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Elevated estuary water temperature drives fish gut dysbiosis and increased loads of pathogenic vibrionaceae

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A B S T R A C T

Marine water temperatures are increasing globally, with eastern Australian estuaries warming faster than predicted. There is growing evidence that this rapid warming of coastal waters is increasing the abundance and virulence of pathogenic members of the Vibrionaceae, posing a significant health risk to both humans and aquatic organisms. Fish disease, notably outbreaks of emerging pathogens in response to environmental perturbations such as heatwaves, have been recognised in aquaculture settings. Considerably less is known about how rising sea surface temperatures will impact the microbiology of wild fish populations, particularly those within estuarine systems that are more vulnerable to warming. We used a combination of *Vibrio*-specific quantitative PCR and amplicon sequencing of the 16S rRNA and *hsp60* genes to examine seawater and fish (*Pelates sexlineatus*) gut microbial communities across a quasi-natural experimental system, where thermal pollution from coal-fired power stations creates a temperature gradient of up to 6 °C, compatible with future predicted temperature increases. At the warmest site, fish hindgut microbial communities were in a state of dysbiosis characterised by shifts in beta diversity and a proliferation (71.5% relative abundance) of the potential fish pathogen *Photobacterium damselae* subsp. *damselae*. Comparable patterns were not identified in the surrounding seawater, indicating opportunistic proliferation within estuarine fish guts under thermal stress. A subsequent evaluation of predicted future warming-related risk due to pathogenic Vibrionaceae in temperate estuarine fish demonstrated that warming is likely to drive opportunistic pathogen increases in the upper latitudinal range of this estuarine fish, potentially impacting adaptations to future warming. These findings represent a breakthrough in our understanding of the dynamics of emerging pathogens in populations of wild aquatic organisms within environments likely to experience rapid warming under future climate change.

1. Introduction

Climate change is impacting estuarine systems globally, with increasing water temperatures documented in estuaries throughout North America (Najjar et al., 2010; Oczkowski et al., 2015), South Africa (James et al., 2008, 2013) and Australia (Scanes et al., 2020; Hallett et al., 2018). It is highly likely that anthropogenic activity is responsible for the rapid warming of the atmosphere and ocean since the mid-20th century, with continued emissions of greenhouse gases predicted to drive further warming and global change (Dowdy et al., 2015). Eastern Australian estuaries are warming faster than rates predicted by global ocean and atmospheric models (Collins et al., 2013), at 0.2 °C year^{-1} (Scanes et al., 2020), which has been attributed in part to the intensification of the East Australian Current (Ridgway, 2007). These rapid increases in water temperature have been predicted to impact the ecology and health of coastal environments in this region (Gillanders et al., 2011; Scanes et al., 2020).

Estuaries and nearshore coastal waters support productive fisheries, contributing a large proportion of commercial and aquaculture catch with a combined annual value of about USD 395.5 billion globally (FAO, 2018). Rapid warming of these systems, however, has the potential to threaten these ecological services (Hallett et al., 2018), and may additionally pose a significant health risk to both human and aquatic organisms through increases in the abundance of pathogenic bacteria, particularly within temperate regions (Baker-Austin et al., 2017). These increasing reports on the effects of climate change on Vibrionaceae abundance and virulence have been primarily focused on the most significant pathogens from a human health perspective: *Vibrio cholera, V. vulnificus, and V. parahaemolyticus* (Baker-Austin et al., 2013, 2017; Froelich and Daines, 2020). Research on Vibrionaceae in fish and shellfish has therefore been focused mostly on seafood contamination and the subsequent human health risk (Balter et al., 2006; Froelich and Daines, 2020; Jones and Oliver, 2009), rather than addressing ecological questions about the health of estuarine systems and the key organisms

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within these in response to global climate change.

Among aquatic pathogens, the Vibrionaceae contain a number of globally significant pathogenic species that cause illness in both humans and aquatic animals (Thompson et al., 2004a). Notably, there is evidence that the abundance and virulence of pathogenic members of the Vibrionaceae are increasing, primarily as a consequence of rising water temperatures (Baker-Austin et al., 2017). Within the Vibrionaceae, the genera *Vibrio* and *Photobacterium* have gained notoriety partly due to the multiple pathogenic species that cause disease in diverse marine organisms including *V. anguillarum, V. salmonicida, V. harveyi* and *Photobacterium damselae* (Terceti et al., 2016; Thompson et al., 2004a). Among these, the species *Photobacterium damselae* subsp. *damselae* (formerly *Vibrio damsela*), has been recognised as an emerging aquaculture pathogen, with outbreaks in fish species including turbot, rainbow trout, seabass and seabream recorded and sometimes related to increased water temperature during summer months (Matanza and Osorio, 2018; Rivas et al., 2013; Terceti et al., 2016). Despite these reports indicating that disease outbreaks are likely to increase in aquatic organisms with future climate induced warming and recent evidence that temperate estuaries such as those along the east coast of Australia may be more vulnerable to warming, little is known about how increasing water temperatures will influence key fish communities within these vulnerable systems.

Fish comprise the largest and most diverse group of vertebrates (Wong and Rawls, 2012), but currently represent an understudied group with regards to understanding the dynamics of their microbiome (Nayak, 2010). Gut microbial diversity has been used as a biomarker of fish health, with low diversity, stability and dysbiosis of gut microbiota closely associated with disease (Li et al., 2017; Nie et al., 2017; He et al., 2017). Fish disease and the impacts of shifts in the fish microbiome have been widely recognised in aquaculture settings (Infante-Villamil et al., 2021), but considerably less focus has been placed on the microbiology of wild fish populations, in particular those from temperate estuarine systems that are particularly vulnerable to rapid warming. Here we employed an urbanised temperate Australian estuary that is subject to thermal pollution from coal-fired power stations as a model experimental system for examining the impacts of elevated water temperature on the gut microbiome of *Pelates sexlineatus*, a common estuarine fish. The temperature gradients produced by these thermal discharges are comparable to predictions of up to 5.7 °C by 2090 for the Sydney region (Dowdy et al., 2015). *Pelates sexlineatus* was selected for this study due to its extensive distribution in estuaries along the east coast of Australia (Smith and Suthers, 2000; Trnski and Neira, 1998), and evidence for high site fidelity based on spatial dietary patterns and isotopic signatures (Suzzi et al., 2022; Sanchez-Jerez et al., 2002). We investigated Vibrionaceae communities associated with seawater and fish hindgut samples using a combination of *Vibrio*-specific quantitative PCR and amplicon sequencing of the 16S rRNA and *hsp60* genes, to examine the hypothesis that seawater and fish gut microbiomes from thermally affected sites will harbour a significantly greater abundance of potentially pathogenic Vibrionaceae. These findings contribute to the growing body of research on host-microbiome interactions and the emergence of aquatic pathogens with relevance to future predictions across coastal temperate regions globally.

2. Methods

2.1. Sample location

Lake Macquarie is a wave-dominated back-barrier coastal estuarine lake, located on the temperate southeast coast of Australia (33.1°S, 151°E) (Roy et al., 1980). This is Australia's largest coastal lake, with a surface area of 110 km^2 and maximum depth of 11 m. The lake has a narrow permanent entrance at Swansea, resulting in poor tidal exchange with the ocean, however, salinity ranges from 30 to 35 ppt due to minimal freshwater inputs (Spencer, 1959). Shallow waters across the width of the lake from Swansea to the western shores limit movement of deep water within the lake, resulting in a division of the lake into northern and southern portions with essentially independent water movements (DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT, 2021). Two coal-fired power stations, Vales Point and Eraring, discharge ~4000 ML/day and ~7000 ML/day of heated outlet water into receiving bays in southern Lake Macquarie at a maximum permissible temperature of 37.5 °C (Delta Electricity, 2020; Origin Energy, 2020), creating a temperature gradient of up to 6 °C across the lake (DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT, 2021). Lake Macquarie therefore provides an interesting and unique model environment, with this thermal pollution serving as an excellent within-environmental experimental system to study the impacts of increasing seawater temperatures on natural estuarine biota. Our sample site selection reflected this temperature gradient, with high temperature sites (HT.1 and HT.2) nearby receiving bays for Eraring and Vales Point respectively, mid temperature sites (MT.1 and MT.2) in the south-east of the lake, and low (ambient) temperature sites (LT.1 – LT.4) in the north of the lake.

2.2. Field sampling

Seawater was collected from each site in sterile bottles (rinsed in 10% bleach solution) for qPCR and amplicon sequencing (n = 5 per site; 30 samples in total). *Pelates sexlineatus*, a common estuarine fish inhabiting seagrass meadows along the south-east coast of New South Wales (Trnski and Neira, 1998; Smith and Suthers, 2000) were collected from each site using a 10 m seine net pulled through seagrass beds (n = 5 per site). Adults are known to move outside of estuaries to spawn (Smith and Suthers, 2000), but *Zostera muelleri* meadows are important nursery habitats for juveniles, where spatial patterns in diet according to local prey availabilities indicate high site fidelity (Sanchez-Jerez et al., 2002). At Mannering Park no fish were caught, likely due to a lack of suitable *Zostera muelleri* habitat, resulting in a total of 25 fish sampled throughout Lake Macquarie.

