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Effect of Time and Temperature Before Preservation on Bacterial eDNA in Sediment Samples

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ABSTRACT

Environmental DNA is being increasingly used for research and regulatory purposes, but currently the lack of standardization is holding it back. There are gaps in our understanding of the eDNA analysis pipeline and the potential impacts of areas of variability within that. Standardization is necessary for all uses of eDNA to allow comparison between sample sets and ensure accuracy and reproducibility. Understanding sample stability is particularly important for planning fieldwork and writing practicable guidance for regulatory compliance monitoring. Samples collected for eDNA analysis are preserved as soon as possible for stability, but practicalities of sampling in the field can lead to delays where the sample temperature may be uncontrolled. We collected eDNA sediment samples along an organic enrichment impact gradient and incubated them at 10°C, 20°C, and 40°C for up to 48 h prior to preservation, then sequenced the bacterial 16S gene. We show that bacteria families responded differently to the incubation temperatures and times to an extent that affected ecological interpretation. Predictions of benthic health using a trained random forest machine learning model were tolerant of incubation up to 20°C, and showed sensitivity to temperature within 3 h of incubation at 40°C. We show that the influence of temperature can depend on the study aim, taxa involved, and analysis used, such that some situations may allow temporary storage up to 20°C but others will be affected by 10°C. We confirm that keeping sediment temperature low is critical for many applications, and that potential temperature deviations must be reported.

1 | Introduction

Environmental DNA (eDNA) is DNA extracted from environmental samples (Taberlet et al. 2012) and is a promising new tool for ecological monitoring purposes. There are many potential methods for eDNA analysis, including qPCR (Hamaguchi et al. 2022) and ddPCR (Mauvisseau et al. 2019) for single-species detection, and metabarcoding (Chariton et al. 2015) for multiple species. eDNA-based approaches are being developed and used for many research and policy areas such as endangered species detection (Mauvisseau et al. 2020), invasive species detection (Dejean et al. 2012; Gargan et al. 2022), and changes associated with anthropogenic pressures, including the effects of expanding human population (Baird and Hajibabaei 2012; Cordier et al. 2017; Dully, Rech, et al. 2021; He et al. 2021). The benefits of eDNA versus traditional monitoring methods include increased speed, lower costs, reduced need for taxonomic specialists, ability to find rare or cryptic species, and less invasive sampling. One of the main issues with the use of eDNA for monitoring is the lack of standardization (Zaiko et al. 2018; Cordier et al. 2021), and while steps have been made there are still some gaps which may have implications for ecological inference and regulatory assessment (Bruce et al. 2021). Standardization requires an understanding of sources of variability.

Variability in any aspect of eDNA sample processing can potentially influence results (Alberdi et al. 2018; Alexander et al. 2023). The process of eDNA metabarcoding broadly consists of sample collection, preservation, DNA extraction, library preparation, and sequencing. One aspect that has not been thoroughly explored is the effect of a delay between sample collection and preservation on bacterial communities, particularly in sediment samples, and the influence of temperature during such delay. Studies of short-term changes in microbial assemblages in environmental samples have focused on soil samples and found the assemblages to be consistent over time even in the absence of a particular preservation strategy (Tatangelo et al. 2014; Frøslev et al. 2022). Coolers are commonly used and when they can maintain temperature, they are comparable to liquid nitrogen storage for up to 24 h (Delavaux et al. 2020). Soil has a relatively low water content, which affects microbial activity and may influence assemblage structure (Borowik and Wyszkowska 2016). Fully dried soil samples can be preserved for years (Clark and Hirsch 2008). In contrast, eDNA preservation/degradation water sample studies have typically focused on single-species methods looking at eukaryotic eDNA rather than bacterial community (Harrison et al. 2019; Mauvisseau et al. 2022). Comparing eukaryotic eDNA persistence in both water and sediment finds it persists far longer in sediment (Ogata et al. 2021). This is likely a protective effect of organic material, which appears to protect extracellular DNA from DNases (Cai et al. 2006). DNases are enzymes produced by many dying cells, including bacteria, that break down free DNA (Singh and Marshall 1966), and their activity correlates with temperature up to the point of denaturation (Khwen et al. 2021). A study on carp eDNA found it persisted longer in dystrophic (high nutrient, low oxygen) water compared to both oligotrophic and eutrophic water, likely due to the higher levels of dissolved organic carbon (DOC) in the former (Eichmiller et al. 2016). Marine sediments can have varied redox potentials (Pereira et al. 2004), which can damage DNA (Shadfar et al. 2023). Ancient eukaryotic eDNA up to 2 million years old has been recovered from permafrost sediment samples (Kjær et al. 2022), suggesting that sediment is highly capable of preserving DNA given the right conditions.

