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Marine Heatwaves, Ocean Warming and Acidification Reshape Reef Fish Gut Microbiomes

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ABSTRACT

Extreme climatic events and gradual climate change are increasingly anticipated to interact and reshape ecological communities. However, the combined effects of ocean warming, acidification and marine heatwaves on host-associated microbial communities and their potential role in host adaptation remain poorly understood. Here, we assessed shifts in gut microbiome communities and their associations with physiological performance in one tropical (*Abudefduf vaigiensis*) and one subtropical (*Microcanthus strigatus*) reef fish species, across three temperate reefs representing natural analogues of climate change: a present-day baseline ('cool reef'), a chronically warmed reef ('warm reef') and a reef experiencing combined warming and extreme acidification ('extreme reef'). We also examined gut microbiome changes in *A. vaigiensis* before and during a severe marine heatwave. *A. vaigiensis* had lower gut microbiome evenness and diversity at the warm (43% and 44% decrease, respectively) and extreme (38% and 31% decrease) reefs compared to the cool reef, and its gut microbiome community shifted at the extreme reef with a 122% increase in abundance of opportunistic bacteria *Vibrio*. *A. vaigiensis* also had lower gut microbiome richness at the warm (42% decrease) and extreme (52% decrease) reefs during the heatwave compared to pre-heatwave individuals. In contrast, *M. strigatus* showed higher microbiome evenness (99% increase) and diversity (98% increase) at the warm reef compared to the cool reef; however, these gains were lost at the extreme reef, with microbiome diversity and evenness returning to cool reef levels. Microbiome changes in both species were generally not associated with their physiological performance (protein content, oxidative stress, antioxidant capacity or body condition). Our findings suggest that marine heatwaves, ocean warming and acidification can reshape reef fish gut microbiomes, driving simplification in *Abudefduf vaigiensis* but distinct restructuring in *Microcanthus strigatus*. We conclude that climate-driven microbiome reshuffling may alter host–microbiome relationships and functions in fishes in a future ocean.

Angus Mitchell and Chloe Hayes should be considered equal first authors.

1 | Introduction

Anthropogenic climate change is facilitating the rapid transformation of marine ecosystems, with ocean warming, acidification and extreme climatic events such as marine heatwaves emerging as primary threats to marine life (Pecl et al. 2017; Smale et al. 2019; IPCC 2021). The effects of climate change on marine ecosystems is set to worsen (García Molinos et al. 2016) and may accelerate the rate of species extinctions in the near future (Urban 2015). Rising ocean temperatures have already altered species redistributions (Pecl et al. 2017; García Molinos et al. 2022), driven ecological phase shifts (Vergés et al. 2014) and reshaped ecosystem functioning (Parmesan and Yohe 2003; Doney et al. 2012), while marine heatwave events have triggered mass mortality events (Cheung et al. 2021; Oliver et al. 2021). Concurrently, ocean acidification is projected to reshuffle and simplify food web architecture, reshape trophic interactions and alter resource availability (Nagekerken et al. 2020; Agostini et al. 2021; Nagelkerken and Connell 2022). While important research has focused on the physiological and ecological consequences of climate change stressors, the role of host-associated microbiomes remains poorly understood, despite its potential to influence species resilience and persistence in future oceans.

Microbiome communities play a fundamental role in host metabolism (Dvergedal et al. 2020), immunity (Gerardo et al. 2020), fitness (Gould et al. 2018), behaviour (Ezenwa et al. 2012) and stress resilience (Sommer et al. 2017; Egerton et al. 2018). Gut microbiome assemblages are shaped by host diet (Miyake et al. 2014), environmental conditions (Clever et al. 2022; Risely et al. 2023; Suzzi et al. 2023; Hayes et al. 2025) and species-specific host–microbiome interactions with the environment (Gould et al. 2018). However, when these influencing factors are disrupted, gut microbiomes enter a state of dysbiosis where an imbalance in the mutualistic relationship between hosts and microbes causes reduced microbial diversity and increases the prevalence of opportunistic or pathogenic taxa (Petersen and Round 2014). While hosts may acclimatise within generations (Donelson et al. 2011) or adapt across generations to climate change (Veilleux et al. 2015), their associated microbiomes could respond more rapidly (over minute to hour timeframes; Kolodny and Schulenburg 2020), potentially facilitating acclimatisation and contributing to adaptation over evolutionary timescales (Jones et al. 2018; Kolodny and Schulenburg 2020; Peterson et al. 2023). Microbiome plasticity has been proposed as a key mechanism for buffering environmental stress (Kolodny and Schulenburg 2020), with dynamic shifts in microbial composition and function potentially enhancing host resilience under climate change (Voolstra and Ziegler 2020). While plasticity of microbiomes may serve as a plastic response to climate change, it remains seldom addressed whether microbial communities mediate host resilience or destabilise instead under climate stress, leading to dysbiosis and increased host vulnerability to climate change.

Climate change is anticipated to reorganise host-associated microbiome communities (Greenspan et al. 2020; Messer et al. 2020). Because gut microbiomes play a central role in modulating host metabolism, immunity and stress tolerance, they have the potential to facilitate resilience to environmental change; however, these same microbial communities are themselves sensitive to climate-driven stressors (Li et al. 2022; Steiner et al. 2022). For example, extreme temperatures and cold stress can reduce microbial diversity (Strano et al. 2023; Bell et al. 2024; Vompe et al. 2024), leading to dysbiosis (Greenspan et al. 2020; Suzzi et al. 2023; Hayes et al. 2025), lowered functional diversity (Bell et al. 2024) and increased susceptibility to disease and environmental stressors (Brown et al. 2012; Lokesh and Kiron 2016). Ocean acidification can also promote opportunistic and pathogenic bacteria while decreasing overall microbial diversity (Scanes et al. 2021) and functioning (Botté et al. 2019). By contrast, moderate ocean warming may increase microbiome diversity when temperatures approach host species thermal optima (Bestion et al. 2017; Li et al. 2022; Steiner et al. 2022). The projected intensification (Cheung et al. 2021) and potential synergies between ocean warming, acidification and extreme heat events could interact to alter microbiomes further (Castro et al. 2024; Huerlimann et al. 2025) and increase host susceptibility to disease (Suzzi et al. 2023). While emerging research shows that ocean warming can restructure gut microbiomes in marine fishes (Li et al. 2022; Hayes et al. 2025), little is known about how multiple climate stressors interactively shape microbiome diversity in reef fishes. Understanding how ocean acidification, warming and heatwaves interact to shape microbial communities in reef fishes is required to predict host adaptation and resilience in a future ocean.

Here, we investigated the interactive effects of ocean warming, acidification and a severe marine heatwave on gut microbiome diversity, variability and composition in two common reef fish species, the tropical Indo-Pacific Sergeant Major (*Abudefduf vaigiensis*) and the subtropical Stripey (*Microcanthus strigatus*), across three natural climate change analogues: (1) a cool reef representing present-day temperate conditions; (2) a warm reef simulating moderate ocean warming; and (3) an extreme reef simulating ocean warming combined with extreme acidification. In addition, we assessed the impact of a widespread severe marine heatwave on *A. vaigiensis* to determine whether rapid temperature anomalies further altered gut microbial communities at reefs already exposed to chronic warming and acidification. Our study focused on assessing the net ecological effects of climate change on fish gut microbiomes by capturing both the direct impacts of ocean warming and acidification and the indirect effects, such as potential shifts in local resource availability that may influence gut microbial communities.

We hypothesised that: (Hypothesis 1) moderate ocean warming (+1°C) at the warm reef relative to the cool reef would increase gut microbiome diversity as temperatures approach both fish species thermal optima; (Hypothesis 2) at the extreme reef, where fishes experience chronic warming and extreme acidification, gut microbial diversity would decline and opportunistic taxa would increase relative to the cool and warm reefs, leading to homogenised microbial communities across individuals; and (Hypothesis 3) the additive effect of a marine heatwave would further simplify gut microbiomes, particularly at the extreme reef where diversity was expected to already be reduced. Given the role of the microbiome in modulating host metabolism, immunity and stress tolerance (Greenspan et al. 2020), we additionally predicted (Hypothesis 4) that reductions in gut microbiome diversity would be associated with reduced short-term physiological condition,

as reflected by body condition, total protein content, oxidative stress or antioxidant capacity, while acknowledging that these metrics may not capture longer-term or delayed fitness consequences. Because the two study species differ in thermal niche, physiology and life-history strategies, we anticipated that microbiome responses to climate stressors could be context-dependent. We therefore examined hypotheses separately within each species, using a tropical and a subtropical reef fish to assess whether microbiome restructuring is broadly consistent or species-specific. By leveraging natural climate analogues during an unprecedented marine heatwave, our study provides a rare in situ test of how gut microbial communities respond to both chronic and acute climate stressors.