2.3. DNA extraction

Prior to dissection, total length (mm) and weight (g) of fish was measured, and Fulton's condition factor (*K*) was calculated using the following formula: $K = 100 \times W/L^3$, where W and L are the recorded weight and length, respectively. *P. sexlineatus* were then dissected, hindgut contents were removed, and fish were observed for physical signs of disease. Seawater samples were filtered via peristalsis onto 0.2 μm Sterivex filter units. Approximately 800 ml was filtered from each site due to particulate matter in water samples. Qiagen DNeasy PowerSoil and PowerWater kits were used to extract DNA from hindguts and seawater respectively, with sample quantity and quality checked using a NanoPhotometer NP80.

2.4. 16S rRNA gene sequencing

The microbial communities associated with seawater and fish hindguts were assessed by amplicon sequencing. The V3–V4 region of the 16S rRNA gene was amplified at the Ramaciotti Centre for Genomics; Sydney, NSW, using universal primers 341F (5′- CCTACGGGNGGCWGCAG-3′) and 805R (5′- GACTACHVGGGTATCTAATCC -3′) attached with Illumina adaptors in PCR under the following conditions: 95 °C for 3 min, then 25 cycles of: 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, then 72 °C for 5 min. PCR products were sequenced using an Illumina Miseq v3 (2 × 300bp) instrument, with resulting amplicons processed using the R pipeline DADA2 with default parameters (Callahan et al., 2016). Sequences were trimmed and denoised, chimeras were removed and contigs assembled. Resulting reads were clustered to produce ASVs (amplicon sequence variants, equivalent to unique bacterial strains) and sequences were aligned to the SILVA v132 database (Yilmaz et al., 2014)

for taxonomic assignment. The dataset was further cleaned by removing singletons and those identified as non-bacterial or chloroplasts. Cleaned data were then rarefied at 1432 reads per sample for hindguts and 159, 128 for seawater. This cut off for the hindgut samples allowed us to retain all samples while also retaining diversity within samples. We performed alpha and beta diversity analyses before and after rarefaction to account for rarefaction biases in diversity analyses (McMurdie and Holmes, 2014; Weiss et al., 2017), and report no differences between analyses carried out before and after rarefaction (see Supplementary Tables 1–4).

2.5. Hsp60 gene sequencing

The *hsp60* gene was amplified from samples in a 50 μl PCR reaction as follows: 10 μl of 5 x Hi-Fi Buffer (Bioline), 5 μl of 10 mM dNTPs, 2 μl of high-fidelity velocity polymerase (0.5 units μl^{-1}; Bioline), 2.5 μl of 10 μM forward primer (Vib-hspF3-23), 2.5 μl of 10 μM reverse primer (Vib-hspR401-422)(King et al., 2019), 2 μl of DNA template, with the remaining volume made up with sterile water. PCR conditions consisted of initial denaturation at 98 °C for 2 min, followed by 30 cycles of 98 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s and a final extension at 72 °C for 10 min. PCR products were purified with a Bioline Isolate II PCR and Gel Kit (catalog: BIO-52059) using the manufacturer's instructions. Amplicons were sequenced on a NovaSeq SP Lane, 500 cycle platform at AGRF. *Hsp60* sequencing reads were processed as outlined in (Kahlke, 2018). Paired-end DNA sequences were joined using FLASH (Magoč and Salzberg, 2011) and trimmed at 420 bp using Mothur (Schloss et al., 2009). Resulting fragments were clustered into OTUs (operational taxonomic units; 97%) and chimeric sequences were identified and removed using vsearch (Rognes et al., 2016). Taxonomy was assigned using QIIME (Caporaso et al., 2010) with the RDP classifier against a *Vibrio-hsp60* reference dataset (King et al., 2019) and custom *Photobacterium-hsp60* reference dataset created for this study. Raw data files in FASTQ format were deposited in NCBI Sequence Read Archive (SRA) under Bioproject number PRJNA893691. *Hsp60* data analysis pipeline is available at https://doi.org/10.17605/OSF.IO/4798P (King et al., 2019), and the custom *Photobacterium-hsp60* reference dataset is available at https://osf.io/qhkv5/?view_only=b951b8c79a10489fa71d0afc7817e720.

2.6. Quantitative PCR (qPCR)

Members of the Vibrionaceae were quantified using the 16S rRNA primer pair Vib1-f and Vib2-r (Thompson et al., 2004a). The primers were designed to amplify the *Vibrio* genus but at melting temperatures below 64 °C also amplify strains with 1–2 mismatches (Thompson et al., 2004b). These include closely related taxa including the *Photobacterium, Catenococcus,* and *Aliivibrio* (as identified by the Silva TestPrime tool https://www.arb-silva.de/search/testprime). We refer to this assay as total *Vibrio* throughout the study. Quantitative PCR was performed on a BIO-RAD CFX384 Touch™ Real-Time PCR Detection System™. Gene copies were calculated using a standard curve on BIO-RAD's CFX MAESTRO™ software version 1.1. Standard curves were generated from known concentrations of the targeted section of the gene. The qPCR was run in triplicate with SYBR assays consisting of 5 μl reaction volumes that consisted of 2.5 μl BIO-RAD iTaq UniversalSYBR® Green Supermix, 1.1 μl nuclease free water, 0.2 mM of each forward and reverse primer and 1 μl of undiluted seawater DNA template and diluted (1:20) hindgut DNA template. A dilution of 1:20 was made to eliminate the influence of DNA inhibitors on PCR amplification. This dilution was chosen after performing qPCR on a range of dilutions on samples and selecting the lowest dilution that provided consistent results. Calibration curves were run with every plate. Plate preparation was conducted using an epMotion® 5075 I Automated Liquid Handling System. QPCR cycling conditions consisted of an initial denaturation step at 95 °C for 3 min and then 45 cycles of: 95 °C for 15 s and then an annealing/extension temperature of 60 °C for 1 min followed by a melt curve to ensure amplification of a single product.

2.7. Mapping future vibrionaceae risk

In order to evaluate the potential risk of Vibrionaceae to the estuarine fish *P. sexlineatus* under future warming, we selected a thermal metric: the number of months exceeding an average sea surface temperature (SST) of 27 °C, given that annual average water temperature in the thermally affected sites sampled in Lake Macquarie where we report significant proliferations of *P. damselae* subsp. *damselae* within *P. sexlineatus* fish gut samples was >27 °C, compared to ~20 °C ambient. This risk metric was calculated for the near-future (2050) and far-future (2100) under RCP4.5 and 8.5 emissions scenarios, using CMIP5 global CSIRO-BOM ACESS1-0 Model (spatial resolution 0.5° × 0.5°), extracted from the Copernicus Climate Change Service (C3S) Climate Data Store (CDS) (Wouters et al., 2021). We interpolated this risk indicator for the available stations in the CMIP5 model over the latitudinal range of *P. sexlineatus* (27°–35° (O'Connor and Booth, 2021)), and produced maps for both near- and far-future horizons under RCP4.5 and 8.5 scenarios. The inverse distance weighting (IDW) interpolation method, which computes average variables in unmeasured sites using values from nearby weighted sites, was used here. The weights are proportional to the distance between the measured and unmeasured sites and are determined by the IDW power coefficient. The larger this coefficient is, the stronger the weights are attributed to the closest locations. A default power of $p = 2$ was selected. The root mean squared error (RMSE, gives an estimate of the standard deviation of the residuals (prediction errors) of the interpolation) for each horizon and RCP scenario was calculated using a leave-one-out procedure (see Supplementary Fig. 1).