Bacterial assemblages can be useful in environmental monitoring in many types of environment (Aylagas et al. 2017; Glasl et al. 2019; Martin et al. 2020; Pearman et al. 2022). Bacteria are key to biogeochemical cycling (Harris 2012) and the assemblages change rapidly in response to environmental change. In aquatic environments, bacteria are most prevalent in sediment (Harris 2012) and as such have been the focus of many eDNA metabarcoding studies (Dully, Balliet, et al. 2021; Frühe et al. 2021; Harrison et al. 2021; Pearman et al. 2022; Gonzalez et al. 2023). The bacterial assemblage is therefore highly informative, but ecological interpretation of the assemblage may be sensitive to anything that affects sequencing results. An essential step in metabarcoding is cell lysis, where cells are broken open to release the DNA. Many environmental bacteria have robust cell walls and require physical methods such as bead-beating for lysis (Luna et al. 2006). The toughness of these cell walls varies by taxa and is not entirely determined by peptidoglycan thickness (Gram positive/negative) (Zhang et al. 2021). The variation means preservation buffers can bias the bacterial taxa recovered from eDNA samples (Gray et al. 2013; Iturbe-Espinoza et al. 2021; Pavlovska et al. 2021). As a result, sediment samples are often frozen (−20°C) for preservation as it is cost-effective, accessible within the laboratory, and reduces the risk of bias. Freezing is often not possible when sampling in the field and therefore must wait for samples to return to the laboratory. Uncontrolled temperatures during this delay may cause changes in the bacterial assemblages.

Bacteria are affected by temperature in several ways. Bacteria found in sediment samples may be alive and active, dormant and able to reactivate, or dead but intact, and the specific fractions depend on the ambient conditions (Luna et al. 2002). The live and dormant bacteria may be affected by temperature, as temperatures exceeding the tolerances of some bacteria may kill them (Zobell and Conn 1940). Over time, temperatures may also influence their dormancy state, metabolism, and replication (Pomeroy and Wiebe 2001); thus biasing the DNA recovered (Adams et al. 2010). Additionally, the exposure to oxygen during sample collection may kill obligate anaerobes (Imlay 2002), which proliferate in some forms of environmental impact. As bacteria die, they slowly lose different functions, including maintenance of cell membrane integrity (Kramer and Thielmann 2016), and potentially cell wall integrity. This loss of integrity is likely to be taxon-specific, and post-sampling change may have profound influences on the interpretation of eDNA-based analysis.

Identifying taxa in metabarcoding data is complicated by these data being compositional (Aitchison 1982; Gloor et al. 2017). Compositional data are collected with a constraint, which means all values are relative abundances. This creates issues such as spurious positive correlations, sub-compositional incoherence of correlation, and negative correlation bias (Gloor et al. 2017); without correction, compositional data cannot provide evidence of real (absolute) change in abundances of the individual taxa on which it is based. There have been numerous attempts to solve this by transforming or normalizing the data based on Aitchison's original centre log-ratio transformation (Aitchison 1982), but we are not aware of any that are able to deal with the structural zeros and extreme asymmetry that are common along the gradients in some environmental monitoring. Rank-based dissimilarity approaches (e.g., non-metric multidimensional scaling (NMDS), based on Bray–Curtis dissimilarity matrices) are useful as they maintain the relationships between taxa, but they are affected by sample size and particularly high abundances. Sequencing data from eDNA samples typically have uneven read depths, despite normalization prior to sequencing, falsely affecting diversity metrics. Data can also be biased by unequal PCR amplification (Suzuki and Giovannoni 1996). The validity of rarefying as a means of correcting uneven sequencing depth is under debate, but it is currently largely accepted and widely used (McMurdie and Holmes 2014; Schloss 2024a, 2024b). ANCOMBC2 (updated from ANCOM-II) (Kaul et al. 2017; Lin and Peddada 2024) is one of the most consistent and reliable methods for differential abundance analysis on compositional data (Nearing et al. 2022).

