Morgans et al., 2019; Rosado et al., 2019; Doering et al., 2021; Peixoto et al., 2021; Santoro et al., 2021; Thatcher et al., 2022). The feces of corallivorous fish contain high densities of potential probiotics, including live Symbiodiniaceae cells (Muller Parker, 1984; Castro-Sanguino and Sánchez, 2012; Grupstra et al., 2021)

and mutualistic coral bacteria including members of the family Endozoicomonadaceae (Clever et al., 2022). The deposition of feces on corals may thus affect colony health in nuanced ways depending on aspects of fecal composition including diversity of microbiota, nutritional value and sediment content. Studying how feces from coral reef fish in different trophic guilds, whose feces contain different microbial, nutritional and sediment compositions, affect coral health will advance coral reef ecology and inform management and conservation plans.

We tested the effect of feces from distinct consumer guilds on coral health and used heat-killed fecal controls to tease apart physical versus biological effects of contact with fecal material. We then characterized bacterial community assemblages and quantified relative abundances of the coral pathogen *V. coralliilyticus* in feces of 10 abundant consumers, ranging from obligate corallivores to grazer/detritivores, to determine whether results from the feces addition experiment are generalizable at the guild level. We tested the following hypotheses: 1) microbiota in grazer/detritivore feces, but not corallivore feces, negatively impact coral health through the development of lesions, 2) microbial communities in feces are consumer guild-specific and grazer/detritivore feces contain higher relative abundances of the coral pathogen *V. coralliilyticus*, whereas corallivore feces contain more coral-associated bacteria that are potentially beneficial.

Methods

Feces addition experimental design

A feces addition experiment was conducted three times (Final N = 11 replicates) using fragments of *Pocillopora* spp. (Johnston et al., 2018) in Moorea, French Polynesia. An initial experimental iteration was performed in October 2020 (four replicates); two additional iterations took place in June 2021 (5 replicates each). For the initial iteration, four colonies of the cryptic coral genus *Pocillopora* spp. were collected from the fore reef at ~10 m depth; these colonies were later identified to be members of the species *Pocillopora meandrina* following methods in Johnston et al. (2018). After 24 h, all corals were cut into five ~10 cm long fragments and left to acclimate for 48 hours, and any macrosymbionts (e.g., *Trapezia* crabs, shrimp) were removed. All fragments were transferred to glass jars containing 500 ml of 0.2 µm-filtered (sterile) seawater with bubblers and air stones to promote aeration and water circulation, photographed under a dissection microscope with 3.5-180X zoom (AmScope SM-1TSZZ-144S-10M). Initial dark-adapted (20 min) photosynthetic efficiency (Fv/Fm) was then measured using an imaging pulse amplitude modulation (I-PAM) fluorometer (WALZ, Germany) under the default settings.

We then blindly assigned each jar containing a coral fragment to one of the following five treatments (such that one fragment from each coral colony was assigned to each treatment, 4 replicate colonies x 5 treatments = 20 coral fragments in separate jars): fresh feces from a corallivorous butterflyfish (FC); fresh feces from a grazer/detritivore (FG); sterilized feces from a corallivorous

butterflyfish (SC); sterilized feces from a grazer/detritivore (SG); no-feces control (C). For the fresh feces treatments (FC, FG), we applied 100 μ l of fresh feces isolated from the hindgut of the butterflyfish *Chaetodon ornatissimus* (FC) or the grazer/detritivore *Ctenochaetus striatus* (FG) directly onto each coral fragment. Briefly, feces were isolated from the hindgut using sterile tools by making an incision from the anus to the pelvic fin and removing the intestinal tract; feces were then squeezed from the hindgut into sterile collection tubes using sterile tweezers and pipetted onto coral fragments using 1 ml filter tips that were modified to widen the tip opening using a sterilized razor blade. For the sterilized feces treatments (SC, SG), fecal pellets were sterilized in a pressure cooker for 40 minutes at 120 °C and then applied in the same manner as fresh feces. The fresh feces used in the experiment were subsampled for DNA extractions by preservation in DNA/RNA Shield (Zymo Research, CA). No manipulation was conducted on the no-feces control fragments. The experiment ran for ~22 hours; this treatment duration is reflective of time periods over which corals may sometimes be in direct contact with fish feces *in situ* (up to 48 hours; Ezzat et al., 2019; Ezzat et al., 2021). All fragments were photographed under the dissection microscope before and after the removal of the fecal pellet, and final Fv/Fm measurements were collected from each coral fragment using imaging-PAM fluorometry following a 20-minute dark adaptation.

Minor modifications were incorporated into the design of the second and third experimental iterations: first, all coral colonies used in the experiment were sampled before collection and their species identity was determined as *Pocillopora verrucosa* using restriction fragment length polymorphism (RFLP) analysis, as outlined in Johnston et al. (2018). For these iterations, five replicate colonies were used instead of four. Additionally, fecal treatments in these iterations were composed of feces from two individuals per fish species that were mixed prior to application (instead of using feces from a single fish individual per treatment as in the first experimental iteration).

The third iteration of the experiment ended after ~15 hours (instead of ~22 hours) because fragments (including control fragments) from three of five colonies were exhibiting signs of tissue loss consistent with stressors outside the scope of our experiment. Tissue loss in some other fragments (that were not selected for use in the experiment) of these coral colonies had started before treatments were applied to experimental fragments. Thus, all data for these three colonies (the entire set of samples—all treatments) were discarded and not included in subsequent analyses. For all subsequent analyses, replicates from all remaining iterations (11 experimental replicates) were analyzed together.

Bacterial culturing

To ensure that the sterilization method for the sterile feces treatments was effective, we streaked agar plates with subsampled material of each fecal treatment in iterations 2 and 3 of the experiment. Briefly, sterile plates (Difco™ Marine Agar 2216, 5.51%) were swabbed with a rice grain-sized mixture of fresh or

sterile feces from *C. ornatissimus* or *C. striatus*. Culture plates were monitored for bacterial growth for five days and transferred to fresh agar plates. Bacterial growth was observed in ten of ten agar plates with fresh fecal samples and one of eight agar plates with sterilized fecal samples.

Analysis of coral lesions and coral fragment photosynthetic efficiency

To test how microbial communities in fish feces affected coral health, we quantified the frequencies and sizes of coral lesions caused by fecal treatments (in addition to measuring the photosynthetic efficiency of each fragment). In brief, fecal pellets were removed from each fragment and photographs were taken using a dissection microscope. Dissection microscope photographs were analyzed in ImageJ v. 1.53f51. Each fragment was binned to one of three categories: “apparently healthy” (no change in fragment compared to before fecal application), “lesion” (fragment contains a novel patch of bare calcium carbonate, where coral tissue died and sloughed off following fecal application), or “dead” (fragment no longer contains live tissue; all tissue sloughed off following fecal application). The size of each lesion (excluding fragments binned as “apparently healthy” or “dead”) was measured by determining the average polyp size on each fragment in ImageJ by counting the number of polyps in a polygon of standardized size in triplicate. Then, the size of the lesion was measured (in pixels), and the lesion size was expressed as the estimated number of polyps that had died.