2.8. Statistical analysis

Statistical analysis was carried out in R Studio (RStudio Team, 2020). To analyse differences in annual (2020) average water quality parameters across sample sites, analysis of variance (ANOVA) with Tukey's HSD were carried out using the 'aov' and 'TukeyHSD' functions in the 'vegan' package (Oksanen et al., 2020). Water quality data for 2020 was collected by Lake Macquarie City Council, as part of their monthly lake health monitoring program, and by a University of Newcastle owned temperature logger deployed at HT.1. Salinity, turbidity and pH was obtained from Lake Macquarie City Council's dataset, with the nearest collection site used as a proxy for salinity, pH and turbidity at each sample site in this study (see Supplementary Fig. 2). From each sample date (11 in total), 5 representative water quality measurements were selected from similar water column depths (~0.5 m), resulting in 55 water quality measurements from each site. Lake Macquarie has limited freshwater inputs as well as low tidal exchange (Spencer, 1959), with salinity only weakly negatively associated with increasing distance from the estuary mouth (see Supplementary Fig. 2). Microbial community data was analysed separately for fish hindgut and seawater samples using the 'phyloseq' package (McMurdie and Holmes, 2013). Data was rarefied using the 'rarefy even depth' function to the smallest sample size, and biodiversity metrics were subsequently calculated using rarefied sequence data. To investigate differences in community composition across sites, PERMANOVA was carried out using the 'adonis' function in the 'vegan' package (Oksanen et al., 2020), with pairwise comparisons carried out using the 'pairwise.perm.manova' function in the 'RVAideMemoire' package (Hervé and Hervé, 2020). ANOVA and Tukey's HSD were used to compare alpha diversity (Shannon's diversity) as well as ASVs most closely matched (National Centre for Biotechnology Information (NCBI) blast search) to *Photobacterium damselae* across sample sites. Pearson correlations were employed to test for significant correlations between alpha (Shannon's diversity) and beta diversity (Bray Curtis similarity) and seawater temperature. Finally, differences in abundances of total *Vibrio* between sites and

sample type (fish hindguts and seawater) were determined using ANOVA and Tukey's HSD, with abundance tested for association with environmental parameters using linear regression and Pearson correlation.

3. Results

3.1. Variation in environmental conditions throughout Lake Macquarie

Significant spatial variation in seawater temperature occurs throughout the lake (one-way ANOVA, $F_{7,432} = 3.362$, $p < 0.01$; Fig. 1; Supplementary Tables 5 and 6), with average annual temperatures at the

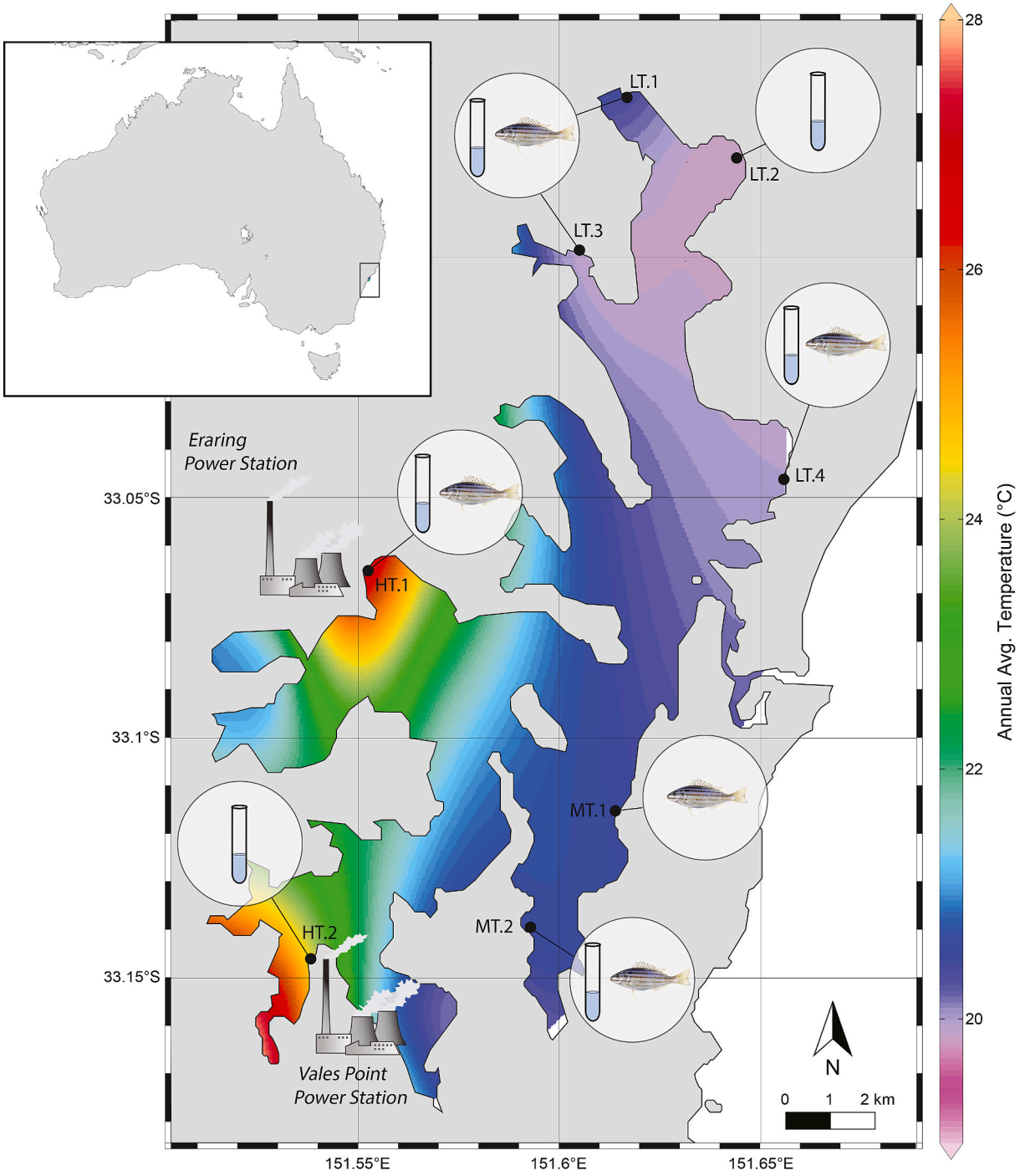

Fig. 1. Sample sites and location of power stations in Lake Macquarie, eastern Australia. Sample sites were selected according to the temperature gradient across the lake, with high temperature sites (HT.1 and HT.2) selected for their proximity to power station outflows, mid temperature sites (MT.1 and MT.2) selected in southern Lake Macquarie, and low (ambient) temperature sites (LT.1 – LT.4) selected in the north. Fish and water test tube icons indicate where *Pelates sexlineatus* and seawater samples were collected, respectively. Fish could not be collected at HT.2, likely due to a lack of suitable seagrass habitat. Base map indicates annual average temperature throughout Lake Macquarie for 2020. Temperature data was collected by Lake Macquarie City Council as part of their monthly lake health monitoring program at 13 sites around the lake, and a University of Newcastle owned temperature logger at HT.1 (map produced in Ocean Data View (Schlitzer, 2021) using these 14 data points).

sites closest to the input of power station effluent (HT.1 and HT.2) up to 7.42 °C warmer (27.30 °C ± 4.70 and 26.55 °C ± 4.57 respectively) than at sites elsewhere in the lake ($p < 0.05$). Annual average salinity and turbidity also differed significantly between sites ($F_{4,270} = 3.362$, $p < 0.01$; $F_{4,270} = 85.17$, $p < 0.01$, respectively), but pH did not ($F_{4,270} = 2.096$, $p = 0.08$; Supplementary Tables 5 and 6). Annual average salinity ranged from 33.41 to 34.23 ppt across sites and turbidity between 0.50 and 2.50 NTU (Supplementary Table 5).

Composition of *Pelates sexlineatus* hindgut bacterial communities differed significantly by site (PERMANOVA, $F_{5,23} = 2.5287$, $p < 0.01$), with samples from HT.1, the site most heavily impacted by thermal pollution, more tightly clustered and distinct from samples collected elsewhere in the lake ($p < 0.05$; Fig. 2A). Bray Curtis similarity further demonstrated a significant positive association with seawater temperature (Pearson correlation, $R = 0.39$, $R^2 = 0.14$, $p = 0.03$), highlighting the influence of temperature on the gut microbiome. Alpha diversity (Shannon's diversity) of *P. sexlineatus* hindgut microbiome did not differ significantly between sample sites (one way ANOVA, $F_{5,23} = 1.754$, $p = 0.16$) but Pearson correlation revealed a significant negative correlation between Shannon's diversity and seawater temperature ($R = -0.44$, $R^2 = 0.19$, $p = 0.01$). Fulton's condition factor did not vary significantly across the lake (one way ANOVA, $F_{5,24} = 1.258$, $p = 0.31$), however fish were generally longer at warmer seawater temperatures in southern Lake Macquarie ($F_{5,24} = 4.615$, $p < 0.01$; Supplementary Table 7).