Aquaculture impact monitoring in Scotland is overseen by the Scottish Environment Protection Agency (SEPA). Fin-fish aquaculture impacts the benthos via the accumulation of feces and uneaten feed of finfish in cages, causing organic enrichment. Organic inputs are rapidly consumed by bacteria, initially using

oxygen. As the oxygen declines, bacteria move to alternatives, including sulphates, which creates toxic hydrogen sulphide (Harris 2012; Choi et al. 2018). Sulfur-oxidizing and sulfur-reducing bacteria are found in aquaculture sites around the world (Bissett et al. 2006; Aranda et al. 2015; Choi et al. 2018; Frühe et al. 2021). In sufficient concentrations, hydrogen sulphide extirpates many benthic taxa in order of their sensitivity to it (Pearson and Rosenberg 1978). Historically, macrobenthic taxa have been used to assess the status of the benthos around fin fish farms (Rosenberg et al. 2004). In the UK and Ireland, macrobenthic tolerances and abundances are used to calculate the Infaunal Quality Index (IQI) (Phillips et al. 2014). SEPA has recently introduced eDNA-based methods into their compliance assessment of benthic impact from Scottish finfish aquaculture sites (SEPA 2023; Wyness et al. 2026). SEPA's eDNA approach uses the bacterial 16S rRNA gene, as it has the largest signal-to-noise ratio for fish farm related metabarcoding (Wilding et al. 2023). The SEPA standard operating procedure requires that samples are frozen within four hours of collection (SEPA 2023), which is challenging and potentially costly to implement where sampling may take several days to complete in remote locations.

This study examined changes in recovered bacterial DNA in sediment samples incubated at different temperatures and durations prior to preservation by freezing. Temperatures and incubation times were chosen based on realistic hypothetical sampling scenarios.

2 | Methods

2.1 | Sample Collection and Incubation

Salmon farming compliance assessment provides a suitable case study to investigate potential causes of variability in eDNA-based environmental monitoring (Wilding et al. 2023). The upper incubation temperature chosen (40°C) was considered a plausible extreme for sampling in temperate areas, particularly during heat waves, and can clarify if these samples may still be viable. The lower incubation temperatures (10°C and 20°C) were intended to give a practical range up to the extreme temperature without going down to refrigerator temperatures (3°C–5°C).

Samples were collected at three stations (Cage Edge, Intermediate, Reference) along an environmental impact gradient at a fish farm in Argyll and Bute, Scotland, in November 2022. Sampling occurred partway through a production cycle, which does not align with typical monitoring sampling which would be near the end of the production cycle, at peak biomass.

A 0.1 m^2 van Veen benthic grab sampler was used to collect sediment samples, and a sterile plastic spatula was used to place surface sediment into a falcon tube. Three 50 mL falcon tubes were filled with sediment from one grab, and four grabs were sampled at each station along the impact gradient, totaling 36 falcon tubes. The falcon tubes were placed into a cooler with ice packs to control temperature until they could be subsampled and incubated. Sample collection started at 09:00 (UTC) on the boat, samples were taken to the laboratory at approximately 12:30, and subsampling began at 13:10. Incubation was in water baths maintained at 10°C, 20°C, and 40°C, with subsampling at 0, 3, 6, 12, 24, and 48 h. Each sample was removed from the cool box, homogenized with a sterile plastic spatula, a subsample put into a sterile 10 mL falcon tube (time zero, unincubated), and the original sample put into the incubation bath. Subsampling was performed at a separate bench away from the water baths to minimize contamination risk. Each sample's incubation period began when the sample was put into the bath. Falcon tubes were supported in the water baths by tube racks, and the water level remained just below the tube lids. Subsequent subsampling rounds were the same, but samples were removed from the incubation bath, and the falcon tubes were dried with clean paper towel before opening to homogenize. Subsamples were frozen within 10 min of removal (−20°C). The time zero subsampling staggered the beginning of each incubation period, and each subsequent round of subsampling was done in the same order, which ensured consistency of the incubation period. Ice packs in the cooler were still frozen at the end of the time zero subsampling. The total time for subsampling was 2 h, which meant the final sample was in the cooler for 2 h more than the first sample, but this is not considered a concern (Delavaux et al. 2020). After subsampling, there were 216 samples: 24 replicates per station/temperature combination, 4 replicates per time point within that.