The effect of experimental treatment on the frequency of lesions and mortality in coral fragments (categories “healthy”, “lesion”, or “dead”, $N = 11$ per treatment) was tested using an ordinal regression (*sensu* Gorczynski and Beaudrot, 2021) in the package ordinal (v. 2022.11-16); colony number was included as a separate fixed effect to control for colony-level effects. Differences in lesion sizes (expressed as the estimated number of dead polyps) among treatments were tested using linear models with lmer (package vegan v 1.1-28), with colony number included as a random effect. An ANOVA in the car package (v3.0-12; Anova command) was used to calculate type II p-values. Values were log-transformed to satisfy the assumptions of the linear model. Fragments that had complete mortality ($N = 4$ total) were excluded from the analysis, as well as one replicate colony (all fragments/treatments) because a lesion in one of the treatments (SG treatment) was not entirely in the image and we were therefore unable to accurately assess its size. Zero values (*i.e.*, fragments that did not have lesions) were not included in the analysis (final N : C, 0; FC, 4; FG, 8; SC, 4; SG, 4). Pairwise comparisons between all treatments were conducted using the package emmeans (v1.7.0) with a Tukey adjustment for multiple comparisons. Model assumptions were visually checked using the check_model function in the package performance v0.9.1.

Photosynthetic efficiency (Fv/Fm) values were extracted from I-PAM fluorometry scans of each coral fragment *post hoc*. Measurements were taken following a transect design: one measurement was taken immediately adjacent to the removed

fecal pellet location, another measurement was taken halfway between the first measurement and the furthest edge away from the fecal pellet, and the final measurement was taken at the furthest edge of the fragment away from the point where feces were placed. We took the mean of these three values for each fragment. The effect of fecal pellets on untransformed Fv/Fm values were tested in the same way as lesion sizes (including colony as a random effect); p-values were generated using type II Wald chi-square tests.

Field sampling of corallivorous and grazer/detritivorous fishes, and other environmental reservoirs of coral-associated microorganisms

To test the extent to which microbial communities in fish feces—and thereby, their effects on coral health—may be broadly generalizable within fish trophic guilds, we collected additional fecal samples from each of the fish species included in the experiment (the obligate corallivore *C. ornatissimus* and the grazer/detritivore *C. striatus*), and other species in the same trophic guilds (grazer/detritivores, obligate corallivores), as well as facultative corallivores. Additionally, samples of corals, algae, sediments, and seawater were collected to test whether bacterial taxa in these samples were also represented in fish feces (N = 5–14 per fish, coral, or algae species/genus, see Table S1). All collections were conducted in October 2020 from the back reef (1–2 m depth) and fore reef (5–10 m depth) in Moorea, between LTER sites 1 and 2 of the Moorea Coral Reef (MCR) Long Term Ecological Research (LTER) site. Obligate corallivores were defined as fish that eat corals nearly exclusively (>90% of stomach content or number of bites; Harmelin-Vivien and Bouchon-Navaro, 1983; Rotjan and Lewis, 2008), and facultative corallivores were defined as fish that were observed to feed on coral for a minor to major part of their diet, while also feeding on algae or other invertebrates (~5–80% of bites from corals; Harmelin-Vivien and Bouchon-Navaro, 1983; Rotjan and Lewis, 2008; Viviani et al., 2019; Ezzat et al., 2020). The selected species included three additional obligate corallivore species (butterflyfishes *Chaetodon lunulatus*, “CHLU”; *Chaetodon reticulatus*, “CHRE”; and the filefish *Amanes scopas*, “AMSC”), three facultative corallivore species (butterflyfishes *Chaetodon citrinellus*, “CHCI”; and *Chaetodon pelewensis*, “CHPE”; and the parrotfish *Chlorurus spilurus*, “CHSP”), and two additional grazer/detritivore species (surgeonfishes *Ctenochaetus flavicauda*, “CTFL”; and *Zebrasoma scopas*, “ZESC”). Coral and algal samples were collected from locally abundant coral and algae genera (Pratchett, 2014; Burkepile et al., 2020), including the corals *Acropora hyacinthus* (“ACR”), *Pocillopora* species complex (“POC”, Gêlin et al., 2017; Johnston et al., 2018), and *Porites lobata* species complex (“POR”, Forsman et al., 2009; Forsman et al., 2015); and mixed communities of turf algae (“Turf”) as well as macroalgae in the genera *Asparagopsis* (“Asp”), *Dictyota* (“Dict”), *Lobophora* (“Lob”), *Sargassum* (“Sarg”), and *Turbinaria* sp. (“Turb”; see Table S1 for replication per species or sample category). Sediment (“SED”; 250 ml) and water (“WAT”; 1.9 L) were collected concomitantly with fish and environmental samples using

sterilized containers. Following collection, all samples (fish, coral, algae, sediments and water) were immediately transported on ice to the lab, where they were processed using sterile methods as described in Grupstra et al. (2021) and preserved in DNA/RNA Shield (Zymo Research, CA).

Sequencing and analysis of 16S rRNA gene amplicons from *in situ* samples

DNA was extracted from all samples using the ZymoBIOMICS DNA/RNA Miniprep kit (Zymo Research, CA) according to the manufacturer’s instructions, but with a 1 hr proteinase K incubation (35°C) before the lysis buffer step. Library preparation and sequencing was conducted at the Genomics Core Lab at the Institute of Arctic Biology of the University of Alaska Fairbanks. All samples were sequenced using the 16S rRNA gene V4 primers 515f (GTGYCAGCMGCCGCGGTAA) and 806rB (GGACTACNVGCGGTCTAAT) on Illumina MiSeq using v3 2x300bp chemistry (Apprill et al., 2015; Parada et al., 2016; Walters et al., 2016). Mock communities (HM-782D, BEI Resources, VA), extraction negatives, and plate negatives were included to facilitate the identification and removal of potential contaminants *in silico* (Davis et al., 2018).

Bacterial reads were processed in RStudio (version 1.1.456) through the DADA2 pipeline (version 1.11.0, Callahan et al., 2016) and using phyloseq (v1.38). The DADA2 pipeline generated a table of amplicon sequence variants (ASVs), and bacteria taxonomy was assigned using the SILVA rRNA database (version 132, Quast et al., 2012). Non-target (e.g., mitochondrial, chloroplast) reads and singletons were removed. A total of 865 potential contaminant ASVs were identified and removed with the decontam package (v. 1.10.0) using the prevalence method (Davis et al., 2018). A total of 160 samples with >1,000 reads remained after these initial quality control and filtering steps (excluding negatives and mock communities), with a mean of 15,263 reads per sample. All analyses outlined below were conducted based on this dataset, but additional ASV and sample filtering steps were included for several of the approaches (see Table S1 for replication and filtering methods).

To visualize the relationship among samples in an ordination plot, we produced a non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis distance values calculated using metaMDS (K = 3; Vegan v. 2.6–4). Because the dataset was highly diverse (24,095 total ASVs), only common ASVs (>200 reads) were included to facilitate NMDS convergence; samples with <1,000 reads after this additional ASV filtering step were removed and libraries were rarefied to 1,188 reads (NMDS N = 135; see Table S1 for sample sizes per species).

To test for differences in microbial community compositions between sampling groups, PERMANOVA tests were conducted using adonis on Bray-Curtis distances calculated from untransformed counts tables that were rarefied to 1,009 reads per sample (no low abundance ASVs were excluded, in contrast to the NMDS). However, tests of multivariate homogeneity of group dispersion (using betadisper) showed significant heterogeneity,

which can increase the probability of Type I errors while using PERMANOVA if the sampling design is unbalanced (Anderson and Walsh, 2013). Because the sampling design was unbalanced—e.g., the obligate corallivore category contained four fish species with 32 samples total, while the facultative corallivore category contained three fish species with 18 samples total (Table S1)—tests among environmental pools (obligate corallivore feces, facultative corallivore feces, grazer/detritivore feces, coral, algae, sediments and water), and among species/sample types (e.g., feces of *A. scopas* vs. feces of *C. striatus*) were conducted separately to facilitate subsampling to create a balanced design. Thus, all the categories (environmental pool or type/species) were randomly subsampled (18 random samples per environmental pool or 5 random samples per type/species; see Tables S3, S4 for sample names) prior to conducting PERMANOVA. Pairwise PERMANOVAs were conducted (with a Bonferroni correction for multiple comparisons between environmental pools, or a Benjamini-Hochberg correction for tests between species/sample types) using the package pairwise Adonis v.0.4 on these subsampled datasets.