3.2. *P. sexlineatus* hindgut microbiome is dominated by vibrionaceae

Across all samples from the Lake Macquarie estuary, the gut microbiome of *P. sexlineatus* was dominated by the bacterial family Vibrionaceae, which made up 63.06 ± 5.92% relative abundance according to 16S rRNA amplicon sequencing analysis (Fig. 2C). However, the relative abundance of Vibrionaceae increased significantly in fish from the thermally affected site HT.1 (99.29 ± 0.23% relative abundance) (Fig. 2C). The Amplicon Sequence Variants (ASVs) responsible for this increase in Vibrionaceae at HT.1 were most closely matched to *Photobacterium damselae* (Accession numbers MT549173.1, CP046752.1, MN049748.1) (one way ANOVA, $F_{5,23} = 10.61$, $p < 0.01$; Fig. 2C, Supplementary Tables 8 and 9). ASVs matched most closely to *Photobacterium damselae* accounted for 71.50 ± 3.23% relative abundance of bacterial communities within *P. sexlineatus* gut microbiome samples at HT.1, compared to only 11.89 ± 6.44 in the northern sites (LT.1, LT.3 and LT.4) and 13.65 ± 4.61% in the south-east sites (MT.1 and MT.2). In contrast to the high levels observed in fish gut samples, Vibrionaceae only comprised 0.60 ± 0.23% mean relative abundance in 16S rRNA

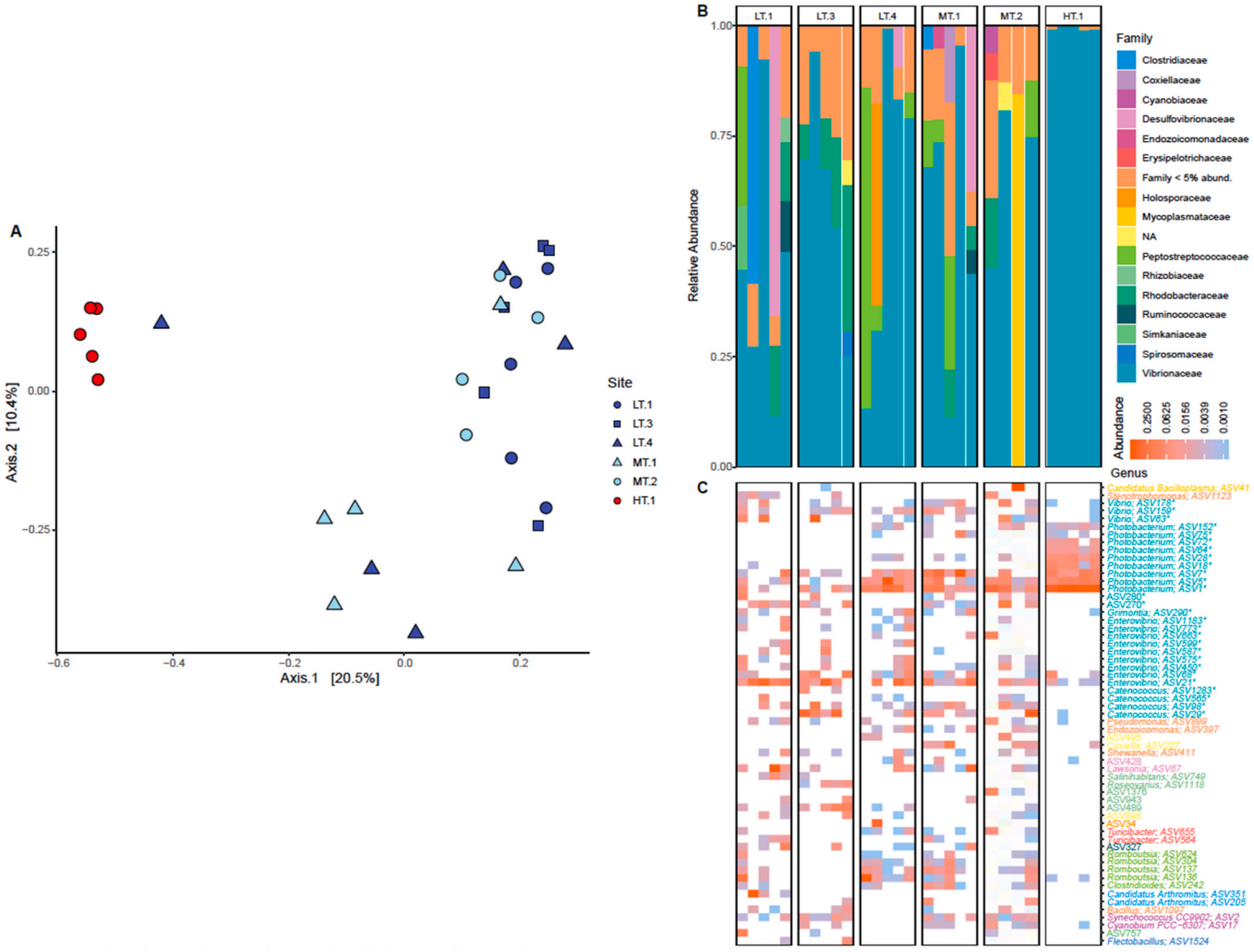

Fig. 2. A) Principal coordinate analysis (PCoA) plot based on Bray Curtis similarity of *Pelates sexlineatus* hindgut microbiome samples throughout Lake Macquarie sample sites, and relative abundance of dominant (>5% sequence abundance) B) bacterial families and C) genera within *P. sexlineatus* hindgut microbiome throughout Lake Macquarie. Names of genera in C are colour coded to match bacterial families in B, and asterisks indicate genera within the Vibrionaceae family. HT.1: high temperature sites, MT.1 and MT.2: mid temperature sites, and LT.1-LT3: low (ambient) temperature sites. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

libraries derived from seawater samples (Fig. 3). Instead, the seawater bacterial communities were dominated by Cyanobiaceae (10.68 ± 0.93%), Rhodobacteraceae (9.30 ± 0.75%), and SAR11 clade I (7.59 ± 0.72%) (Fig. 3).

To acquire greater taxonomic resolution and discrimination of species from the Vibrio and Photobacterium genera than 16S rRNA sequencing, we next applied a Vibrio-specific hsp60 amplicon sequencing assay (King et al., 2019). This analysis confirmed the dominance of Photobacterium damselae subsp. damselae in fish gut microbiome samples from all sites, with P. angustum, Vibrio alfacsensis, V. campbelli, V. diabolicus, V. ponticus, and V. mediterranei also detected in fish from HT.1, and in fish elsewhere in the lake (Fig. 4A). Within seawater samples, 20 Vibrio species were identified via hsp60 sequencing (Fig. 4B) with no patterns identified between thermally affected sites (Fig. 4B). No Photobacterium damselae subsp. damselae were detected in any seawater samples throughout the study.

3.3. Abundance of vibrio increases with elevated water temperature

Given the sequencing analyses delivered evidence for a shift toward a *Vibrio* dominated bacterial assemblage in the fish gut samples, we next quantified the total abundance of *Vibrio* within samples using quantitative PCR. Total *Vibrio* gene copies in *P. sexlineatus* hindgut samples varied significantly across sample sites (one way ANOVA, $F_{5,23} = 9.647$, $p < 0.01$; Fig. 4C), and on average, were 1.33×10^{11} g^{-1} greater in fish from the thermally affected site than elsewhere in Lake Macquarie ($p < 0.05$). Total *Vibrio* gene copies in seawater samples did not differ significantly between sites throughout Lake Macquarie ($F_{6,28} = 1.023$, $p = 0.431$; Fig. 4D), and were significantly lower than in fish hindgut samples ($F_{1,46} = 5.207$, $p < 0.05$), ranging from 0–1.71×10^5 gene copies ml^{-1}. While temperature is most strongly associated with Vibrionaceae abundance and growth, salinity and turbidity were also found to differ significantly among sites in Lake Macquarie and were therefore further investigated for individual and combined effects on total *Vibrio* gene copies using multiple linear regressions (Supplementary Tables 10 and 11). For both fish hindgut and seawater samples, annual average water temperature was the strongest driver of variation in total *Vibrio* abundance (fish hindgut: $p < 0.01$, $R^2 = 0.54$; seawater: $p < 0.05$, $R^2 = 0.17$; Supplementary Tables 10 and 11), while salinity and turbidity were not identified as significant drivers.

3.4. Risk scenarios of vibrionaceae across P. sexlineatus latitudinal range

We next estimated future risk of Vibrionaceae proliferation for this estuarine fish by calculating the number of months with an average SST exceeding this threshold of 27 °C as a risk indicator. Then, we interpolated values of this risk indicator for near-future (2050) and far-future (2100) horizons under both RCP4.5 and 8.5 scenarios for the latitudinal range of *P. sexlineatus* along the east-coast of Australia (O'Connor and Booth, 2021) (Fig. 5). The results of this risk interpolation suggest that for the 2050 horizon, Vibrionaceae growth risk in the fish gut microbiome is likely to increase within the northern limits of *P. sexlineatus* range, with 25% of the mapped latitudinal range predicted to exceed monthly SST averages of 27 °C for at least 3 months of the year