2.2 | Sequencing

DNA was extracted using the soil modular extraction protocol Mu-DNA (Sellers et al. 2018), with 0.25 g of sample sediment. Library preparation followed the Illumina 16S metabarcoding protocol, as in Wilding et al. (2023), using only Promega ProNex for PCR clean-up. Amplicon PCR used 2.5 μL of elution and 9 μL of water; due to some weaker extractions, some samples were added to the PCR as 4 μL of elution and 7.5 μL of water to compensate for the lower concentration. Where required, some samples were also concentrated during PCR clean up.

Extraction and PCRs were run in batches, with a negative control (molecular grade water) added to each batch. Sequencing was performed in two separate runs on an Illumina MiSeq using V3 600 cycle chemistry. Sequences were demultiplexed and uploaded to BaseSpace by the MiSeq, where they were downloaded from for further analysis. Negative controls were checked throughout library preparation for detectable DNA indicating contamination; none was detected at a meaningful level; therefore, no further action was taken.

2.3 | Bioinformatics and Data Analysis

Analysis was performed in R v4.4.1, and graphics were produced using ggplot2. Raw FASTQ files were denoised and taxa allocated using the eDNA2IQI v2.4.8 pipeline with default parameters (Wyness et al. 2026), which uses cutadapt (Martin 2011) for primer removal and DADA2 (Callahan et al. 2016) for both denoising into amplicon sequence variants (ASVs) and taxonomic assignment with the SILVA 138 database (Quast et al. 2013; Yilmaz et al. 2014). The pipeline produces a list of taxa at family level. eDNA2IQI was also used

to predict IQI values, which were plotted to visualize changes. eDNA2IQI is highly consistent in predictions, therefore predicted IQI values were used as a sense check for each sample, and those that misaligned considerably with other samples from the same grab were removed as outliers.

Data were rarefied to 5000 reads, as this is deemed appropriate for benthic bacterial communities (Dully, Wilding, et al. 2021) and is used by the eDNA2IQI package for predictions. Rarefaction was used to check that 5000 reads were appropriate for alpha diversity metrics for this dataset (Schloss 2024a), by rarefying to 5000 and calculating metrics 100 times, then averaging and calculating the coefficient of variation. A coefficient of 0.05 or below is typically considered acceptable and will be used here. Reported results are not averages from rarefaction; they are from rarefied data once the read count has been deemed appropriate. The alpha diversity metrics used were Shannon's, complement Simpson's (hereafter referred to as Simpson's), and richness. Vegan (Oksanen et al. 2024) was used to rarefy, calculate diversity metrics (except richness, which was just calculated with base R), and generate non-metric multidimensional scaling (NMDS) ordinations using Bray–Curtis dissimilarity matrices. The dissimilarity matrices were based on rarefied and fourth-root transformed data as recommended by Gloor et al. (2017) to manage the impact of taxa with particularly high read counts. Alpha and beta diversity metrics were plotted to enable visual comparison.

Variance partitioning was explored using non-parametric multivariate analysis of variance (PERMANOVA) (Anderson 2001), through the adonis2 function from vegan. The following model was used, where temperature was treated as a factor

$$\text{Model: Rarefied data} \sim \text{Station}^{*}\ \text{Time}^{*}\ \text{Temperature}^{*}\ \text{Grab}$$

Phyloseq (McMurdie and Holmes 2012) was used to create aggregated taxa plots with detection and prevalence rates set to maximize both taxa and readability, and for differential abundance analysis. Aggregated taxa plots were used to identify dominant taxa and visualize the trends in dominance across stations and incubations. ANCOMBC2 was run with default parameters, and rank = "Family" and fix_formula = "Station_Time_Temperature". Only the temperature log fold changes are reported. The top twelve taxa identified by ANCOMBC2 were overlaid on the NMDS plot to provide further context.