ASVs shared between the feces of consumers and tissues of resource species (corals and algae), and sediments and water, were identified using the intersect command. To reduce noise and potential “transient” bacterial taxa, low abundance ASVs (<100 reads) were removed; samples with <1,000 reads after this additional ASV filtering step were excluded from the analysis (final N = 149; see Table S1 for replication per species), and samples were rarefied to 1,008 reads.

Lastly, we tested for differences in the relative abundances of members of the family Endozoicomonadaceae, some of which are coral mutualists (Neave et al., 2016; Neave et al., 2017a; Neave et al., 2017b; Tandon et al., 2020), among environmental pools (obligate and facultative corallivore feces, grazer/detritivore feces, corals, algae, sediment and water). All libraries were rarefied to 1,009 reads and normalized to 1. Kruskal-Wallis tests were used to test for differences in relative Endozoicomonadaceae read abundances because the residuals were not normally distributed when using linear models. Pairwise tests between environmental pools were conducted using Dunn tests in the package dunn.test v. 1.3.5. Kruskal-Wallis tests and Dunn tests were also conducted to test for differences between individual sample types/species within each environmental pool (e.g., within algae, *Turbinaria* sp. vs. turf algae).

Quantitative PCR of *Vibrio coralliilyticus* genes in fish feces

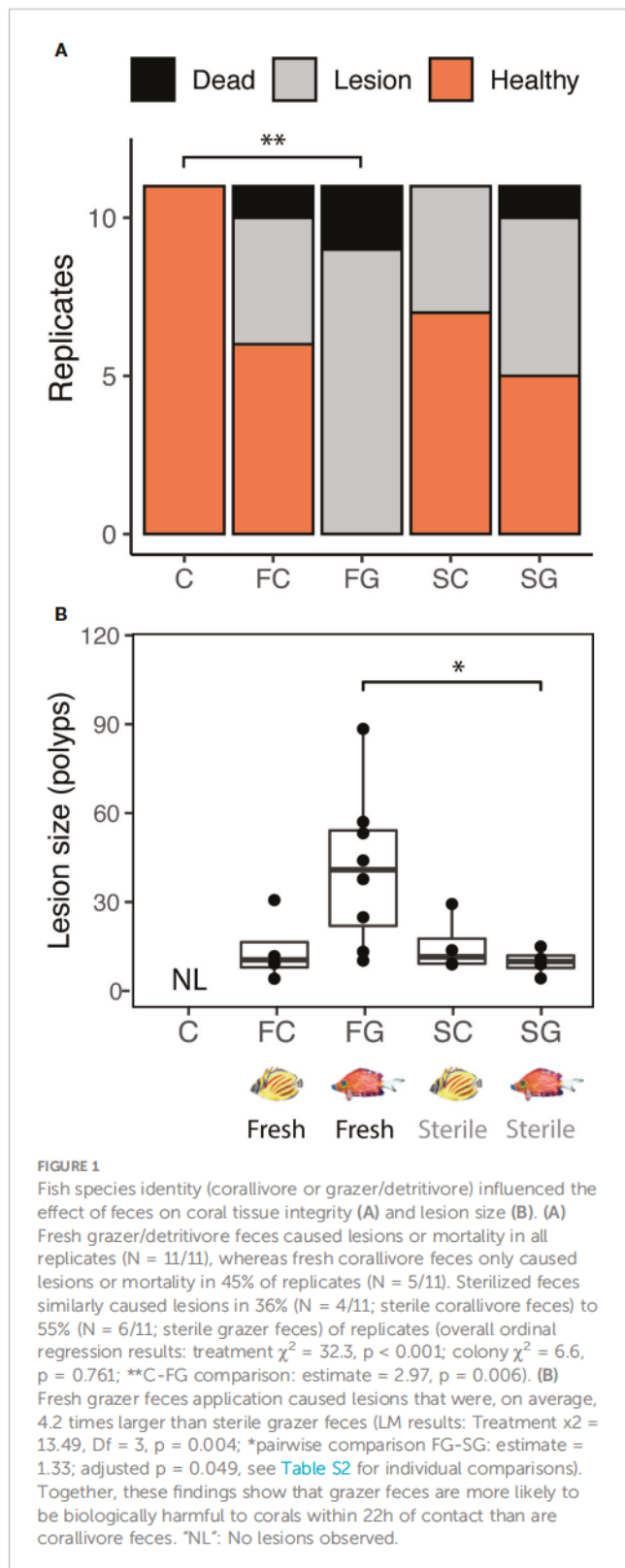
Quantitative PCR (qPCR) was conducted using vcpARTF and vcpARTR qPCR primers developed for the bacterial coral pathogen *Vibrio coralliilyticus* (Wilson et al., 2013) and results were standardized using primers for general bacteria 967F and 1046R (Sogin et al., 2006; Chen et al., 2011; Shiu et al., 2020). Standard curves (for *V. coralliilyticus* and general bacteria) were made from a *Vibrio coralliilyticus* culture (AS008) isolated from corals collected in the Flower Garden Banks National Marine Sanctuary (northwest Gulf of Mexico). Sequencing of the full-length 16S gene region of

bacterial DNA with primers 8F and 1513R resulted in 97.5% percent identity with *V. coralliilyticus* strain U2 (accession MK999891.1) with 100% query cover. The primers vcpARTR and vcpARTF were used to amplify metalloprotease genes and the amplicon was then cleaned using a genejet PCR purification kit (Thermo Fisher, MA); resultant DNA concentrations were acquired using Qubit (Thermo Fisher, MA). A standard curve was made using serial dilutions from 10^9 to 10^0 gene copies per μ l template; the standard curve for *V. coralliilyticus* primers vcpARTR and vcpARTF had an efficiency of 106.2%; the standard curve for general bacteria primers 967F and 1046R had an efficiency of 92.3%. Sanger sequencing of gene fragments amplified using vcpARTF and vcpARTR primers from DNA extracted from *C. striatus* feces resulted in a top hit against a *V. coralliilyticus* strain P4 metalloprotease gene (accession JQ345042.1) with an e-value of 3×10^{-12} (query cover 58%, percent identity 85%). Amplification of the *V. coralliilyticus* target gene at <30 cycles was counted as a positive detection, which roughly corresponded to amplification of the 10^2 gene copies μ l⁻¹ standard (Ct=30.4). Delta cycle threshold (dCT) values were calculated by subtracting the cycle threshold at which the signal from general bacteria primers was detected from the cycle threshold at which *V. coralliilyticus* was detected. Higher dCT values indicate lower relative abundances of *V. coralliilyticus*. Differences in dCT values between fecal samples of each trophic guild were tested with an ANOVA, followed by Tukey tests for multiple comparisons (using a Tukey correction). The assumptions for the test were visually assessed.