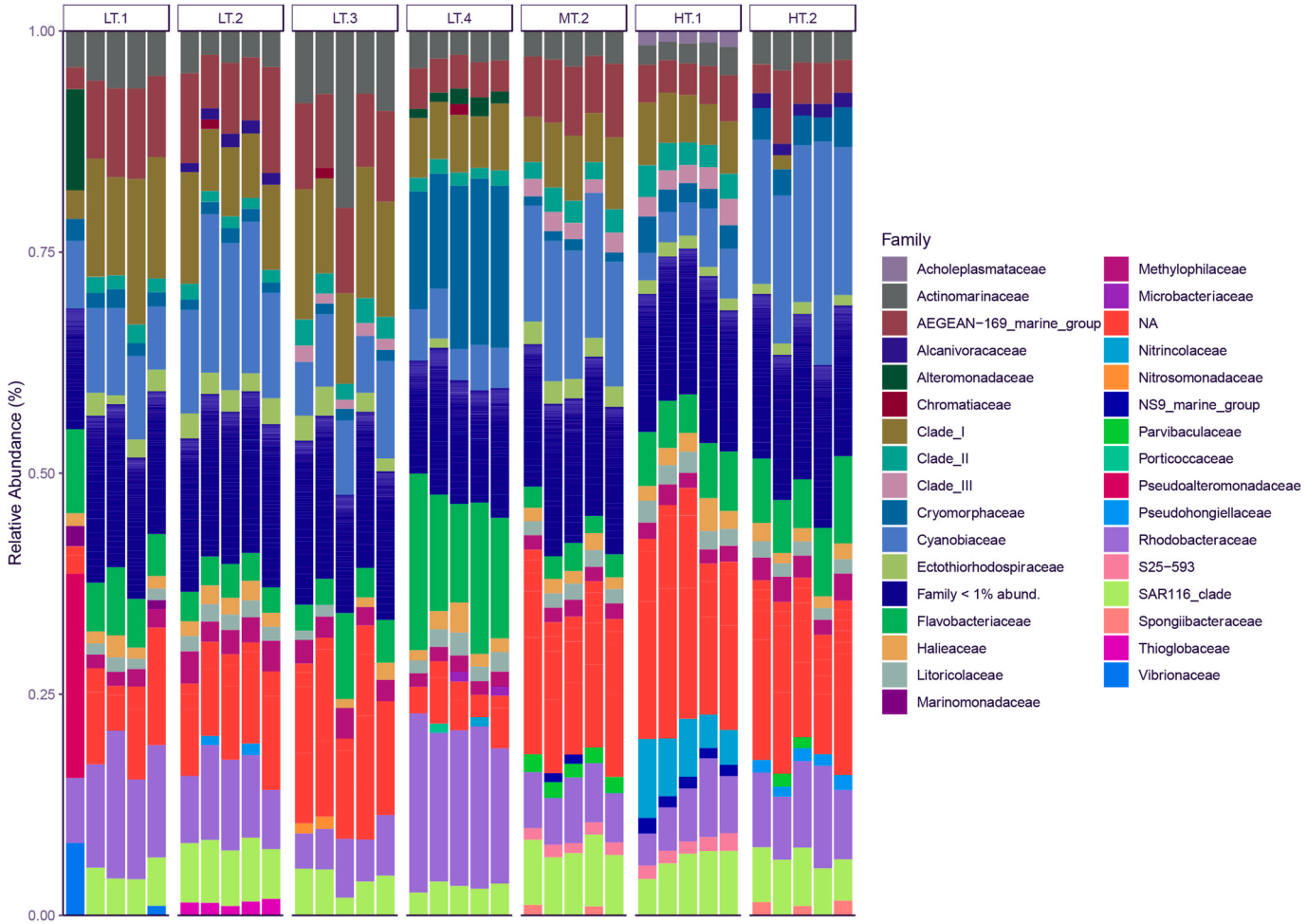

Fig. 3. Relative abundance of dominant bacterial families (>1% relative sequence abundance) within seawater samples collected from sites throughout Lake Macquarie (n = 5). HT.1 and HT.2: high temperature sites, MT.2: mid temperature site, and LT.1-LT4: low (ambient) temperature sites.

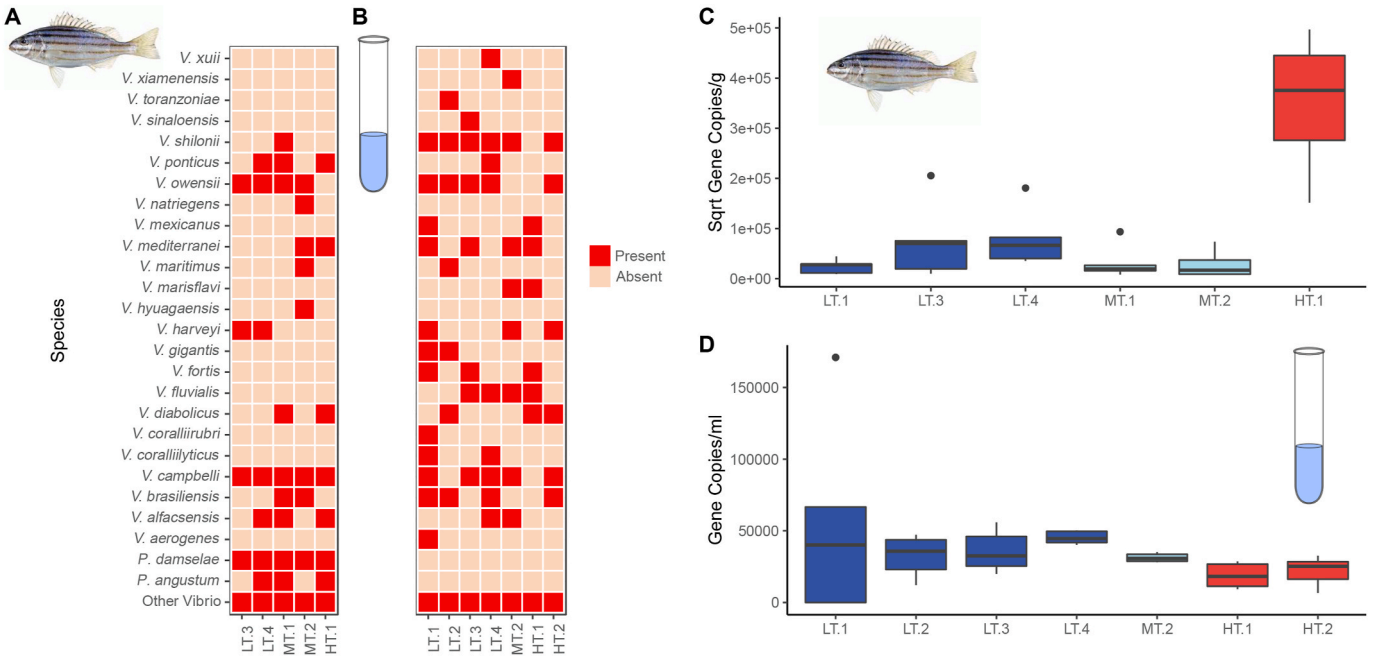

Fig. 4. Presence/absence of *Vibrio* and *Photobacterium* species in A) *Pelates sexlineatus* hindgut samples and B) seawater samples determined by *hsp60* sequencing; and total *Vibrio* gene copies in C) *P. sexlineatus* hindgut and D) seawater samples determined by qPCR. HT.1 and HT.2: high temperature sites, MT.1 and MT.2: mid temperature site, and LT.1-LT4: low (ambient) temperature sites.