2 | Methods

2.1 | Study Species

Our study focused on two reef fish species spanning contrasting biogeographic affinities: a tropical fish (*Abudefduf vaigiensis*) and a subtropical fish (*Microcanthus strigatus*). *A. vaigiensis* is widely distributed across the tropical Indo-Pacific and has extended its range into temperate reefs in both Australia and Japan (Booth et al. 2011; Froese 2020). Across its range, this species typically inhabits water temperatures between 21.9°C and 29.3°C with a thermal optimum of 28.2°C (Froese 2020). *M. strigatus* occurs from southern China to northern Japan and along the western coast of Australia, preferring water temperatures between 17.5°C and 26.4°C with a thermal optimum of 23.7°C (Froese 2020). Both species are site-attached omnivores found across diverse habitats, including natural CO_2 seeps and ocean warming hotspots (Cattano et al. 2020; Hayes et al. 2024), making them ideal study species for assessing microbiome responses to climate stressors.

2.2 | Study Sites

We sampled both fish species at three shallow (0.5–3 m depth) nearshore rocky reefs exposed to the warm Kuroshio current and a natural carbon dioxide (CO_2) seep in Japan (Figure 1). The studied reefs represent contemporary ecosystems that currently experience differential thermal and carbonate chemistry regimes, rather than fixed proxies for future ocean states and may follow divergent ecological trajectories as climate change progresses. One reef is situated off the Izu Peninsula in Japan (34.67°N, 138.95°E) and is representative of a temperate reef ecosystem (hereafter referred to as 'cool reef'). The other two reefs are located offshore of Shikine Island, Japan, 50 km south of the cool reef and are warmer than the cool reef due to the warm Kuroshio Current causing different coastal temperature regimes over short distances (Murazaki et al. 2015), which may contribute to site-specific thermal histories and ecological contexts for resident fish populations. One of these reefs is a natural analogue for ocean warming (hereafter: 'warm reef'; 34.32°N, 139.211°E; Agostini et al. 2021) and was on average +2.3°C warmer than the cool reef (Table 1). The second reef experiences warming as well as ocean acidification from a CO_2 seep to levels beyond all SSP 2100 climate projections (pCO_2 = ~1612–8333 during sampling periods; Agostini et al. 2015) and was on average +4.3°C warmer than the cool reef during the sampling periods (Table 1), making it a natural analogue for extreme ocean acidification and warming (hereafter: 'extreme reef'; 34.32°N, 139.20°E; Agostini et al. 2015).

During the study period, the warm and extreme reefs experienced a widespread strong marine heatwave classified as Category II (strong intensity) under the Hobday et al. (2016) framework, with a cumulative intensity of 78.87 degree-days and a peak anomaly of +3.51°C above the 30-year climatological mean. The event persisted for 30 consecutive days, commencing on 14 July 2023 and formed part of an unprecedented, large-scale marine heatwave affecting extensive regions of the Pacific Ocean (Sato et al. 2024). In contrast, the cool reef did not experience marine heatwave conditions during this same period, with heatwave onset occurring approximately 10 days later than at the warm and extreme reefs and was therefore not sampled during heatwave exposure. Marine heatwave metrics were extracted from marineheatwavetracker.org and defined following Hobday et al. (2016), which characterises marine heatwaves as discrete warm-water events lasting at least 5 days and exceeding the 90th percentile of a 30-year historical baseline.

2.3 | Fish Collections From Natural Analogues of Climate Change

Juvenile *M. strigatus* and *A. vaigiensis* were collected from all three reefs using hand nets and a 1:6 clove oil to ethanol spray solution. At the cool reef, collections occurred on 14 June, 19 June and 3 July 2023, which was 13–33 days before the onset of the marine heatwave event. Sample sizes for gut microbiome analysis at the cool reef were *A. vaigiensis* ($n = 16$) and *M. strigatus* ($n = 10$). At the warm and extreme reefs, collections took place on 7 and 8 June 2023, 39–40 days before the marine heatwave. Samples sizes for gut microbiome analysis were as follows: warm reef *A. vaigiensis* ($n = 5$), *M. strigatus* ($n = 10$); Extreme reef *A. vaigiensis* ($n = 9$), *M. strigatus* ($n = 11$). We then collected *A. vaigiensis* during the marine heatwave on days 10 and 12 of heatwave exposure, specifically on 25 and 27 July 2023, at the warm ($n = 12$) and extreme reefs ($n = 7$; see Table S1 for gut microbiome sample size and fish body wet weight and total lengths).

Collected fishes were then euthanised using *ike jime*, a rapid humane brain spike (Diggles 2016) and their wet weight (±0.01 g) and total length (±0.01 mm) were recorded (Table S1). All fish appeared visibly healthy at the time of capture and dissection, with no external lesions, abnormal behaviour (e.g., irregular swimming) or obvious internal signs of disease based on gross examination conducted by experienced researchers.

Fish were first sprayed with 70% ethanol to avoid cross-contamination of skin and gut microbial communities, and the stomach section of the gut was dissected and stored in RNA*later* Stabilisation solution (Thermo Fisher Scientific Inc). Samples were first kept at 4°C to allow RNA*later* stabilisation solution to penetrate sampled tissues, then frozen and maintained at −80°C until further processing. Since ocean warming and acidification can affect fish physiology and health (Frommel et al. 2012; Alfonso et al. 2021),