Results

Microbial activity in grazer/detritivore feces increased coral lesion frequencies and sizes

Fecal treatments resulted in the formation of lesions or complete mortality in some coral fragments, whereas other fragments remained visually healthy (Figures 1A; S1; Supplementary Data File 1; overall ordinal regression results: treatment $\chi^2 = 32.3$, $p < 0.001$; colony $\chi^2 = 6.6$, $p = 0.761$). Fresh grazer/detritivore feces resulted in the formation of lesions or complete mortality in all replicates (N = 9 and 2, respectively) and differed significantly from control (no feces) fragments (estimate = 2.97, $p = 0.006$). The fresh corallivore feces treatment resulted in lesions or total mortality in only some fragments (N = 4 and 1, respectively) while most fragments remained visually healthy (N = 6) and did not differ significantly from control fragments (estimate = 1.02 $p = 0.249$). Effects of both sterile feces treatments on fragment health also did not differ from controls (sterile corallivore estimate = 1.31, $p = 0.140$; sterile grazer estimate = 1.39, $p = 0.120$) and were comparable to those caused by fresh corallivore feces (i.e., lesions or total mortality in only some fragments; sterile corallivore: N = 4 and 0; sterile grazer: N = 5 and 1). All analyzed control fragments (no feces treatment, N = 11) remained visually healthy during the experiment. Lesion sizes caused by fecal pellets also differed significantly among treatments (Figure 1B; Df = 3, $\chi^2 = 13.49$, $p = 0.004$; Supplementary Data File 2). Pairwise



comparisons revealed that mean ($\pm$ SD) lesion sizes in the fresh grazer feces treatment (41.1 ± 25.8 polyps) were, for example, 4.2 times larger than those in the sterile grazer feces treatments (9.76 ± 4.46 polyps; estimate = 1.33; $p = 0.049$, see Table S2 for all pairwise comparisons). Lesions caused by grazer feces were also three times larger than those caused by fresh corallivore feces (13.9 ± 11.6

polyps), but this pairwise comparison was not significant, potentially due to the low number of lesions caused by fresh corallivore feces (N = 4). Lesion sizes caused by fresh corallivore feces did not differ from sterile corallivore feces (15.3 ± 9.6 polyps). Finally, coral photosynthetic efficiency was not significantly affected by the fecal treatments (Figure S2; lmer results: treatment: $Df = 4$, $\chi^2 = 9.09$, $p = 0.059$).

Bacterial communities differed between environmental pools

To determine whether the microbiota in feces of the corallivore and the grazer/detritivore fish species used in the feces addition experiment are representative of microbiota at the guild-level (and may thus have similar effects on coral health), we quantified the bacterial diversity in feces of three fish trophic guilds (four species of obligate corallivore, three species of facultative corallivore, and three species of grazer/detritivore; see Table S1 for replication; Supplementary Data Files 3–5). Bacterial communities in fish feces were also compared to those in corals, algae, sediment and water to characterize the extent to which fish feces contain the microbial taxa associated with their main food sources (all samples combined, N = 160; see Table S1 for replication in each analysis).

Bacterial communities in all environmental pools (i.e., obligate and facultative corallivore feces, grazer/detritivore feces, corals, algae, sediment and water) were significantly different from one another (Figure 2; overall PERMANOVA results: $R^2 = 0.17$, $F_{(5, 107)} = 4.1$, $p < 0.001$, pairwise PERMANOVA results: $R^2 \geq 0.08$, $F > 2.6$, Bonferroni-adjusted p -values ≤ 0.005 ; see Table S3). Bacterial communities associated with many of the individual species/sample types also differed from each (e.g., bacteria in feces from *C. ornatissimus* versus those in *Ctenochaetus flavicauda*; Figure 2; overall test results $R^2 = 0.29$, $F = 3.3$, $p < 0.05$). Out of all pairwise comparisons among species/sample types (Table S4; 210 pairwise comparisons total), most (189, 90%) were significant, including all pairwise comparisons between obligate corallivore species and grazer/detritivore species. Out of the pairwise comparisons that did not differ significantly (21, 10%), most comparisons (14/21) were among different types of algae, and the coral *Acropora hyacinthus*. Interestingly, several of the obligate and facultative corallivores did not differ significantly from each other (CHLU and CHOR, CHOR and CHRE, CHOR and CHSP; Table S4). Comparisons between two grazers and between a facultative corallivore and a grazer were also insignificant (CTST and ZESC, CHSP and CTST, respectively; Table S4).

Consumers disperse bacterial taxa associated with the resource that they consume

Feces of individual obligate corallivore species contained up to 92.1% of the distinct ASVs found in *Porites lobata* species complex (*C. reticulatus*; 223 of 242 ASVs), and up to 80.4% of ASVs found in *Pocillopora* species complex (*C. reticulatus*; 74 of 92 ASVs; Figure 3;

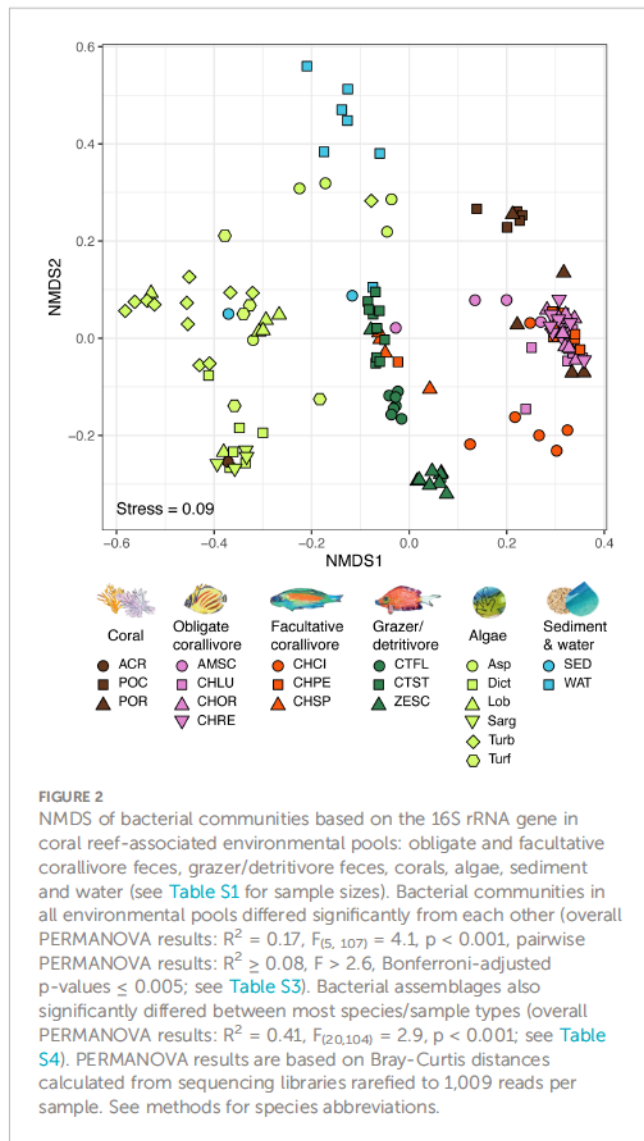


Table S5). By comparison, grazer/detritivore feces contained only up to 9.5% of distinct ASVs associated with *Porites lobata* species complex (*C. striatus*; 23 of 242) and up to 6.5% of the ASVs identified in *Pocillopora* species complex (*C. striatus*; 6 of 92). ASVs associated with *A. hyacinthus* were much less frequently identified in corallivore feces (up to 2.7%; *C. spilurus*), but these corals were relatively uncommon at our study site during the sampling period and were not frequently consumed by corallivores (Grupstra et al., 2021).