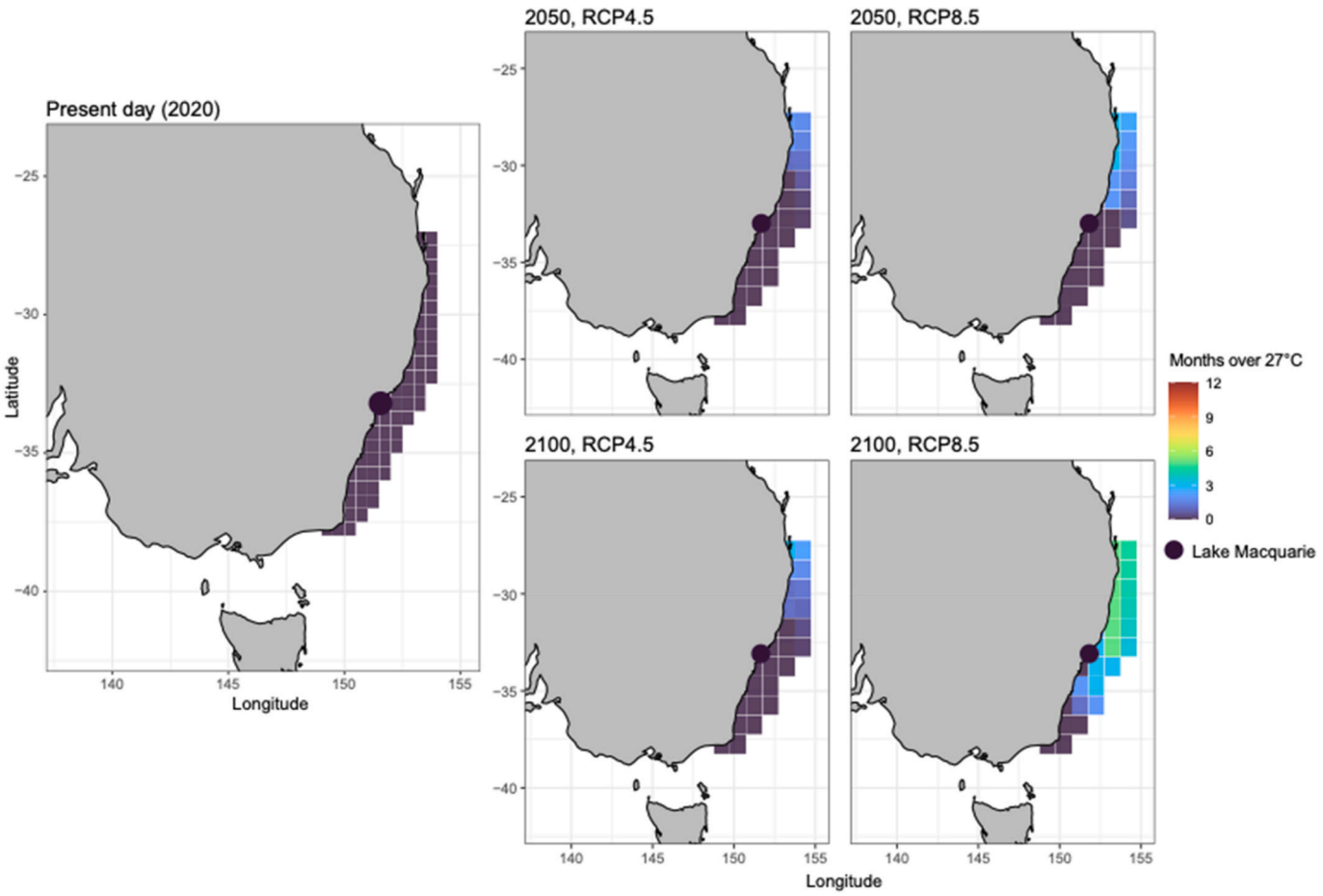

Fig. 5. Inverse distance weighting (IDW) interpolation of risk indicator (number of months exceeding an average monthly sea surface temperature (SST) of 27 °C) across the latitudinal range of *Pelates sexlineatus* under Representative Concentration Pathway (RCP) 4.5 and 8.5 climate scenarios for the near-future (2050) and far-future (2100) horizons.

under RCP8.5 scenario and 7.14% under RCP4.5. The risk indicator increases for the far-future horizon in the mid – northern limits of *P. sexlineatus* range regardless of the RCP scenario, with projected monthly mean SSTs exceeding 27 °C for over 3 months of the year for 10.71% of the mapped latitudinal range under RCP4.5 scenario and 53.57% of the range under RCP8.5.

4. Discussion

There is growing evidence that rapid climate induced warming of temperate coastal systems is increasing the abundance and virulence of pathogenic members of the Vibrionaceae, posing a significant risk to diverse aquatic organisms (Baker-Austin et al., 2017; Thompson et al., 2004b). We demonstrate here that for wild Pelates sexlineatus populations, elevated estuary water temperatures drive shifts in beta diversity coupled with a significant proliferation of an emerging aquaculture pathogen Photobacterium damselae subp. damselae. Abundances of Vibrionaceae in seawater samples were not influenced by elevated water temperatures, indicating opportunistic proliferation within estuarine fish guts under favourable environmental conditions. Risk projections of Vibrionaceae growth indicate that future warming is likely to drive increases of potential pathogens in the upper latitudinal range of this estuarine fish under future climate scenarios with likely implications for adaptations to future warming.

4.1. Elevated water temperatures drive shifts in the hindgut microbiome

Sea surface temperatures for the East Coast region nearby Sydney (151.23°E, −33.86°S) are predicted to increase by between 1.2 and 2.9 °C under RCP (Representative Concentration Pathway, a greenhouse gas concentration trajectory adopted by the IPCC) 4.5 and 2.8–5.7 °C under RCP8.5 by the year 2090 (Dowdy et al., 2015). The elevated temperatures recorded in southern Lake Macquarie at sites subject to power station thermal pollution are consistent with these projected increases for the region, and we demonstrate that these elevated temperatures are associated with increased homogeneity of the fish gut microbiome at the thermally affected site. This shift in beta diversity is consistent with that frequently observed for stressed or diseased host organisms, however it is currently unclear whether these shifts are a cause or consequence of disease (Xiong et al., 2019). Nevertheless, such shifts in the gut microbiome have the potential to decrease resilience against potential pathogens given that more diverse microbial communities are hypothesized to exert greater protective effects on the host, reducing colonization by opportunistic pathogens (Li et al., 2017; Xiong et al., 2019). Exposure to thermal stress in experimental settings has led to similar reductions in gut microbiome diversity as well as dominance of potential pathogens (typically the *Vibrio* genus) (Hassenrück et al., 2020; Nie et al., 2017). These shifts at the thermally affected site further indicate that there may be thermal thresholds or trigger values for temperature, above which there are shifts in diversity, stability and immune response of the fish gut microbiome, leading to dysbiosis. Thermal thresholds or trigger values have been reported for other marine hosts including sea anemones, sponges and corals (Hartman et al., 2019; Ramsby et al., 2018; Savary et al., 2021; Webster et al., 2008), where associated microbiomes are generally stable until a thermal threshold is surpassed, after which richness, beta diversity and relative abundance of taxa is affected. In laboratory-based warming experiments, *P. sexlineatus* growth rate has been shown to increase at seawater temperatures of 26 °C potentially due to its latitudinal range extending into subtropical regions, suggesting the ability to acclimate to future temperature predictions in temperate regions (O'Connor and Booth, 2021). While Fulton's condition factor did not vary significantly here across the lake our findings are consistent with increased fish length at warmer seawater temperatures in southern Lake Macquarie, and the shifts in gut microbial diversity reported here offer a unique perspective for thermal thresholds which may influence host health and future responses to climate induced warming in estuarine fish.

4.2. Elevated water temperatures drive opportunistic proliferation of P. damselae subsp. Damselae within fish

In several fish species, *P. damselae* subsp. *damselae* causes haemorrhaging and ulcerative lesions around the mouth and pectoral fins and may be fatal in short time frames (i.e., within 24 h) (Rivas et al., 2013; Terceti et al., 2016), but in some species it also appears to be a commensal member of the gut microbiome (Grimes et al., 1985). Similar findings have been reported in wild mullet, where high occurrences of this bacterium were found in otherwise healthy individuals (Serracca et al., 2011). Unfavourable environmental conditions such as further increases in temperature are hypothesized to lead to disease outbreaks, given the role of elevated temperature in driving rapid increases in abundance to bacterial populations capable of causing disease, as well as upregulating virulence-related factors in *P. damselae* subsp. *damselae* (Matanza and Osorio, 2018) and several *Vibrio* species (Kimes et al., 2012; Mahoney et al., 2010; Oh et al., 2009). These observations are significant given that we observed *P. damselae* subsp. *damselae* in the gut microbiome of *P. sexlineatus* at control sites, yet at sites characterised by elevated temperatures the *P. sexlineatus* gut microbiome demonstrated a significant disruption in community structure characterised by low diversity and the proliferation of *P. damselae* subsp. *damselae* ASVs. Notably, seawater is thought to be an important vector for transmission of *P. damselae* (Fouz et al., 2000), however we did not detect this species in any seawater samples throughout the study, indicating opportunistic proliferation within the fish gut microbiome under elevated water temperatures.

4.3. Implications under future warming

While we did not observe any physical signs of disease in the fish sampled from HT.1, dysbiosis of the gut microbiome has repeatedly been shown to impact host metabolism and immunity (Xiong et al., 2019). Elevated water temperature promoting the overgrowth of *P. damselae* subsp. *damselae* is also a cause of concern, given that additional stressors could influence pathogenicity or virulence, resulting in disease outbreaks (Serracca et al., 2011; Matanza and Osorio, 2018; Ben-Haim et al., 2003; Kimes et al., 2012). Given the potential for sublethal effects and development of disease, we propose that a shift towards a Vibrionaceae-dominated hindgut community under elevated water temperature poses a risk to fish populations. Despite increased growth rates of *P. sexlineatus* at seawater temperatures of 26 °C indicating an ability to acclimate to future warming scenarios (O'Connor and Booth, 2021), the results of the Vibrionaceae risk projections indicate that in the upper limits of their range, *P. sexlineatus* individuals are likely to be at increased risk of Vibrionaceae growth, with potential implications for host fitness, immunity and subsequent ability to adapt to future change. Considering the significance of *P. damselae* subsp. *damselae* as an emerging aquaculture pathogen along with the rapid warming of temperature coastal systems, these findings represent advancements in our knowledge of the emergence and dynamics of globally significant pathogens in key host-microbe interactions within vulnerable estuarine systems.