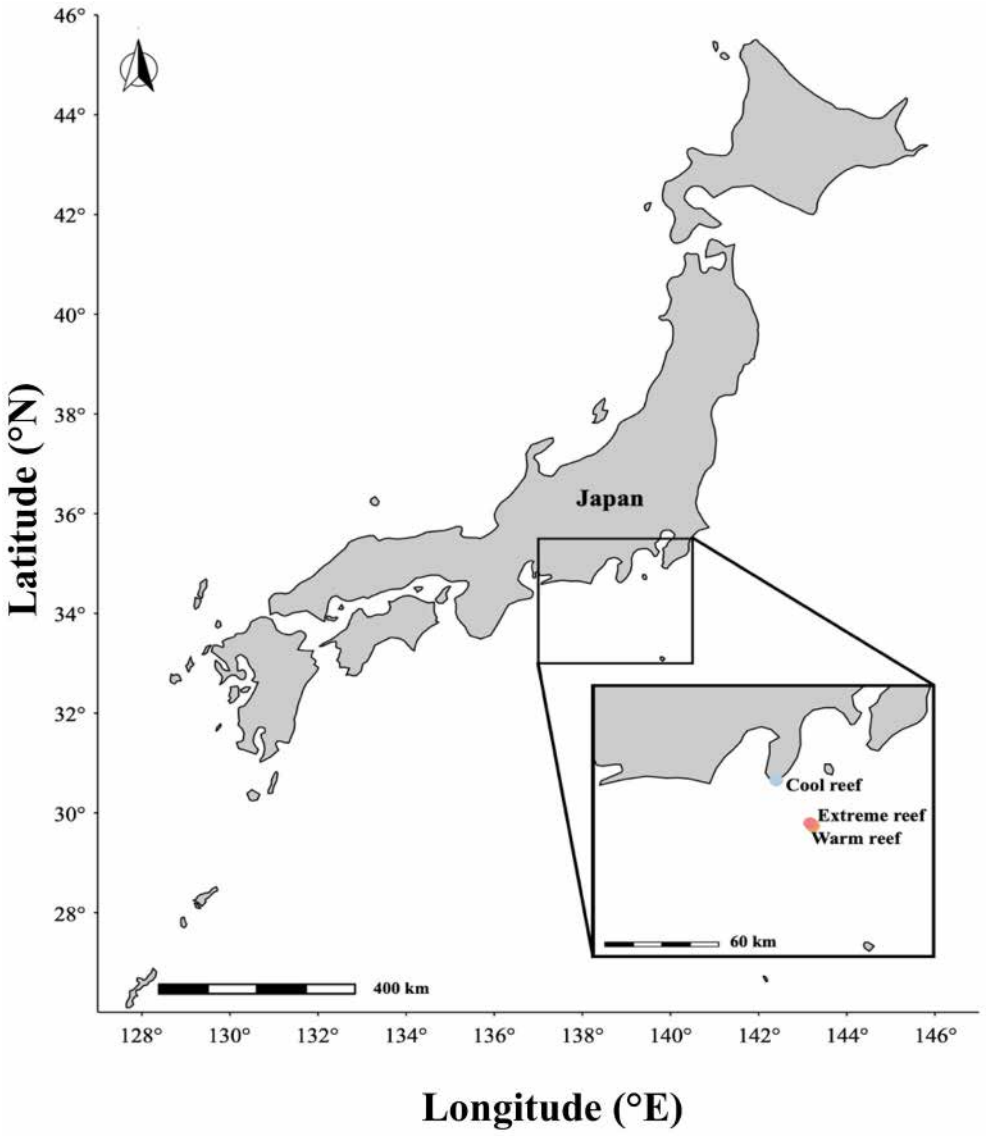

FIGURE 1 | Map of Japan showing the locations of the three shallow nearshore rocky reef study sites exposed to the warm Kuroshio Current and a natural CO_2 seep. The cool reef (sky blue; 34.67° N, 138.95° E) is situated off the Izu Peninsula and represents a present-day temperate reef ecosystem. The warm (orange; 34.32° N, 139.211° E) and extreme reefs (red; 34.32° N, 139.20° E) are located near Shikine Island. The inset map shows the ~50 km distance between the cool reef and the warm and extreme reefs on Shikine Island. Scale bars are approximate.

TABLE 1 | Seawater chemistry at the cool, warm and extreme reef localities before and during the marine heatwave (June–July 2023).

Reef	pH (NBS)	Temperature (°C)	Salinity (ppt)	pCO_2 (μatm)
June 2023: Before Heatwave				
Cool reef	8.128 (±0.003)	21.13 (±0.06)	34.0 (±< 0.1)	445
Warm reef	8.097 (±0.010)	22.42 (±0.26)	34.2 (±< 0.1)	489
Extreme reef	6.963 (±0.130)	24.60 (±0.06)	34.3 (±< 0.1)	8333
July 2023: During Heatwave				
Cool reef + heatwave	—	—	—	—
Warm reef + heatwave	8.189 (±0.063)	27.08 (±0.34)	34.4 (±< 0.1)	388
Extreme reef + heatwave	7.661 (±0.094)	28.91 (±0.06)	34.4 (±< 0.1)	1613

Note: Values are presented as means ± standard error. Temperature, pH and salinity have been reproduced in part from Mitchell et al. (2026). '–' denotes no abiotic data available for the cool reef during the heatwave period due to delayed heatwave onset at that site.

we correlated gut microbiome with the following physiology measures: cellular defence (TAC; total antioxidant capacity), oxidative stress (MDA; malondialdehyde concentration), total protein content (TP; total protein) and body condition (Fulton's body condition factor). TP, TAC and MDA measures were quantified using Elabscience assay kits (catalogue numbers: E-BC-K168-S, E-BC-K136-S and E-BC-K025-S) following methodology from Hayes et al. (2025). These physiological metrics were selected because they are known to be sensitive to environmental stressors such as ocean warming and acidification in fish (Frommel et al. 2012; Alfonso et al. 2021). These measures were used to assess whether the gut microbiome is associated with climate-mediated physiological changes, or whether gut microbiome shifts occur independently of physiological performance.

2.4 | Background Seawater Sampling From Natural Analogues of Climate Change

To assess whether ambient seawater microbial communities influenced fish gut microbiomes, water samples were collected from each reef at a depth of 1 m during July 2023 ($n = 3$ per reef). Each water sample was drawn up into a sterile 50 mL syringe and pushed through a 0.22 μm pore size Sterivex filter unit (MF-Millipore Membrane). Once sealed at one end, the filter unit was then filled with DNA/RNA Shield solution (Zymo Research). Samples were briefly stored at 4°C to allow DNA/RNA Shield solution to penetrate the filter paper, then frozen and maintained at −80°C until further processing.

2.5 | DNA Extraction and PCR Amplification

DNA was extracted using the Maxwell RSC Faecal Microbiome DNA kit, following the manufacturer's protocol with minor modifications to increase yield, described in Hayes et al. (2025) and Cruaud et al. (2017). For water samples, DNA/RNA Shield solution was first drawn up from the Sterivex filter unit with a syringe. The Sterivex casing was then opened with PVC tube cutters, the filter removed, cut into small pieces with sterilised scissors and added to a 2 mL microcentrifuge tube alongside lysis buffer and 100 μL of DNA/RNA Shield solution from the respective sample. Water and gut samples then underwent bead-beating and enzymatic digestion before extraction. Blanks were used to assess any potential contamination, and ZymoBIOMICS cellular standard was used as a positive control to assess extraction biases. V3 and V4 hypervariable regions of the 16S ribosomal RNA gene were amplified using Illumina primer pairs 341F (5′ TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG) and 805R (5′ GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGA CTA CHV GGG TAT CTA ATC C). Each polymerase chain reaction (PCR) contained 20 μL Q5 HotStart Polymerase, 0.4 μL of each primer (20 μM), 17.6 μL of nuclease-free water and 1.6 μL of either extracted template DNA or ZymoBIOMICS Microbial Community DNA Standard. Thermocycling conditions were an initial denaturation at 98°C for 30 s; followed by 35 cycles of 98°C for 10 s, 60°C for 30 s and 72°C for 30 s; with a final elongation step at 72°C for 2 min. Amplicon libraries were prepared following Illumina's 16S Metagenomic Sequencing Library Preparation protocol and sequenced at the Okinawa Institute of Science and Technology (OIST) Sequencing Section (Japan) on an Illumina MiSeq platform using a V3 600-cycle kit, generating 2×300 bp paired-end reads.

2.6 | Sequence Analysis

Raw 16S rRNA sequence data were processed in R (version 4.4.3; R Core Team 2025) using the 'DADA2' package (version 1.34; Callahan et al. 2016). Paired-end reads were quality filtered, trimmed and denoised. Reads were discarded if they contained ambiguous bases (maxN = 0), exceeded three expected errors for forward reads or four for reverse reads (maxEE = 3, 4) or included bases with low-quality scores (truncQ = 2). To eliminate low-quality ends, forward and reverse reads were truncated to 240 bp. Reads were then dereplicated, merged and denoised to infer amplicon sequence variants (ASVs), followed by chimera removal. Taxonomic assignment was conducted using the SILVA reference database (v138.1; Quast et al. 2013; Yilmaz et al. 2014). Non-targeted sequences, including those identified as mitochondrial, chloroplast or unclassified at the phylum level, were excluded from further analysis.

2.7 | Statistical Analysis

All statistical analyses and data visualisation were conducted in R using the packages 'phyloseq' (v1.44; McMurdie and Holmes 2013), 'vegan' (v2.6–4; Oksanen et al. 2007), 'ANCOMBC' (v2.9.1; Lin and Peddada 2024) and 'ggplot2' (v3.5.1; Wickham 2016). Because *M. strigatus* was not collected during the marine heatwave event, analyses were conducted separately for each species since combining species in a single analysis could confound results due to differences in sampling design. Seawater microbial communities were also analysed to assess the influence of environmental microbial load on fish gut-associated communities across reef sites. Alpha diversity was calculated using the 'estimate_richness' function in the 'phyloseq' package for three metrics: richness (the observed number of bacterial ASVs), community evenness (Pielou's evenness, calculated as Shannon diversity divided by log richness) and Shannon diversity index (which captures both richness and relative abundance of ASVs within a sample).