Grazer/detritivore feces contained high representation of ASVs that were associated with different types of algae (Figure 3; Table S5). For example, 24.0% of turf algae ASVs were identified in feces of *C. striatus* (87 of 363 ASVs), whereas only up to 3.6% of turf algae ASVs were found in obligate corallivore feces (*A. scopas*; 13 of 363 ASVs). In addition, feces of the grazer/detritivores *C. flavicauda* and *C. striatus* contained higher numbers of the ASVs associated with all other categories of algae (up to 21.4%) than did feces of obligate (up to 5.0%) or facultative corallivores (up to 12.9%; Figure 3; Table S5). Feces of these species also contained more of the ASVs found in sediment and water samples (*C. striatus*; 25.4%, 53 of 209 ASVs and 29.3%, 95 of 324 ASVs), than did other trophic guilds (but note that the obligate corallivore *A. scopas* and the facultative corallivore *C. spilurus* also had high representation of ASVs found in sediment and water). Interestingly, while feces from the grazer/detritivore *Z. scopas* also contained more ASVs associated with algae (1.8–6.6%) than feces of the obligate corallivores (0.0–3.8%), the representation of algal ASVs in *Z. scopas* feces was noticeably lower than in feces of the other grazer/detritivores (4.6–16.8% for *C. flavicauda*; 6.5–24.0% for *C. striatus*). In fact, *Z. scopas* appeared more similar to two of the sampled facultative corallivores (*C. pelewensis*: 2.3–12.9% and *C. spilurus*: 2.7–9.1%; see Figure 3; Table S5) than to grazer/detritivores in terms of the ASVs it shared with algae.

Facultative corallivore feces contained a mix of ASVs associated with corals and algae. For example, *C. pelewensis* contained 88.8% of the ASVs found in *Porites lobata* species complex (215 of 242 ASVs;

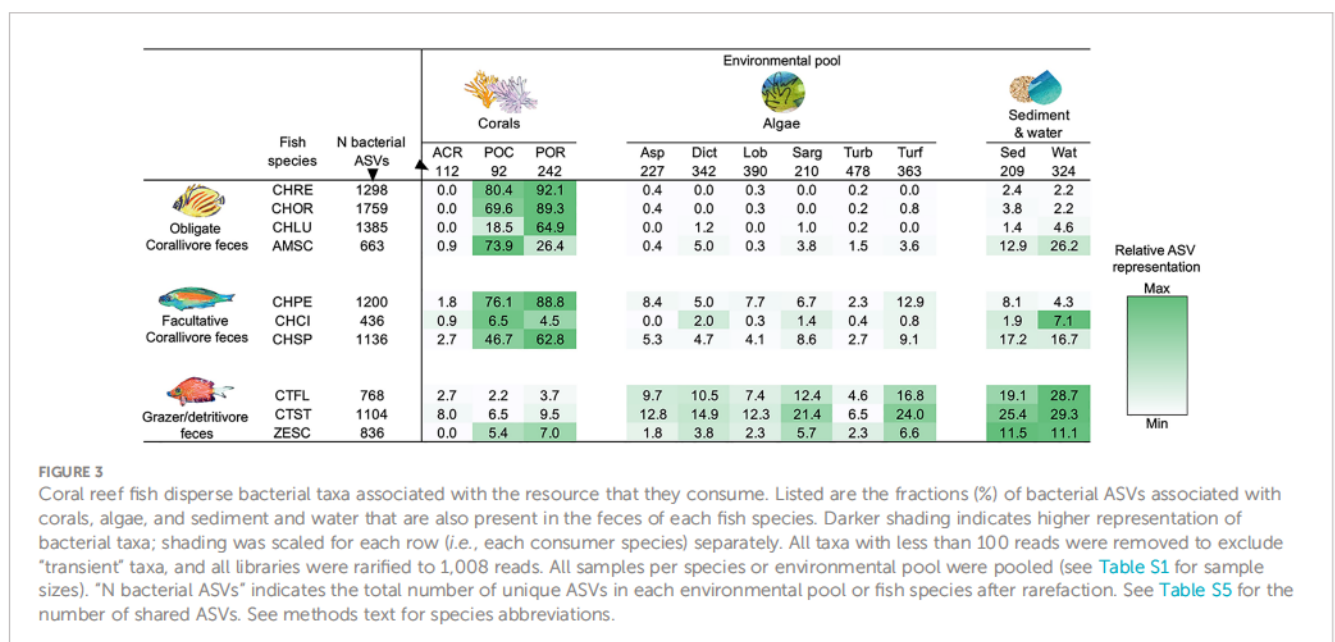


Figure 3; Table S5), as well as up to 12.9% of the ASVs associated with algal samples (47 of 363 ASVs associated with turf algae). Interestingly, feces of the facultative corallivore *C. citrinellus* contained fewer of the ASVs associated with corals or algae than other members of its guild; fecal samples of this species contained only 0.9–6.5% of ASVs associated with coral samples and 0.0–2.0% of ASVs associated with algae.

Relative abundances of the coral mutualist Endozoicomonadaceae were higher in feces of obligate corallivores than grazer/detritivores

Relative abundances of Endozoicomonadaceae reads in amplicon sequencing libraries significantly differed among environmental pools (Figures 4, S3; Kruskal-Wallis test, $df = 5$, $\chi^2 = 96.2$, $p < 0.001$). Pairwise tests demonstrated that mean ($\pm$ SE) relative abundances of Endozoicomonadaceae reads were significantly higher ($|Z| > 2.8$, adjusted p -values < 0.05 , see Table S6) in corals ($44.3 \pm 7.5\%$), obligate corallivore feces ($24.4 \pm 1.7\%$), and facultative corallivore feces ($26.8 \pm 2.6\%$) than in grazer feces ($11.5 \pm 0.9\%$), algae ($3.7 \pm 0.4\%$), or sediment and water ($6.0 \pm 1.3\%$). Relative Endozoicomonadaceae read abundances were also higher in feces of the obligate corallivore *C. ornatissimus* (24.7 ± 1.8) than the grazer/detritivore *C. striatus* (10.2 ± 1.5), which were the two species used for the feces addition experiment (Kruskal-Wallis test results: $\chi^2 = 15.5$,

$df = 1$, $p < 0.001$). Relative read abundances of Endozoicomonadaceae further differed among species/sample types within environmental pools or trophic guilds (Figure S3; Table S7). For example, relative abundances of Endozoicomonadaceae reads in feces differed among obligate corallivore species; they were lower in feces of *A. scopas* than in feces of any other obligate corallivores sampled (Kruskal-Wallis test results: $\chi^2 19.26$, $df = 3$, $p < 0.001$; pairwise Dunn test results $|Z| > 2.0$, $p < 0.05$; see Table S7).

Vibrio coralliilyticus relative abundances were higher in feces of grazer/detritivores than in corallivore feces

The *Vibrio coralliilyticus* metalloprotease gene was amplified using qPCR of DNA extracted from feces of each sampled fish species (Figure 5). Using this method, *V. coralliilyticus* genes were identified ($Ct < 30$; see Methods) in 54% ($N = 20/37$) of obligate corallivore feces, 50% of facultative corallivore feces ($N = 9/18$), and in 65% ($N = 20/31$) of grazer/detritivore feces. Relative abundances of *V. coralliilyticus* genes differed between fish guilds (ANOVA: $F_{(2,45)} = 16.14$, $p < 0.001$; Supplementary Data File 6). A Tukey *post-hoc* test revealed that relative abundances of *V. coralliilyticus* genes were significantly higher in grazer/detritivore feces than in obligate corallivore feces (Figure 5; difference = 4.61, $p < 0.001$) and facultative corallivore feces (Figure 5; difference = 2.44, $p = 0.046$). Relative *V. coralliilyticus* abundances did not