This study focused on sea surface temperatures for the ocean as temperature projection data is not available for estuaries, and warming rates in estuaries vary depending on estuary type due to variables such as depth, volume, retention factor and flushing time (Scanes et al., 2020). While east Australian estuaries are generally warming more rapidly than oceans, most of this warming is concentrated to smaller systems (lagoons, rivers), with larger lake systems experiencing less change (Scanes et al., 2020). Temperature data collected across Lake Macquarie indicates that warming is occurring at approximately the same rate as ocean warming from 1970 to 2010 (+0.015 °C per year) (DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT,

2021), validating our use of projected ocean SST to predict the risk that Vibrionaceae may pose to estuarine fish along the east coast of Australia in similar systems. However, we note that smaller estuarine systems warming more rapidly are likely to be under greater threat. We also note that these findings are based on a single sampling event, and here we focused on water temperature as the main factor affecting Vibrionaceae proliferation given that Lake Macquarie generally experiences low volumes of freshwater inputs and salinity within the system is typically close to marine values (DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT, 2020). Increased storm events and rainfall associated with global climate change have also been recognised as factors influencing Vibrionaceae growth and abundance (Takemura et al., 2014). Further investigations into the combined effects of climate change on estuarine host-microbiome interactions over time are therefore recommended to better predict future risk of Vibrionaceae proliferation within temperate systems.

5. Conclusion

Climate change induced warming and emerging pathogens are two of the greatest threats to the stability of wildlife populations globally (Aguirre and Tabor, 2008). Temperature driven increases in the occurrence of the Vibrionaceae have drawn attention to the potential risk that future warming has on aquatic coastal species, particularly in temperate systems experiencing rapid warming such as south-east Australia (Baker-Austin et al., 2017). Here, thermal pollution gradients in a coastal lake provided a natural experimental system for assessing the likely impacts of climate change induced warming, and we demonstrate that this thermal pollution had a profound impact on both the composition and diversity of *P. sexlineatus* hindgut microbiome, with a significant increase in total *Vibrio* gene copies and an overrepresentation of the marine fish pathogen *Photobacterium damselae* subsp. *damselae*. Conversely, microbial communities and abundance of the Vibrionaceae associated with seawater samples were not affected by thermal pollution, and *P. damselae* subsp. *damselae* were not identified in seawater throughout the study, indicating that *P. damselae* subsp. *damselae* undertakes opportunistic proliferation within estuarine fish guts under thermal stress (Matanza and Osorio, 2018). By establishing links between warming and dysbiosis of the fish gut microbiome in a natural experimental estuarine system, our results provide a unique perspective on potential future interactions between climate induced warming and the dynamics of emerging pathogens in wild aquatic organisms.

Credit author statement

Alessandra L Suzzi: Conceptualisation, Investigation, Formal analysis, Writing – original draft, Writing – review & editing, Funding acquisition. Michael Stat: Conceptualisation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. Troy F Gaston: Conceptualisation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition. Nachshon Siboni: Investigation, Resources, Writing – review & editing. Nathan L R Williams: Investigation, Resources, Writing – review & editing. Justin R Seymour: Resources, Writing – review & editing. Megan J Huggett: Conceptualisation, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw sequence data in fastq format is available in the NCBI SRA and hsp60 data analysis pipeline and reference datasets are available at OFS. Links are provided in the methods of the mansucript.

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Appendix A. Supplementary data

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References

Aguirre, A.A., Tabor, G.M., 2008. Global factors driving emerging infectious diseases: impact on wildlife populations. Ann. N. Y. Acad. Sci. 1149, 1–3.
Baker-Austin, C., Trinanes, J.A., Taylor, N.G.H., Hartnell, R., Siitonen, A., Martinez-Urtaza, J., 2013. Emerging Vibrio risk at high latitudes in response to ocean warming. Nat. Clim. Change 3, 73–77.
Baker-Austin, C., Trinanes, J., Gonzalez-Escalona, N., Martinez-Urtaza, J., 2017. Non-cholera vibrios: the microbial barometer of climate change. Trends Microbiol. 25, 76–84.
Balter, S., Hanson, H., Kornstein, L., Lee, L., Reddy, V., Sahl, S., Stavisky, F., Fage, M., Johnson, G., Bancroft, J., 2006. Vibrio parahaemolyticus infections associated with consumption of raw shellfish–three states, 2006. MMWR (Morb. Mortal. Wkly. Rep.) 55, 854–857.
Ben-Haim, Y., Zicherman-Keren, M., Rosenberg, E., 2003. Temperature-regulated bleaching and lysis of the coral Pocillopora damicornis by the novel pathogen Vibrio coralliilyticus. Appl. Environ. Microbiol. 69, 4236–4242.
Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583.
Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Peña, A.G., Goodrich, J.K., Gordon, J.I., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7, 335–336.
Collins, M., Knutti, R., Arblaster, J., Dufresne, J.-L., Richefet, T., Friedlingstein, P., Gao, X., Gutowski, W.J., Johns, T., Krinner, G., Shongwe, M., Tebaldi, C., Weaver, A. J., Wehner, M., 2013. Long-term climate change: projections, commitments and irreversibility. In: STOCKER, T.F., QIN, D., PLATTNER, G.-K., TIGNOR, M., ALLEN, S.K., DOSCHUNG, J., NAUELS, A., XIA, Y., BEX, V., MIDGLEY, P.M. (Eds.), Climate Change 2013: the Physical Science Bases. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.
DELTA ELECTRICITY, 2020. Vales Point Power Station Monthly Environmental Data Summary. EPA Licence 761.
DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT, 2020. State of the Estuary Lake Macquarie 2020. State of NSW and Department of Planning, Industry and Environment.
DEPARTMENT OF PLANNING INDUSTRY AND ENVIRONMENT, 2021. State of the Estuary Lake Macquarie 2020. State of NSW and Department of Planning, Industry and the Environment.
Dowdy, A., Abbs, D., Bhend, J., Chiew, F., Church, J., Ekström, M., Kirono, D., Lenton, A., Lucas, C., Mcinnes, K., Moise, A., 2015. East coast cluster report. In: Climate Change in Australia Projections for Australia's Natural Resource Management Regions: Cluster Reports.
FAO, 2018. The State of World Fisheries and Aquaculture. Food and Agriculture Organisation of the United Nations, Rome, Italy.
Fouz, B., Toranzo, A.E., Milan, M., Amaro, C., 2000. Evidence that water transmits the disease caused by the fish pathogen Photobacterium damselae subsp. damselae. J. Appl. Microbiol. 88, 531–535.
Froelich, B.A., Daines, D.A., 2020. In hot water: effects of climate change on Vibrio–human interactions. Environ. Microbiol. 22, 4101–4111.
Gillanders, B.M., Elsdon, T.S., Halliday, I.A., Jenkins, G.P., Robins, J.B., Valesini, F.J., 2011. Potential effects of climate change on Australian estuaries and fish utilising estuaries: a review. Mar. Freshw. Res. 62, 1115–1131.
Grimes, D.J., Brayton, P., Colwell, R.R., Gruber, S.H., 1985. Vibrios as autochthonous flora of neritic sharks. Syst. Appl. Microbiol. 6, 221–226.
Hallett, C.S., Hobday, A.J., Tweedley, J.R., Thompson, P.A., Mcmahon, K., Valesini, F.J., 2018. Observed and predicted impacts of climate change on the estuaries of south-western Australia, a Mediterranean climate region. Reg. Environ. Change 18, 1357–1373.
Hartman, L.M., VAN, O.P.P.E.N., , M. J. H., Blackall, L.L., 2019. The effect of thermal stress on the bacterial microbiome of Exaiptasia diaphana. Microorganisms 8, 20.