Levene's test confirmed homogeneity of variance for each metric, permitting parametric testing. For *A. vaigiensis*, a two-way analysis of variance (ANOVA) was used to test for effects of reef (cool, warm and extreme) and marine heatwave (before and during), while for *M. strigatus* and background seawater communities, a one-way ANOVA was performed with reef as the sole factor. Post hoc Tukey HSD tests were used to determine differences in alpha diversity across factors.

To assess whether microbiome alpha diversity was influenced by fish body size and physiological condition, generalised linear models (GLMs) were fitted with each diversity metric as the response variable and body condition, total antioxidant capacity (TAC), malondialdehyde (MDA), total protein (TP), reef type

and marine heatwave as predictors. Model selection was based on corrected Akaike's Information Criteria (AIC), the best-fit models were identified, and McFadden's R^2 formula was calculated for each model (log-likelihood ratio between top-ranked model and the null model; see Table S7).

Microbiome beta diversity measures were assessed using both phylogenetic (Weighted and Unweighted UniFrac) and non-phylogenetic (Bray-Curtis) distance metrics calculated with the 'distance' function in 'phyloseq'. Bray-Curtis measures compositional dissimilarity based on relative abundance without accounting for phylogeny, while Weighted UniFrac accounts for both abundance and phylogenetic distance, and Uniweighted Unifrac highlights differences in presence-absence, especially among rare taxa. Differences in community composition across reef and marine heatwave conditions were testing using permutational multivariate analysis of variance (PERMANOVA) with 9999 permutations, using 'adonis2' and 'pairwise. adonis' in the package 'vegan'. Microbiome variability (i.e., dispersion) was assessed using permutational analysis of multivariate dispersion (PERMDISP2) using 'betadisper' in the package 'vegan', with bias correction applied to account for differences in sample size across reefs. For these analyses, dispersion reflects within-group variance and average distance of samples to their group centroids in multivariate space. Principal coordinate analysis (PCoA) was used to visualise community dissimilarities and to distinguish whether observed differences were driven by centroid separation or dispersion. Microbiome beta diversity analyses (PERMANOVA, PERMDISP and PCoA visualisations in Figure 2) were conducted using ASV level data and not grouped at the genus level.

Relative abundances of microbiome bacterial taxa were visualised using stacked bar graphs at the genus level, showing the ten most dominant bacterial taxa across reef types and marine heatwave conditions. Taxon abundances were calculated within each sample and then averaged across samples per reef or marine heatwave. Differential abundance analysis was performed using ANCOMBC-2, which estimates log fold changes (LFC) in taxon abundance across reefs and marine heatwave while correcting for compositional bias. Genera with a false discovery rate (FDR)-adjusted q-value <0.05 were considered significantly different.

Heatmaps were used to visualise log-fold changes but were not produced for marine heatwave conditions because no significant differences were detected for *A. vaigiensis* during the marine heatwave, or for *M. strigatus* across reefs.

3 | Results

3.1 | Sequencing Data Summary and Depth

Sequencing of the 16S rRNA gene V3-V4 region of 80 samples resulted in 10,194,708 raw sequences obtained. After quality filtering, denoising and merging of paired-end reads, a total of

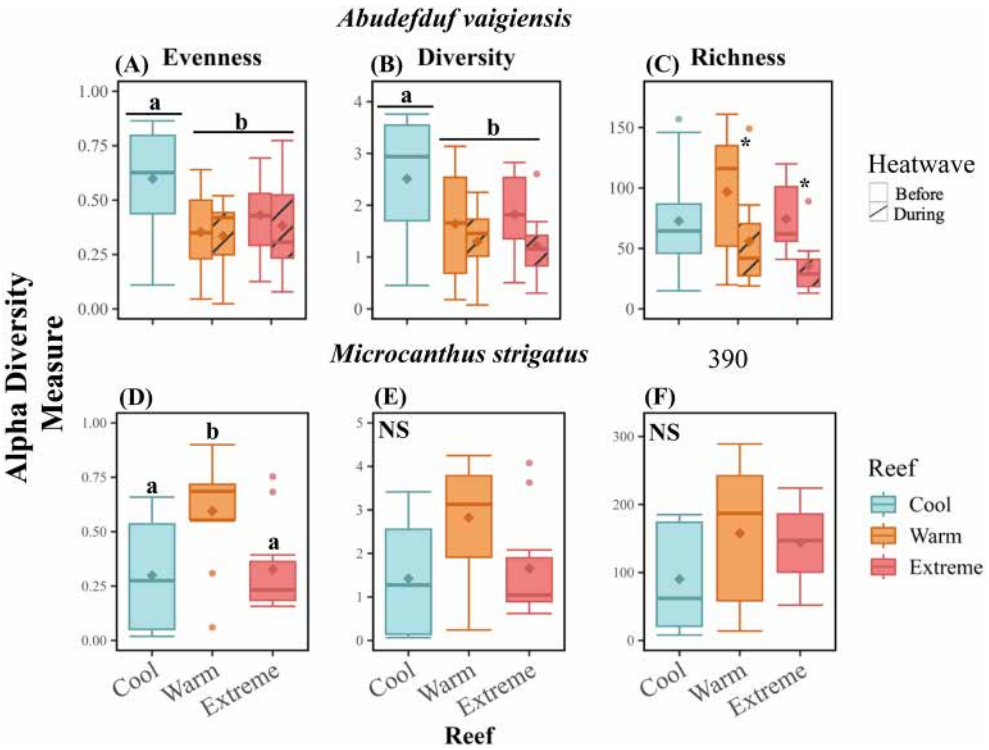

FIGURE 2 | Boxplots showing differences in the alpha diversity measures: Evenness (A, D), diversity (B, E) and richness (C, F) of *Abudefduf vaigiensis* (A–C) and *Microcanthus strigatus* (D–F) gut microbiomes across the different reefs [Cool (skyblue), Warm (orange), Extreme (red)]. The boxes represent the lower and upper quartile, whiskers extend to values within 1.5× the interquartile range, horizonal lines show the median and diamonds show the mean. The different letters indicate Post hoc Tukey HSD test significant differences between reefs and across marine heatwave periods ($p<0.05$). * denotes significant ($p<0.05$) marine heatwave effect at the warm and extreme reefs for *A. vaigiensis*. NS, non-significant ($p>0.05$).

8,407,112 non-chimeric reads for the samples were obtained and ranged from 72,548 to 129,844 reads per sample. The total number of ASVs detected for the samples was 17,759.

3.2 | Gut Microbiome Diversity Under Ocean Warming, Acidification and Marine Heatwaves

In *Abudefduf vaigiensis*, gut microbiome evenness and diversity were lower at the warm (evenness: −43%, diversity: −44%) and extreme reefs (evenness: −31%, diversity: −38%) compared to the cool reef ($p \leq 0.041$; Figure 2A,B; Table S2). Gut microbiome richness was similar across all reefs ($p \geq 0.515$; Figure 2C) but was 42% lower at the warm reef and 52% lower at the extreme reef during the marine heatwave compared to pre-heatwave individuals ($p = 0.008$; Table S2). Microbiome variability (beta dispersion) was higher at the cool reef than at the warm and extreme reefs, both before and during the heatwave (Bray–Curtis: $p < 0.001$; Weighted UniFrac: $p = 0.011$; Figure 3G–I; Table S4).

In *Microcanthus strigatus*, gut microbiome evenness was 99% higher at the warm reef compared to the cool reef, and 82% higher compared to the extreme reef ($p \leq 0.040$; Figure 2E; Table S2), while gut microbiome richness and diversity did not differ across reefs ($p \geq 0.053$; Figure 2D,F; Table S2). Microbiome variability was consistent across reef localities, except for lower dispersion at the extreme reef relative to the warm reef based on Weighted UniFrac ($p = 0.007$; Figure 3J–L; Table S4).

3.3 | Gut Microbiome Composition Under Ocean Warming, Acidification and Marine Heatwaves

In *A. vaigiensis*, gut microbiome community composition differed across the cool, warm and extreme reefs (Bray–Curtis $R^2 = 0.21$; Unweighted UniFrac $R^2 = 0.13$; Weighted UniFrac $R^2 = 0.21$; all $p < 0.001$; Figure 3A–C; Table S3), and also differed between before and during heatwave periods at the warm and extreme reefs (Bray–Curtis and Weighted UniFrac, $p \leq 0.04$).