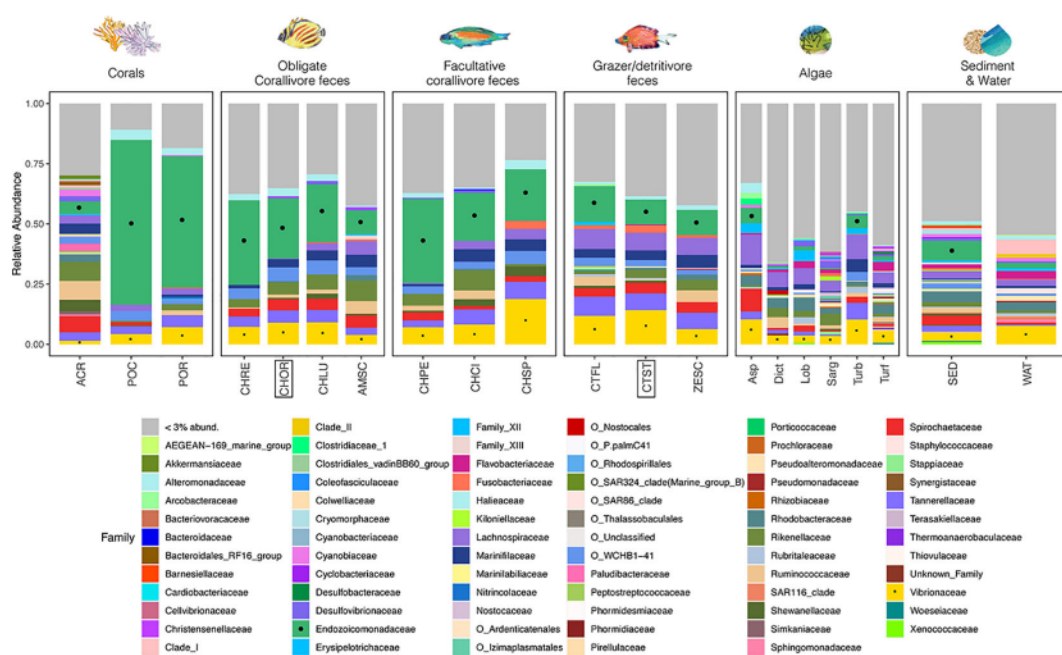
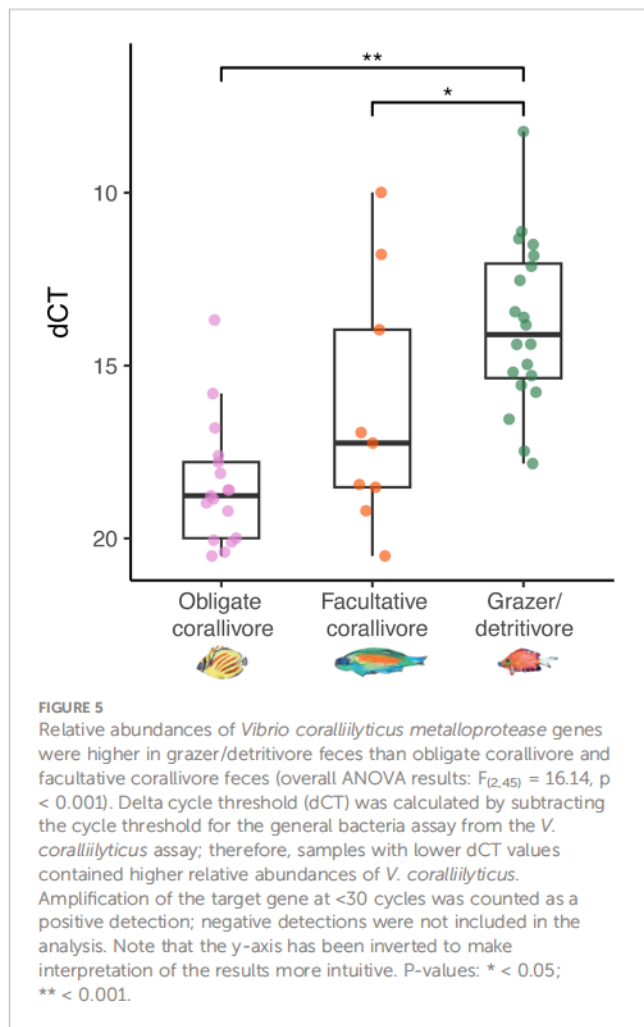


FIGURE 4

A stacked bar plot illustrates that relative abundances of bacterial families differ among environmental pools, consumer trophic guilds, and species (see Table S1 for sample sizes). Notable families include Endozoicomonadaceae (green, marked with black circles where possible), which are coral mutualists and (in this study) are abundant in the feces of obligate and facultative corallivores (see Figure S3; Tables S6, S7 for pairwise comparisons of relative abundances). The family Vibrionaceae (yellow, marked with black squares), which includes some notable coral pathogens, are present in all sample types. Fish species included in the feces addition experiment (CHOR, CTST; Figure 1) are indicated with boxes. Bacterial families that represent $< 3\%$ of total reads were collapsed (" $< 3\%$ abund."). Taxa that are not classified at the family level are listed with their order names (indicated with "O_").



significantly differ between obligate and facultative corallivores (difference = 2.18, $p = 0.09$).

Discussion

The production of waste products is ubiquitous across the animal kingdom; this waste is a key component of nutrient cycles (Roman and McCarthy, 2010; Le Mézo et al., 2022) as well as an important way in which microbiota are transported throughout environments (Grupstra et al., 2022). Animal diets determine the composition of waste products, including their associated microbiota, and thus influence how the waste may affect other organisms in the environment (Barron Pastor and Gordon, 2016; Reese and Dunn, 2018; Clever et al., 2022). Testing how feces derived from animal species in diverse trophic guilds affect cohabitating organisms can inform agriculture, ecosystem management, and restoration. Here, we demonstrate that feces from distinct trophic guilds of coral reef fish—grazer/detritivores and corallivores—affect the health of reef-building corals in distinct ways. We demonstrated that microbiota in grazer/detritivore, but not corallivore, feces are detrimental to coral health. Subsequent characterization of bacterial diversity in grazer/detritivore and corallivore feces indicated that these communities were trophic

guild-specific, suggesting that results from the fecal addition experiment presented here are generalizable to trophic guild. Together, these findings highlight an underexplored, indirect result of consumers on resource species, which has potentially important ramifications for ecosystem health and functioning.

Microbiota in feces of grazer/detritivore fish, but not corallivorous fish, are detrimental to coral health

Intact coral reef ecosystems are characterized by high abundances of diverse fishes that release feces, containing up to 2.6×10^{11} bacterial cells g^{-1} (dry weight), as they move through their territories (Hughes, 1994; Hughes et al., 2003; Bellwood et al., 2004; Smriga et al., 2010). This frequent deposition of feces is likely to affect coral microbiota assembly, and thereby, colony health. The feces addition experiment presented here demonstrated that fresh feces from the grazer/detritivore *C. striatus* caused detrimental effects to coral health (higher frequency of lesions and larger lesions) compared to no-feces controls or sterilized feces from the same species (Figures 1B, 2; Table S2); this suggests that microbial activity in fresh grazer/detritivore feces is driving observed detrimental effects to coral health. This may be caused by higher relative abundances of pathogens like *V. coralliilyticus* in *C. striatus* feces, or the presence of other potential pathogens that may be associated with the algae that these fish feed on (Figures 3–5; Table S5; Nugues et al., 2004; Dinsdale et al., 2008; Sweet et al., 2013). By comparison, fresh feces from the obligate corallivore *C. ornatus* did not cause significantly larger lesions on corals than sterilized feces from the same species, suggesting that the microbiota in feces of this species were not detrimental to coral health (Figures 1, 2).