Hassenrück, C., Reinwald, H., Kunzmann, A., Tiedemann, I., Gärdes, A., 2020. Effects of thermal stress on the gut microbiome of Juvenile Milkfish (Chanos chanos). Microorganisms 9, 5.
He, S., Wang, Q., Li, S., Ran, C., Guo, X., Zhang, Z., Zhou, Z., 2017. Antibiotic growth promoter olaquindox increases pathogen susceptibility in fish by inducing gut microbiota dysbiosis. Sci. China Life Sci. 60, 1260–1270.
Infante-Villamil, S., Huerlimann, R., Jerry, D.R., 2021. Microbiome diversity and dysbiosis in aquaculture. Rev. Aquacult. 13, 1077–1096.
James, N.C., Whitfield, A.K., Cowley, P.D., 2008. Preliminary indications of climate-induced change in a warm-temperate South African estuarine fish community. J. Fish. Biol. 72, 1855–1863.
James, N.C., VAN Niekerk, L., Whitfield, A.K., Potts, W.M., Götz, A., Paterson, A.W., 2013. Effects of climate change on South African estuaries and associated fish species. Clim. Res. 57, 233–248.
Jones, M.K., Oliver, J.D., 2009. Vibrio vulnificus: disease and pathogenesis. Infect. Immun. 77, 1723–1733.
Kahlke, T., 2018. Ampli-Tools. Zenodo, Geneva, p. 10, Version 1.0.
Kimes, N.E., Grim, C.J., Johnson, W.R., Hasan, N.A., Tall, B.D., Kothary, M.H., Kiss, H., Munk, A.C., Tapia, R., Green, L., 2012. Temperature regulation of virulence factors in the pathogen Vibrio coralliilyticus. ISME J. 6, 835–846.
King, W.L., Siboni, N., Kahlke, T., Green, T.J., Labbate, M., Seymour, J.R., 2019. A new high throughput sequencing assay for characterizing the diversity of natural Vibrio communities and its application to a Pacific oyster mortality event. Front. Microbiol. 10, 2907.
Li, T., Li, H., Gatesoupe, F.-J., She, R., Lin, Q., Yan, X., Li, J., Li, X., 2017. Bacterial signatures of "Red-Operculum" disease in the gut of crucian carp (Carassius auratus). Microb. Ecol. 74, 510–521.
Magoč, T., Salzberg, S.L., 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27, 2957–2963.
Mahoney, J.C., Gerding, M.J., Jones, S.H., Whistler, C.A., 2010. Comparison of the pathogenic potentials of environmental and clinical Vibrio parahaemolyticus strains indicates a role for temperature regulation in virulence. Appl. Environ. Microbiol. 76, 7459–7465.
Matanza, X.M., Osorio, C.R., 2018. Transcriptome changes in response to temperature in the fish pathogen Photobacterium damselae subsp. damselae: clues to understand the emergence of disease outbreaks at increased seawater temperatures. PLoS One 13, e0210118.
Mcmurdie, P.J., Holmes, S., 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8, e61217.
Mcmurdie, P.J., Holmes, S., 2014. Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput. Biol. 10, e1003531.
Najjar, R.G., Pyke, C.R., Adams, M.B., Breitburg, D., Hershner, C., Kemp, M., Howarth, R., Mulholland, M.R., Paolisso, M., Secor, D., 2010. Potential climate-change impacts on the chesapeake bay. Estuar. Coast Shelf Sci. 86, 1–20.
Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. Aquacult. Res. 41, 1553–1573.
Nie, L., Zhou, Q.J., Qiao, Y., Chen, J., 2017. Interplay between the gut microbiota and immune responses of ayu (Plecoglossus altivelis) during Vibrio anguillarum infection. Fish Shellfish Immunol. 68, 479–487.
O'Connor, C., Booth, D.J., 2021. Response of estuarine fishes to elevated temperatures within temperate Australia: implications of climate change on fish growth and foraging performance. J. Exp. Mar. Biol. Ecol. 544, 151626.
Oczkowski, A., Mckinney, R., Ayvazian, S., Hanson, A., Wigand, C., Markham, E., 2015. Preliminary evidence for the amplification of global warming in shallow, intertidal estuarine waters. PLoS One 10, e0141529.
Oh, M.H., Lee, S.M., Lee, D.H., Choi, S.H., 2009. Regulation of the Vibrio vulnificus hupA gene by temperature alteration and cyclic AMP receptor protein and evaluation of its role in virulence. Infect. Immun. 77, 1208–1215.
Oksanen, J., Simpson, G.L., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., DE Caceres, M., Durand, S., Evangelista, H.B.A., 2020. Vegan: community ecology package. R Package Version 2 6.
ORIGIN ENERGY, 2020. Eraring Power Station EPA Licence 1429 - Environmental Monitoring Data December 2020.
Hervé, M., Hervé, M.M., 2020. Package 'RVAideMemoire'. https://CRANR-projectorg/package= RVAideMemoire.
Ramsby, B.D., Hoogenboom, M.O., Whalan, S., Webster, N.S., 2018. Elevated seawater temperature disrupts the microbiome of an ecologically important bioeroding sponge. Mol. Ecol. 27, 2124–2137.
Ridgway, K.R., 2007. Long-term trend and decadal variability of the southward penetration of the East Australian Current. Geophys. Res. Lett. 34.
Rivas, A.J., Lemos, M.L., Osorio, C.R., 2013. Photobacterium damselae subsp. damselae, a bacterium pathogenic for marine animals and humans. Front. Microbiol. 4, 283.
Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 4, e2584.
Roy, P., Thom, B., Wright, L., 1980. Holocene sequences on an embayed high-energy coast: an evolutionary model. Sediment. Geol. 26, 1–19.
Rstudio TEAM, 2020. RStudio: Integrated Development for R. In: RSTUDIO. PBC, Boston, MA.
Sanchez-Jerez, P., Gillanders, B., Kingsford, M., 2002. Spatial variation in abundance of prey and diet of trumpeter (Pelates sexlineatus: teraponidae) associated with Zostera capricorni seagrass meadows. Austral Ecol. 27, 200–210.
Savary, R., Barshis, D.J., Voolstra, C.R., Cárdenas, A., Evensen, N.R., Banc-Prandi, G., Fine, M., Meibom, A., 2021. Fast and pervasive transcriptomic resilience and acclimation of extremely heat-tolerant coral holobionts from the northern Red Sea. Proc. Natl. Acad. Sci. USA 118, e2023298118.
Scanes, E., Scanes, P.R., Ross, P.M., 2020. Climate change rapidly warms and acidifies Australian estuaries. Nat. Commun. 11, 1803.
Schlitzer, R., 2021. *Ocean Data View* [Online]. Available: https://odv.awi.de [Accessed].
Schloss, P.D., Westcott, S.L., Ryabin, T., Hall, J.R., Hartmann, M., Hollister, E.B., Lesniewski, R.A., Oakley, B.B., Parks, D.H., Robinson, C.J., 2009. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl. Environ. Microbiol. 75, 7537–7541.
Serracca, L., Ercolini, C., Rossini, I., Battistini, R., Giorgi, I., Prearo, M., 2011. Occurrence of both subspecies of Photobacterium damselae in mullets collected in the river Magra (Italy). Can. J. Microbiol. 57, 437–440.
Smith, K.A., Suthers, I.M., 2000. Consistent timing of juvenile fish recruitment to seagrass beds within two Sydney estuaries. Mar. Freshw. Res. 51, 765–776.
Spencer, R., 1959. Some aspects of the ecology of Lake Macquarie, NSW, with regard to an alleged depletion of fish. II. Hydrology. Mar. Freshw. Res. 10, 279–296.
Suzzi, A.L., Stat, M., Macfarlane, G.R., Seymour, J.R., Williams, N.L., Gaston, T.F., Alam, M.R., Huggett, M.J., 2022. Legacy Metal Contamination Is Reflected in the Fish Gut Microbiome in an Urbanised Estuary. Environmental Pollution, 120222.
Takemura, A.F., Chien, D.M., Polz, M.F., 2014. Associations and dynamics of Vibrionaceae in the environment, from the genus to the population level. Front. Microbiol. 5, 38.
Terceti, M.S., Ogut, H., Osorio, C.R., 2016. Photobacterium damselae subsp. damselae, an emerging fish pathogen in the Black Sea: evidence of a multiclonal origin. Appl. Environ. Microbiol. 82, 3736–3745.
Thompson, F.L., Iida, T., Swings, J., 2004a. Biodiversity of vibrios. Microbiol. Mol. Biol. Rev. 68, 403–431.
Thompson, J.R., Randa, M.A., Marcelino, L.A., Tomita-Mitchell, A., Lim, E., Polz, M.F., 2004b. Diversity and dynamics of a North Atlantic coastal Vibrio community. Appl. Environ. Microbiol. 70, 4103–4110.
Trnski, T., Neira, F.J., 1998. Terapontidae. In: NEIRA, F.J., MISKIEWICZ, A.G., TRNSKI, T. (Eds.), Larvae of Temperate Australian Fishes. University of WA Press, Nedlands, WA.
Webster, N.S., Cobb, R.E., Negri, A.P., 2008. Temperature thresholds for bacterial symbiosis with a sponge. ISME J. 2, 830–842.
Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R., Vázquez-Baeza, Y., Birmingham, A., 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. Microbiome 5, 1–18.
Wong, S., Rawls, J.F., 2012. Intestinal microbiota composition in fishes is influenced by host ecology and environment. Mol. Ecol. 21, 3100–3102.
Wouters, H., Berckmans, J., Maes, R., Vanuytrecht, E., DE Ridder, K., 2021. Global bioclimatic indicators from 1950 to 2100 derived from climate projections. In: Copernicus Climate Change Service (C3S) Climate Data Store (for [sea surface temperature], [ACESS1-0 (BoM-CSIRO, Australia], [r1i1p1], [RCP4.5; RCP8.5]), version 1.0 (CDS).
Xiong, J.-B., Nie, L., Chen, J., 2019. Current understanding on the roles of gut microbiota in fish disease and immunity. Zool. Res. 40, 70.
Yilmaz, P., Parfrey, L.W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W., Glöckner, F.O., 2014. The SILVA and "all-species living tree project (LTP)" taxonomic frameworks. Nucleic Acids Res. 42, D643–D648.