In *M. strigatus*, microbial composition differed between the extreme reef and both the cool and warm reefs (Bray–Curtis $R^2 = 0.19$; Unweighted UniFrac $R^2 = 0.12$; Weighted UniFrac $R^2 = 0.16$; all $p \leq 0.002$; Figure 3D–F; Table S3).

3.4 | Altered Taxonomic Composition of Reef Fish Gut Microbiomes Under Ocean Warming, Acidification and Marine Heatwaves

Differential abundance analysis (ANCOM-BC2; Figure S4) identified several bacterial genera with significant differences in relative abundance across reefs in the gut microbiome of *A. vaigiensis* (Table S5; Figures 3 and 4), while the top ten most abundant bacterial taxa did not significantly differ in abundance across reefs in *M. strigatus* ($q > 0.05$; Data S1). In *A. vaigiensis*, *Mycoplasma* spp., *Endozoicomonas* spp., *Vibrio* spp. and *Catenococcus* spp. were significantly more abundant at the extreme reef than the cool reef (LFCs = 4.01, 2.40, 3.58 and 3.30 respectively; all $q < 0.001$; Figure S4). *Photobacterium* spp. and

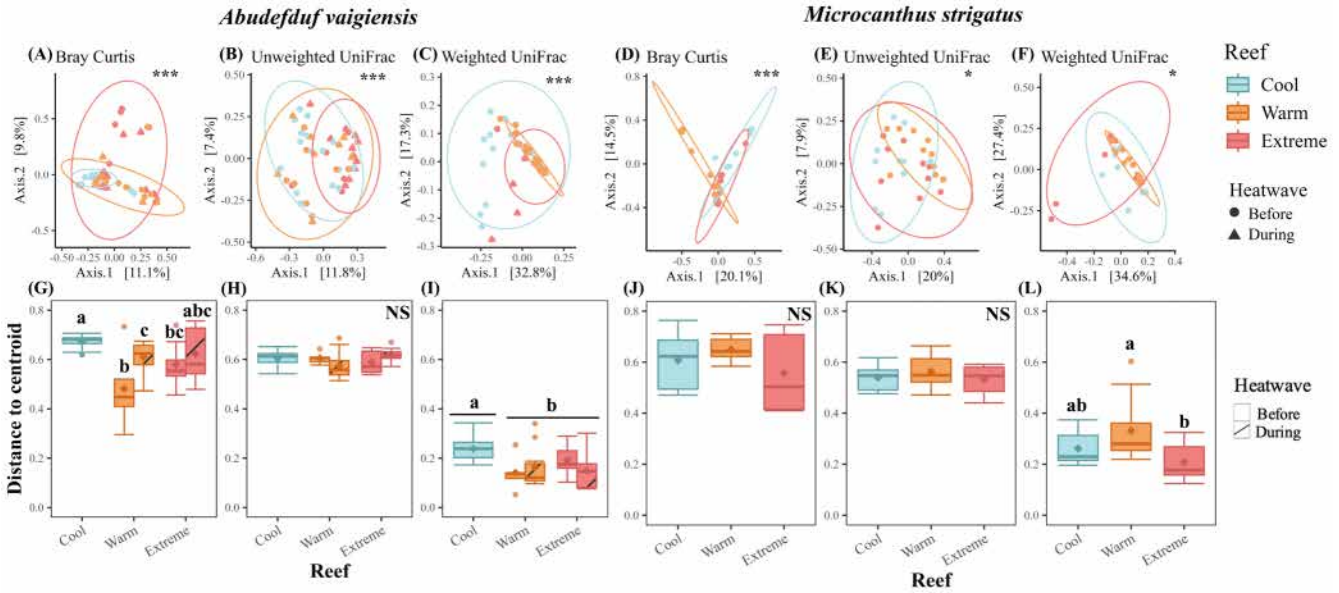

FIGURE 3 | Beta diversity of gut microbiome composition (A–F) and compositional variability (distance to centroid; G–L) visualised using principal coordinate analysis (PCoA) for *Abudefduf vaigiensis* and *Microcanthus strigatus* across the Cool, Warm and Extreme reefs. For *A. vaigiensis*, plots include samples collected before and during the marine heatwave. In panels A–C, circles indicate individuals sampled before the heatwave, and triangles indicate individuals sampled during the heatwave. Significance in panels A–F reflects differences detected by permutational multivariate analysis of variance (PERMANOVA). Compositional variability (G–L) represents the distance of samples to their group centroid; boxes show the interquartile range (IQR), whiskers extend to 1.5× IQR, horizontal lines denote medians and diamonds indicate means. Different letters in G–L denote significant differences in multivariate dispersion between reefs. Asterisks indicate significance levels: *$p < 0.05$, ***$p < 0.001$. Analyses were conducted using Bray–Curtis dissimilarity (A, D), Weighted UniFrac (B, E) and Unweighted UniFrac (C, F): Bray–Curtis reflects ASV abundance; Weighted UniFrac incorporates phylogenetic relatedness and abundance; Unweighted UniFrac considers presence–absence and phylogeny.