To test whether the findings from the feces addition experiment are broadly generalizable, we compared the bacterial assemblages in feces from 10 fish species in three trophic guilds: obligate corallivores, facultative corallivores, and grazer/detritivores. Bacterial communities significantly differed between trophic guilds, as well as between individual species within trophic guilds (Figures 2, 4; Tables S3, S4). While grazer/detritivore feces contained more bacterial taxa associated with algae (Figure 3; Table S5), most corallivore feces contained a higher percentage of coral-associated bacterial ASVs (Figure 3; Table S5) and higher relative abundances of the coral mutualist family Endozoicomonadaceae (Figures 4, S3; Tables S6, S7). Notably, grazer/detritivore feces also contained higher abundances of *V. coralliilyticus* (Figure 5), which is a known coral pathogen (Ben-Haim et al., 2003). Together, these lines of evidence strongly suggest that bacterial assemblages in fish feces are an important determinant of how feces affect coral health, and that feces from different fish trophic guilds are likely to impact coral health in distinct ways.

Herbivorous fishes support coral dominance through the removal of algal competitors, thereby contributing to coral reef health and resilience (Hughes et al., 2003; Littler et al., 2006; Burkepile and Hay, 2009; Littler et al., 2009). While the grazer/detritivores and facultative corallivores studied here (with the exception of *C. spilurus*) are not recognized as removing functionally important algae that compete for space and resources

with corals, some grazer/detritivores (including *C. striatus*) graze on early successional turf algae and may thereby promote post-disturbance recovery of reefs (Marshall and Mumby, 2012; Tebbett et al., 2017a; Nalley et al., 2022). Additionally, some grazer/detritivores remove sediments from dense late successional algal turf assemblages (Goatley and Bellwood, 2010; Perry et al., 2022). This process may be vital to reef health because sediments in the algal matrix can inhibit coral recruitment (Birrell et al., 2005; Fabricius, 2005). Removal of sediments from algal turfs also increases the palatability of the algae for other herbivores, increasing top-down control on algal communities (Goatley and Bellwood, 2010; Tebbett et al., 2017a; Tebbett et al., 2017b; Tebbett et al., 2018). While grazer/detritivores and other herbivores are thus functionally important, our research (as well as two previous studies, Ezzat et al., 2019; Ezzat et al., 2021) illuminates an additional dimension of this relationship: grazer/detritivores can disperse pathogens in their feces, which can cause lesions in coral tissues. More work is needed to test the extent to which different (herbivorous) fish species contribute to the dispersal of viable coral pathogens (Grupstra et al., 2022).

Biotic and abiotic factors mediating coral-feces interaction frequencies and outcomes

Corals may acquire nutrients (Coma et al., 2001; Mills and Sebens, 2004; Allgeier et al., 2017; Shantz et al., 2023) and beneficial or harmful microbiota from fish feces (Muller Parker, 1984; Castro-Sanguino and Sánchez, 2012; Ezzat et al., 2019; Umeki et al., 2020; Ezzat et al., 2021; Grupstra et al., 2021; Velásquez-Rodríguez et al., 2021). As shown here (sterile feces treatments in Figure 1), particulate matter in fish feces may also sometimes smother coral polyps, resulting in mortality of the underlying coral tissue (Rogers, 1990; Krone et al., 2011). The frequency and nature of interactions between corals and fish feces are additionally influenced by biotic and abiotic factors. Examples of potentially relevant biotic factors include fish species' range sizes and spatiotemporal foraging and defecation patterns (Shantz et al., 2015). Relevant abiotic factors include water flow and wave action (Wisniski and Lennon, 2022), which can move fecal pellets onto or away from corals and may affect the consistency and integrity of fecal pellets.

The distribution of feces by fish varies based on species- and individual-level traits (e.g., territoriality, defecation frequency). Many reef fish are territorial, and the area over which these fish disperse feces is influenced by factors affecting territory size, including species identity, location, depth, and competition with other fish (Wrathall et al., 1992; Cox, 1994; Pratchett et al., 2014). For example, many of the corallivores examined here (obligate corallivores: *C. lunulatus*, *C. ornatus*, *C. reticulatus*; facultative corallivores: *C. pelewensis*, *C. citrinellus*) are butterflyfishes, many of which live in mating pairs and maintain and defend territories that are often small in size (50 m² or larger) but can range up to 1,000 m² depending on the species and location (Wrathall et al., 1992; Cox, 1994; Pratchett et al., 2014). Other butterflyfishes, including *C. lunulatus*, are less restricted in their movements and may travel between and through territories of

other fish (Pratchett, 2005). Mature individuals of the facultative corallivorous parrotfish *C. spilurus* are solitary and also territorial, but their territories are generally larger (mean territory size ~250m² in Moorea; Davis et al., 2017). Home ranges for grazer/detritivores vary strongly by species; *C. striatus* territories can be small (8–16 m²; Krone et al., 2008; Goatley and Bellwood, 2010), whereas territories of some *Zebrasoma* species can be large (up to 0.6 km²; Claisse et al., 2009; Giffin et al., 2019).

Defecation frequencies and patterns also differ among species. Three of the butterflyfish species in this study were observed to defecate around once per hour (every 45–60 minutes; Grupstra et al., 2021), whereas parrotfishes and some grazer/detritivores may defecate more than four times per hour (Goatley and Bellwood, 2010; Grupstra et al., unpublished data). Latrine use also likely affects the distribution of fish feces (Polunin and Koike, 1987; Nicholson and Sikkil, 2018). For example, *C. striatus* individuals have been observed to defecate in small sandy areas at the edge of their territories (Krone et al., 2008; Goatley and Bellwood, 2010). They have also been documented to defecate on coral heads at our study site (Ezzat et al., 2019; Grupstra et al., unpublished data), increasing the likelihood of interactions between corals and fish feces. Studies integrating fish species-specific information on fecal qualities and their influence on coral health with spatiotemporal patterns of defecation are needed.

Although it has received relatively little research attention, coprophagy by fish appears common on coral reefs and may reduce the frequency of interactions between corals and fish feces. Reports from the Pacific and Caribbean suggest that 0–92% of the feces released by some abundant reef fish may be eaten by other fish (Bailey and Robertson, 1982; Robertson, 1982; Rempel et al., 2022). The probability that a given fecal pellet will be consumed relates to its nutritional content; protein and lipid-rich feces from some zooplanktivores and other invertebrate feeders (including some obligate corallivores) may be consumed at higher rates than carbohydrate-rich feces from herbivores (Bailey and Robertson, 1982; Rempel et al., 2022). Despite high reported levels of coprophagy from some reef regions, recent observations from Moorea suggest that some (partial) segments of fish feces landed on coral heads in ~91% (20 of 22) of observed defecations (Grupstra et al., 2021). Additional species-specific efforts to quantify coprophagy will improve our understanding of the frequencies at which fish feces come into contact with corals.

The physical integrity of fecal pellets also likely influences the outcome of interactions between corals and fish feces. In our fecal addition experiment, feces placed on coral fragments mimicked intact feces, yet *in situ*, fecal pellets sometimes fall apart in the water column (Grupstra et al., 2021), releasing silt to rice grain-sized particles. Such particle sizes may be less likely to smother coral polyps, and might be more readily removed or ingested by corals (Hankins et al., 2018; Grillo et al., 2021); this may be especially the case in areas with high water current or wave action, where fecal pellets are more likely to disintegrate. In our experiment, we did not explicitly examine whether coral polyps consumed parts of fecal pellets. Future studies of coprophagy behavior by *Pocillopora* spp., and corals in general, are needed to further our understanding of how contact with consumer feces influences coral health.