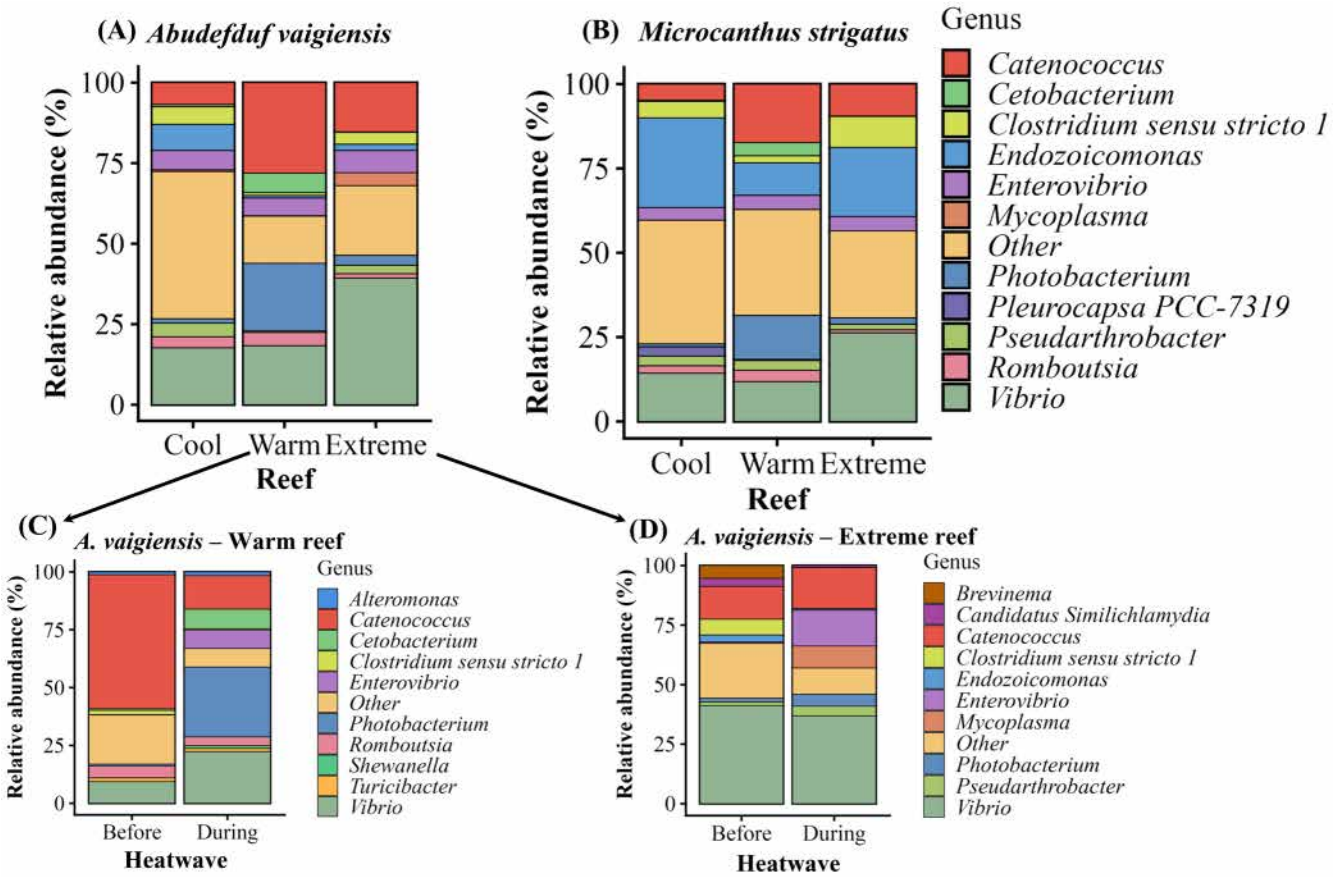

FIGURE 4 | Stacked bar plots showing the relative abundance (%) of the ten most abundant bacterial genera in the gut microbiome of *Abudefduf vaigiensis* (Panel A) and *Microcanthus strigatus* (Panel B) across the reefs (Cool, Warm, Extreme), irrespective of the marine heatwave event. Panels C and D show the relative abundance of the top 10 most relatively abundant (%) genera in *A. vaigiensis* sampled before and during the marine heatwave at the Warm (C) and Extreme (D) reefs. 'Other' represents the combined relative abundance of all genera that were not among the ten most abundant across reefs (A and B) and at Warm (C) and Extreme reefs (D) before and during the heatwave event for *A. vaigiensis*. The key for panels A and B includes 11 genera because the ten most abundant taxa were not identical between *A. vaigiensis* and *M. strigatus*. Each panel displays ten genera, but the combined key reflects all taxa that ranked within the top ten for either species across reefs.

Catenococcus spp. were also more abundant at the warm reef compared to the cool reef (LFCs = 3.76 and 3.84; $q < 0.001$), while *Pseudarthrobacter* spp. was less abundant at the warm reef than the cool reef (LFC = −1.76, $q = 0.015$) and more abundant at the extreme reef than the warm reef (LFC = 2.08, $q = 0.010$). No significant changes in the relative abundance of *Romboutsia* spp., *Enterovibrio* spp., *Clostridium sensu stricto 1* spp. or *Cetobacterium* spp. were observed across reefs ($q > 0.05$; Table S5).

The relative abundance of the top ten most abundant bacterial taxa in *A. vaigiensis* did not differ significantly before and during the marine heatwave at either the warm or extreme reefs ($q > 0.05$; Data S1).

3.5 | Physiological Correlations With Microbiome Diversity

In both fish species, gut microbiome diversity, evenness and richness were not associated with fish body condition, total antioxidant capacity (TAC), oxidative stress (MDA) or total protein content (Figure S2; $p \geq 0.067$; Table S8), except for a positive association between microbial richness and total protein content in *A. vaigiensis* across all three reefs (Figure S2; $p = 0.004$; $R^2 = 0.17$; Table S8).

Body condition was the only physiological response that differed across reefs (Figures S2 and S3; Table S8), with higher body condition at the cool reef compared to the warm reef in *A. vaigiensis* (Figure S3; $p \leq 0.012$; Table S8) and compared to the extreme reef in *M. strigatus* (Figure S3; $p < 0.001$; Table S8). All other physiological metrics did not differ significantly across reefs for either species.

3.6 | Background Seawater Biome Diversity and Composition Across Reefs

Seawater microbial evenness and diversity were significantly higher at the cool reef than the warm reef (evenness: $p = 0.040$; diversity: $p = 0.026$; Table S2), and evenness was also higher at the cool reef than the extreme reef ($p = 0.045$). However, richness did not differ significantly across reefs (Figure S5; $p = 0.414$; Table S2). Microbial community composition was similar across reefs (Figure S6A–C; $p > 0.05$ for all comparisons; Table S3).

Seawater microbial dispersion (i.e., beta diversity variance) was significantly higher at the cool and extreme reefs than at the warm reef based on Unweighted UniFrac distances (Figure S6E; $p < 0.05$; Table S4) but did not differ among reefs when assessed using Bray-Curtis (Figure S6D, $p = 0.632$, Table S4) and Weighted UniFrac metrics (Figure S6F, $p = 0.209$, Table S4). Despite spatial variation in diversity and dispersion, the seawater biome did not significantly differ in the relative abundance of the top ten most common genera across reef sites (ANCOM-BC2, $q = 1$; Figures S7 and S8; Table S6). Furthermore, the most abundant taxa in seawater were entirely distinct from the top ten gut microbiome taxa of either *A. vaigiensis* or *M. strigatus* (Figure 4, Table S6), suggesting minimal overlap in community composition between environmental and host-associated microbiomes.

4 | Discussion

Here we reveal that a severe marine heatwave and ocean warming and acidification can simplify and restructure reef fish gut microbiomes. Across natural climate analogues of climate change, both *A. vaigiensis* and *M. strigatus* showed significant shifts in gut microbial community composition, yet the magnitude and direction of these shifts varied markedly between species and climate stressors. The tropical fish species (*A. vaigiensis*) had lower microbiome evenness and diversity at reefs experiencing warming and combined warming and extreme acidification, alongside higher relative abundances of dominant (*Mycoplasma*, *Endozoicomonas*, *Catenococcus*, *Vibrio*) taxa. Thus, moderate warming did not increase gut microbiome diversity in *A. vaigiensis*, contrary to Hypothesis 1, whereas chronic warming and extreme acidification reduced microbiome diversity and drove community simplification, supporting Hypothesis 2. The marine heatwave event further degraded gut microbiome diversity by lowering gut microbiome richness and variability without altering dominant bacterial taxa in *A. vaigiensis* at the warm and extreme reefs, supporting Hypothesis 3. In contrast, the subtropical fish species (*M. strigatus*) had a distinct microbial community, but largely maintained gut microbiome diversity and variability, across reef localities. Our results align with growing evidence that climate extremes (e.g., heatwaves) and gradual climate change (e.g., ocean warming and acidification) can trigger microbial dysbiosis or community reshuffling across a range of marine taxa (Li et al. 2022; Strano et al. 2023; Bell et al. 2024; Vompe et al. 2024; Hayes et al. 2025). While previous studies have suggested that microbiome reorganisation may buffer hosts against environmental stress (Voolstra and Ziegler 2020; Baldassarre et al. 2022), changes in microbial community structure can also destabilise beneficial interactions and disrupt host–microbiome homeostasis (Zaneveld et al. 2017). Our findings provide rare in situ evidence that ocean warming, acidification and marine heatwaves can drive both reorganisation and simplification of reef fish microbiomes, with the potential to alter host–microbiome relationships and functional stability in a rapidly changing ocean.

We found that *A. vaigiensis* juveniles sampled during a severe marine heatwave at reefs experiencing ocean warming and acidification had lower gut microbial richness and beta diversity than pre-heatwave individuals, supporting Hypothesis 3, despite no detectable change in their physiological performance. Comparable restructuring has been observed in benthic invertebrates, where heatwaves can trigger microbial collapse and host mortality in temperate sponges (Bell et al. 2024), while in corals, bleaching susceptibility was linked to microbiome ecological memory across repeated heatwaves (Vompe et al. 2024). Our results suggest that marine heatwaves act as homogenising forces on gut microbiome, inducing short-term shifts in community structure and decreases in microbial diversity which may filter for thermally sensitive microbial taxa (Castro et al. 2024). However, whether this represents a compensatory response, or an indicator of latent functional loss remains unclear. As marine heatwaves intensify in frequency and duration, their ability to alter host-microbe relationships may pose an increasing threat to reef fish resilience as oceans continue to acidify and warm.

Despite pronounced restructuring of gut microbiomes in both fish species at natural climate analogues of ocean warming and acidification, these changes were not generally associated with physiological costs. Across the studied natural analogue reefs and heatwave periods, we found limited evidence to support Hypothesis 4, as gut microbial diversity did not significantly correlate with proxies of physiological condition (body condition, total protein content, oxidative stress and antioxidant capacity) in either fish species, with the only exception being a positive correlation between total protein content and gut microbiome richness in *A. vaigiensis*. Previous research shows that low microbial diversity can impair host performance (Bestion et al. 2017; Greenspan et al. 2020), and microbial diversity is often used as a proxy for host health (Greenspan et al. 2020). However, our results suggest that substantial shifts in microbiome composition can occur without immediate physiological consequences in reef fishes. While this study does not directly test mechanistic links between microbiome restructuring and host physiological outcomes, we show that climate stressors reshape fish microbial communities in ways that may precede or underlie longer-term functional impacts. It is important to note that our physiological metrics captured only a subset of possible traits and we did not assess longer-term effects such as digestion efficiency, disease resistance or reproductive success. Thus, while we interpret microbiome reorganisation as a plastic response to climate stress, it remains uncertain whether this plasticity is ultimately adaptive or whether it conceals latent costs that may emerge with increasingly frequent and extreme marine heatwaves in a future ocean.

Importantly, we show that ocean warming and acidification not only restructure the gut microbiome of *Abudefduf vaigiensis* but also shift the relative abundance of its dominant taxa. In *A. vaigiensis*, several dominant bacterial taxa responded strongly to ocean warming and acidification. At the extreme reef, *Mycoplasma*, *Endozoicomonas*, *Vibrio* and *Catenococcus* were all more abundant than at the cool reef. Similarly, *Photobacterium* and *Catenococcus* were more abundant at the warm reef than the cool reef. These patterns support environmental filtering (Shade et al. 2012) and are consistent with climate-driven directional shifts in microbiome composition in *A. vaigiensis* (Zaneveld et al. 2017). The higher abundance of *Vibrio* in *A. vaigiensis*, a genus known to include thermotolerant opportunistic strains associated with environmental stress (Austin and Zhang 2006; Lokmer and Wegner 2015), suggests a potential rise in opportunistic taxa in fish residing on reefs experiencing ocean warming and acidification. However,

since 16S rRNA sequencing has limited taxonomic resolution for *Vibrio* spp. (King et al. 2019), we cannot distinguish between potentially pathogenic and non-pathogenic strains in this context. Additionally, as with all amplicon-based studies, relative abundance reflects proportional shifts in community composition and may not capture absolute changes in microbial load. Nonetheless, the increased presence of *Mycoplasma*, *Endozoicomonas* and *Catenococcus*, genera commonly associated with mutualistic and nutrient-processing roles in fish guts (Egerton et al. 2018; Brown et al. 2019), indicates that warming and acidification also facilitate the proliferation of pre-existing dominant taxa in *A. vaigiensis*. Furthermore, while no shifts in dominant microbial taxa were detected in *A. vaigiensis* before and during the marine heatwave, overall microbiome richness and variability declined, suggesting that marine heatwaves can lower diversity without necessarily altering the taxonomic identity of dominant community members. Together, our findings demonstrate that ocean warming and acidification are key drivers of dominant microbial taxa changes in reef fishes, while marine heatwaves primarily reduce microbial diversity and variability.

We show that fish gut microbiomes are not primarily shaped by background water microbiota, even under climate-driven changes in environmental conditions. While we observed differences in seawater microbial diversity and dispersion across reefs, background seawater communities were compositionally distinct from reef fish gut microbiomes, suggesting that ambient environmental microbiota do not primarily structure fish gut communities, as previously observed in other reef fish species (Jones et al. 2018). Instead, gut microbiomes are more actively assembled through host immune regulation (McFall-Ngai et al. 2013), gut physiology (Egerton et al. 2018) and dietary inputs (Miyake et al. 2014). Nonetheless, climate change stressors can simultaneously modify abiotic conditions (e.g., temperature, pH) and biotic structures (e.g., prey availability and food webs; Nagekerken et al. 2020; Agostini et al. 2021). Therefore, regardless of the specific mechanisms shaping gut microbiome structure, the shifts observed in our study likely reflect the cumulative effects of interacting environmental and ecological changes associated with ocean warming, acidification and marine heatwaves likely to be experienced by reef fishes in a future ocean.

It is important to acknowledge that our study design lacks spatial replication within reef categories, and that some gut microbial patterns attributed to warming, acidification or heatwave exposure may partially reflect site-specific environmental variation rather than climate stressors alone. Nevertheless, we observed strong and consistent directional changes in gut microbiome structure during the marine heatwave across both the warming and extreme reefs, suggesting a general response to marine heatwaves on fish gut microbiomes despite the absence of spatial replication. Future work combining multiple natural analogue reef systems with manipulated climate experiments will be important for determining the mechanisms, generality and consistency of climate-driven fish microbiome shifts across broader spatial scales in the world's oceans (Hayes et al. 2026).

We found that gut microbiome reorganisation under climate change can be species-specific in reef fishes. Unlike *Abudefduf vaigiensis*, whose gut microbiome showed proliferation of dominant taxa and lower diversity, richness and evenness at reefs experiencing ocean warming, acidification and under a severe heatwave, *Microcanthus strigatus* showed compositional restructuring of their gut microbiomes without significant changes in the relative abundance of dominant bacterial genera. Additionally, *M. strigatus* had higher microbiome evenness at the warm reef compared to the cool reef, a pattern that may reflect microbial diversification as temperatures approach the species' thermal optimum (~23.7°C, Froese 2020). In contrast, *A. vaigiensis* showed lower microbial diversity and higher abundances of opportunistic taxa (e.g., *Vibrio*) under the same warming conditions, revealing divergent microbial responses between species exposed to identical thermal regimes. These contrasting patterns point to differences to the two study species preferred thermal ranges (Froese 2020), host-specific ecological or physiological traits, including thermal sensitivity or microbial flexibility as key drivers of microbial restructuring under climate change (Llewellyn et al. 2014; Brown et al. 2012). Additionally, the gut microbiome diversity gains observed in *M. strigatus* at the warm reef were tempered at the extreme reef, where the additive effect of extreme ocean acidification suppressed microbiome richness and evenness. We suggest that microbial gains facilitated by warming can be undermined by co-occurring acidification stress, leading to gut microbial community reshuffling, a pattern mirrored at broader ecological scales under ocean acidification (Nagelkerken and Connell 2022). Combined, our findings provide rare in situ evidence that climate change will restructure fish gut microbiomes, but the direction and magnitude of these changes are modulated by host identity to interacting climate stressors.

5 | Conclusions

Our findings reveal that ocean warming, acidification and marine heatwaves can reshape and, in some cases, simplify reef fish gut microbiomes. While warming and acidification drove distinct microbiome reorganisation, marine heatwaves degraded one reef fish species' gut microbial communities, transiently lowering microbial diversity and variability in their gut microbiomes. Together, our findings suggest that ocean warming, acidification and heatwaves can combine to reshape reef fish gut microbiomes, which may alter host-microbe relationships in a future ocean.

Author Contributions

I.N., A.M. and C.H. conceptualised the study. C.H. led the formal analysis with support from C.J.H. and A.M. A.M., C.J.H., S.D.C., I.N., B.P.H., S.A. and J.J. conducted the fieldwork. J.J. conducted laboratory analyses, and T.R. contributed laboratory and field materials. A.M. and C.H. wrote the original draft. All authors reviewed and edited the manuscript. I.N., S.D.C., D.J.B., T.R., B.P.H. and S.A. acquired funding.

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Ethics Statement

Fish collections were approved by the University of Adelaide and University of Tsukuba animal ethics committee (permits S-2023-043 and 23–426, respectively). Fish were collected under sampling permits for Shizuoka Prefecture permit number 5–10 (2023) and under Tokyo Prefecture permit number 5–11 (2023).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available at Figshare: https://figshare.com/s/47a0313b0bfd2c1854e9. Raw sequence reads are uploaded and available on the NCBI Sequence Read Archive (PRJNA1290829).