Finally, many branching corals harbor diverse macrosymbionts including brittle stars, Christmas tree worms, crabs, and small fish (e.g., coral gobies) that can promote coral health (Stier et al., 2012; Doo et al., 2018; Moeller et al., 2023), and these organisms may interact with fish feces that land on coral heads. We observed *Trapezia* crabs, common macrosymbionts of branching corals, breaking up and consuming corallivore feces applied to coral fragments *in situ* as well as in the lab (Grupstra et al. personal observation, but note that *Trapezia* crabs were removed from coral fragments in this experiment). Through such activity, macrosymbionts may in some cases prevent lesions from forming when feces fall directly on coral surfaces. Empirical tests of the extent to which fecal particle size and macrosymbiont activity influence the outcomes of fish fecal contact with corals are needed.

Corallivores promote the dispersal of coral-associated microbiota

Through the dispersal of microbiota, consumer activities affect the assembly of microbiota in resource species (Vašutová et al., 2019; Ezzat et al., 2020; Vannette, 2020; Zhu et al., 2021; Grupstra et al., 2022; Fowler et al., 2023). Here, we show that feces from reef fish contain a high diversity of bacterial taxa (ASVs) that are associated with their food sources. For instance, obligate corallivore feces contained up to 92.1% of the bacterial ASVs found in a locally abundant coral species (*Porites lobata* species complex; Figure 3; Table S5). We therefore posit that the presence and activity of corallivores promotes the dispersal of bacterial taxa associated with corals. Based on experimental demonstration of the impact of microbial activity on coral health (this study; Ezzat et al., 2019; Ezzat et al., 2021), we infer that at least some bacteria in fish feces are viable. Empirical tests of this (e.g., through culturing, or membrane permeability-based stains and cell sorting or viability PCR; Grupstra et al., 2022) will help reveal the extent to which passage through fish digestive tracts affects the viability of microbiota dispersed in the feces of reef fish.

While some corals provide their offspring with key bacteria (Sharp et al., 2012; Leite et al., 2017; Zhou et al., 2017), most corals acquire at least some, or most, microbial taxa *de novo* each generation from the environment (Sharp et al., 2010; Apprill et al., 2012; Epstein et al., 2019; Strader et al., 2022). In many coral species, juveniles may take up diverse microbial taxa from environmental pools, followed by a winnowing process in which microbial assemblages are “refined” (Apprill et al., 2009; Littman et al., 2009; Lema et al., 2014; Damjanovic et al., 2019; Epstein et al., 2019; Van Oppen and Blackall, 2019). Corallivore feces may represent hotspots of homologous bacteria and facilitate the acquisition of locally beneficial microbiota by corals and other hosts. While the transfer of beneficial bacteria between coral colonies *via* fish feces has not been demonstrated, earlier studies have shown that anemones in the genus *Aiptasia*—which are closely related to corals—can take up Symbiodiniaceae cells from the feces of obligate corallivorous butterflyfishes (Muller Parker, 1984); coral juveniles have also been demonstrated to initiate symbiotic partnerships with Symbiodiniaceae cells from the feces of giant

clams (Umeki et al., 2020). Hence, it is likely that juvenile corals can acquire beneficial bacteria from fish feces as well.

We hypothesize that the dispersal of coral-associated bacteria by corallivores is beneficial to stressed adult coral colonies in some cases. Environmental stress can result in disruption of the symbiosis between coral animals and their populations of microorganisms (Ziegler et al., 2016; Grottoli et al., 2018; but see Pogoreutz et al., 2018; Ziegler et al., 2019), yet experimental inoculations with engineered “probiotic” cocktails consisting of cultured coral-derived bacteria and Symbiodiniaceae have increased photosynthetic efficiency and colony health in thermally stressed corals within hours to days after inoculation, potentially by providing nutrition, mitigating toxins and/or deterring pathogens (Peixoto et al., 2017; Morgans et al., 2019; Rosado et al., 2019; Peixoto et al., 2021; Santoro et al., 2021). For example, inoculations with microbiota from heat tolerant coral colonies were shown to reduce bleaching rates in recipient heat-susceptible colonies of the coral species *Pocillopora* sp. and *Porites* sp. in Thailand (Doering et al., 2021). Given that corallivore feces contained many of the bacterial ASVs associated with locally abundant corals in this study, and previous work has shown that such feces contain high densities of live coral-associated Symbiodiniaceae cells (Muller Parker, 1984; Castro-Sanguino and Sánchez, 2012; Grupstra et al., 2021), microbiota in corallivore feces may in some cases be expected to have a “stabilizing” effect on the partnership between corals and their microbiomes. This is especially likely given reports that some corallivorous fish preferentially feed on heat tolerant corals during bleaching events (Cole et al., 2009; MacDonald et al., 2021; Rotjan et al., 2022), thereby potentially transferring microbiota associated with stress-resistant colonies to surrounding stress-susceptible colonies. While this experiment did not generate evidence that microbiota in corallivore feces improved coral health (e.g., photosynthetic efficiency was not increased by corallivore feces addition; Figure S2), additional empirical studies are needed to comprehensively test this hypothesis under varied conditions (e.g., size and integrity of fecal pellets, water flow rate, presence/absence of ectosymbionts), as well as with experimentally stressed (e.g., bleaching, diseased) coral colonies.

Conclusions

Our results indicate that microbiota in the feces of grazer/detritivores, but not corallivores, have negative effects on coral health when they come in direct contact with colonies. Feces from grazer/detritivore fish contain higher proportions of bacterial ASVs associated with locally abundant algae, as well as higher relative abundances of some coral pathogens, than feces from corallivores. These findings add a new dimension to our understanding of how fish trophic guilds influence coral health: although grazer/detritivores may support coral dominance in various ways (e.g., promoting coral recruitment, as well as herbivory), they also disperse pathogens that can harm coral health. Corallivore predation can wound corals, yet corallivores also disperse bacteria in their feces that may be beneficial to corals. Future studies are needed to test how consumers in additional fish trophic guilds affect coral health, as well as to disentangle how global stressors impact

the outcomes of interactions between corals and fish feces. More broadly, studying how different trophic guilds of consumers contribute to the dispersal of microbiota can aid ecosystem management and conservation, for example through the identification of consumer trophic guilds that promote or harm resource species in different environmental contexts.

Data availability statement

Sequence reads have been uploaded to NCBI's sequence read archive (SRA) under BioProject PRJNA935035; all other data have been included as supplementary data files. R code to replicate the analyses has been deposited on GitHub: <https://github.com/CorreaLab/graco>.

Ethics statement

The animal study was reviewed and approved by The institutional animal care and use committee (IACUC) at Rice University [IACUC-21-019].

Author contributions

CG and AC designed the experiment, with input from AV, LH-K, and SC; all authors contributed to data collection; CG and JM processed samples; CG led data analysis, with contributions from all authors; CG wrote the first draft of the manuscript, with contributions by all authors; AC and CG contributed funds for the study. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2023.1110346/full#supplementary-material>

SUPPLEMENTARY DATA FILES 1 AND 2

Data pertaining to the lesion frequencies and sizes, respectively (Figures 1A, B).

SUPPLEMENTARY DATA FILES 3–5

A sample data table, an ASV table and a taxonomy table, respectively, from the 16S V4 rDNA amplicon sequencing analysis (Figures 2–4) that can be combined into a phyloseq object. Provided data are post quality control and filtering of potential contaminant sequences.

SUPPLEMENTARY DATA FILE 6

qPCR data (Figure 5).

SUPPLEMENTARY DATA FILE 7

All supplementary tables and figures.

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