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** mec70275-sup-0001-DataS1.xlsx. **Table S1:** Sample sizes (*n*) for gut microbiome sampling, mean fish total length (mm ± standard deviation) and wet weight (g ± standard deviation) of *Abudefduf vaigiensis* and *Microcanthus strigatus* juveniles collected at the cool, warm and extreme reefs before and during (only *Abudefduf vaigiensis*) the marine heatwave. N/A denotes no fish sampled during the marine heatwave at the specified reef locality. TL, total length (mm); WW, wet weight (g). **Table S2:** Results of ANOVA and pairwise tests for alpha diversity metrics (evenness, diversity and richness) across reef conditions (cool, warm, extreme) and marine heatwave phases (before, during). Significant *p*-values (<0.05) are shown in bold. Tests were conducted using 9999 permutations. **Table S3:** Results for PERMANOVA and pairwise tests for beta diversity measures (Bray-Curtis, Unweighted UniFrac and Weighted UniFrac) across reef conditions (cool, warm, extreme) and marine heatwave phases (before, during) for *Abudefduf vaigiensis* and *Microcanthus strigatus* and background seawater samples. Significant *p*-values (<0.05) are shown in bold. Tests were conducted with 9999 permutations. **Table S4:** Results of PERMDISP analysis for beta diversity dispersion (Bray-Curtis, Unweighted UniFrac and Weighted UniFrac) based on distance to centroid across reef conditions (cool, warm, extreme) and marine heatwave phases (before, during) for *Abudefduf vaigiensis*, *Microcanthus strigatus* and background seawater samples. Significant values (<0.05) indicate uneven dispersion, suggesting high variability in community composition among samples. Tests were performed with 9999 permutations, and significant *p*-values are shown in bold. **Table S5:** Analysis of composition of microbiomes with bias correction (ANCOM-BC) results for the top ten differentially abundant taxa across reef conditions (cool, warm, extreme). Statistically significant differences ($q<0.05$) are shown in bold. The W statistic represents the strength of evidence for differential abundance, with higher values indicating stronger significance. *p*-values reflect the unadjusted statistical significance, while *q*-values are false discovery rate (FDR) adjusted *p*-values for multiple comparisons. **Table S6:** Analysis of composition of seawater microbiomes with bias correction (ANCOM-BC) results for the top ten differentially abundant Genera across reef conditions (cool, warm, extreme). Statistically significant differences ($q<0.05$) are shown in bold. The W statistic represents the strength of evidence for differential abundance, with higher values indicating stronger significance. *p*-values reflect the unadjusted statistical significance, while *q*-values are false discovery rate (FDR) adjusted *p*-values for multiple comparisons. **Figure S2:** Linear regressions

between alpha diversity metrics: evenness (left column), diversity (middle column) and richness (right column) and physiological response variables: wet weight (A–C), total protein (TP) content (D–F), total antioxidant capacity (TAC) (G–I) and malondialdehyde (MDA) levels (J–L) in fish species *Abudefduf vaigiensis*. Data are grouped by reef type: cool (blue), warm (orange) and extreme (coral), with marine heatwave (MHW) conditions indicated by before (circles) and during (triangles). Solid lines represent linear regressions for each reef type. Null indicates the null model was the best fit model. Asterisks (*) indicate significant relationships. See Table S8 for statistical outputs. Reported *p*-values indicate the strength of the relationship between each physiological proxy (response variable) and microbiome richness, evenness or diversity (predictor variables). **Figure S3:** Linear regressions between alpha diversity metrics: evenness (left column), diversity (middle column) and richness (right column) and physiological response variables: wet weight (A–C), total protein (TP) content (D–F), total antioxidant capacity (TAC) (G–I) and malondialdehyde (MDA) levels (J–L) in fish species *Microcanthus strigatus*. Data are grouped by reef type: cool (blue), warm (orange) and extreme (coral). Solid lines represent linear regressions for each reef type. Null indicates the null model was the best fit model. Asterisks (*) indicate significant relationships. See Table S8 for statistical outputs. Reported *p*-values indicate the strength of the relationship between each physiological proxy (response variable) and microbiome richness, evenness, or diversity (predictor variables). **Table S7:** Akaike Information Criterion (AICc) rankings for models predicting alpha diversity metrics (evenness, diversity and richness) across reef conditions (cool, warm, extreme) and marine heatwave phases (before, during) for *Abudefduf vaigiensis* and *Microcanthus strigatus*. Models include different explanatory variables: Fulton's condition factor, Total Protein (TP), Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA). *k* represents the number of model parameters, LL is the log-likelihood, AICc is the corrected Akaike Information Criterion, and ΔAICc indicates the difference from the top-ranked model. The best-supported models are ranked at the top and shown in bold. Where AICc supported the null model, statistical outputs are not shown or interpreted. **Table S8:** Generalised linear model (GLMs) summaries for top-ranked models (see Table S6 for AIC_c best-fit models) predicting evenness, diversity and richness for *Abudefduf vaigiensis* and *Microcanthus strigatus* across reef conditions (cool, warm, extreme) and marine heatwave phases (before, during). Models include different explanatory variables: Fulton's condition factor, Total Protein (TP), Total Antioxidant Capacity (TAC) and Malondialdehyde (MDA). Statistically significant predictors ($p < 0.05$) are shown in bold. **Figure S4:** Differential abundance of top ten most abundant gut microbial taxa in tropical *A. vaigiensis* across reefs based on ANCOM-BC2 analysis. Each cell shows the log fold change for pairwise comparisons across reefs (Cool, Warm, Extreme reefs). Zero values indicate no significant difference in relative abundance (FDR $q > 0.05$). Significant differences in abundance of the genera are shown by * (FDR $q < 0.05$). The red cells show increased abundance (log fold change > 0), and the blue cells show decreased abundance (log fold change < 0) compared to the cool reef, and to the warm reef when compared to the extreme reef. Log fold changes were calculated using the first reef in each comparison as the baseline (e.g., in the Cool–Warm comparison, Cool is the reference and Warm is the comparison). Positive values (red) indicate higher abundance in the comparison group, and negative values (blue) indicate lower abundance. **Figure S5:** Boxplots showing differences in alpha diversity measures: evenness (A), diversity (B) and richness (C) of seawater microbial communities across the different reefs [Cool (skyblue), Warm (orange), Extreme (red)]. The boxes represent the lower and upper quartile, whiskers extend to values within 1.5× the interquartile range, horizontal lines show the median and diamonds indicate the mean. Different letters indicate significant differences between reef sites based on post hoc Tukey HSD tests ($p < 0.05$). Corresponding statistical outputs are reported in Table S2. **Figure S6:** Principal coordinate analysis (PCoA) plots showing microbial community composition (A–C) and compositional variability (D–F) of seawater microbial communities across the Cool, Warm and Extreme reef sites. PCoA plots (A–C) are based on three different dissimilarity metrics: Bray-Curtis (A), Unweighted UniFrac (B) and Weighted UniFrac (C), where each point represents an individual seawater sample. Significance values in panels A–C are derived from Permutational Analysis of Variance (PERMANOVA) testing for differences in microbial community composition across reef sites. Panels D–F represent the multivariate dispersion (i.e., distance to centroid in multivariate space) based on Bray-Curtis (D), Unweighted UniFrac (E) and Weighted UniFrac (F) dissimilarity metrics. Boxplots in D–F represent the lower and upper quartiles, whiskers show 1.5× the interquartile range, horizontal lines indicate the median and diamonds represent the mean. Different letters indicate significant differences in multivariate dispersion between reef sites. Significance is denoted by * for $p < 0.05$. Bray-Curtis considers ASV abundance, Unweighted UniFrac considers presence-absence and phylogenetic structure, and Weighted UniFrac incorporates both ASV abundance and phylogenetic relatedness. Corresponding statistical outputs are reported in Tables S3 and S4. **Figure S7:** Stacked bar plot showing the relative abundance (%) of the ten most abundant bacterial genera in seawater samples collected from the Cool, Warm and Extreme reef sites. Bars represent the average composition of replicate seawater samples collected at each reef ($n = 3$ per site). The 'Other' category includes all genera outside the top ten most abundant across reefs. Corresponding statistical outputs are reported in Table S6. **Figure S8:** Differential abundance of top ten most abundant seawater microbial taxa across reefs based on ANCOM analysis. Each cell shows the log fold change for pairwise comparisons across reefs (Cool, Warm, Extreme reefs). Zero values indicate no significant difference in relative abundance. There were no significant differences in abundance of the genera (FDR $q > 0.05$, Table S6). The red cells show increased abundance (log fold change > 0), and the blue cells show decreased abundance (log fold change < 0) compared to the cool reef, and to the warm reef when compared to the extreme reef. Log fold changes (LFC) are calculated using the first reef in each comparison as the baseline (e.g., in the Cool–Warm comparison, Cool is the reference and Warm is the comparison). Positive values (red) indicate higher abundance in the comparison group, and negative values (blue) indicate lower abundance.