3 | Results

Samples were sequenced on two runs. The first run generated 5,429,943 reads with an average Q30 of 80.66%, which left 3,627,442 ASVs after denoising. The second run generated 2,634,364 reads with an average Q30 of 84.64%, and 1,630,117 ASVs after denoising. Both runs were processed separately, and the taxa lists and predicted IQI lists were combined. One sample failed to sequence successfully (Cage Edge, grab 2, 20°C, 6 h). Samples were rarefied to 5000 reads for analysis, which removed all negative controls and three samples (Cage Edge, grab 1, 10°C, 0 h; Cage Edge, grab 4, 10°C, 24 h; Intermediate, grab 1, 20°C, 6 h). Five additional samples were removed as outliers due to predictions that were extremely inconsistent with other predictions from the same grab (Figure S1) (Cage Edge, grab 3, 20°C, 12 h; Cage Edge, grab 4, 20°C, 12 h; Intermediate, grab 2, 10°C, 0 h; Intermediate, grab 4, 40°C, 3 h; Reference, grab 2, 20°C, 12 h). This left 207 samples.

Alpha diversity metrics varied by station for T = 0 (unincubated) samples and were then affected by both time and temperature to varying extents depending on the station (Figure 1). Rarefaction (rarefying and averaging resultant metrics) to 5000 reads resulted in a maximum coefficient of variation of 0.029 for richness, 0.006 for Shannon, and 0.003 for Simpson's diversities, indicating that variation between rarefied replicates was sufficiently small (< 0.05). In all stations, there was increased variability and a decline in diversity at 40°C, which was especially evident in the Cage Edge samples (Figure 1). The decline in all diversity metrics at 40°C begins as early as T = 3, with a non-linear rate of decline that varied across all the stations and diversity metrics. At 10°C and 20°C, the Simpson and Shannon metrics remained consistent over incubation periods regardless of station, with some variability at T = 48, particularly in Intermediate samples. Richness was less consistent at 10°C and 20°C within Intermediate and Reference samples, where there was increased variability across the whole incubation period (Figure 1).

There were clear differences in the family structure in each station. Based on visual assessment of relative abundances, some families were considered dominant. Cage Edge samples had the fewest families shown on the plots (six, four of which were dominant), compared to Intermediate and Reference samples, which had more families (nine) and much more even relative abundances. Sulfurovaceae was clearly dominant in Cage Edge samples, Sva1003 was dominant in most Reference samples but to a lesser extent than Cage Edge, and Intermediate samples were much more even and lacked an overall dominant taxon (Figure 2). Intermediate samples were generally very similar to Reference samples, typically distinctive from Reference samples by the higher relative abundances of both Sulfurovaceae and Desulfocapsaceae. The established taxa dominance changed in the 40°C incubation samples from 12 h onwards; Oceanospirillaceae dominated Cage Edge samples and Rhodobacteraceae and Vibrionaceae became predominant in Reference samples, while Intermediate samples had a combined response with all three increasing (Figure 2).

ANCOMBC2 identified 58 taxa as differentially abundant across temperature, and the top 12 by largest log-fold change (modeled log difference in bias-corrected absolute abundances, Table 1) were included in the NMDS plot and are discussed in the text (see Table S1 for full list). This included Vibrionaceae and Oceanospirillaceae, but not Rhodoboacteraceae despite it standing out in the family plot (Figure 2). Oceanospirillaceae exhibited the highest log-fold change, which concurs with the dramatic increase seen in the taxa plot (Figure 2). Of the 17 taxa identified by ANCOMBC2, nine had log-fold changes that increased in higher temperatures, and the remaining eight taxa reduced with higher temperatures with smaller log-fold change values (Table 1), indicating smaller changes in absolute values when taxa decline with higher temperatures. The largest reduction was seen in Chitinvibrionaceae. Two of the top 12 taxa exhibited larger changes at 20°C than at 40°C (Exiguobacteraceae and Thermotalaceae), whilst the

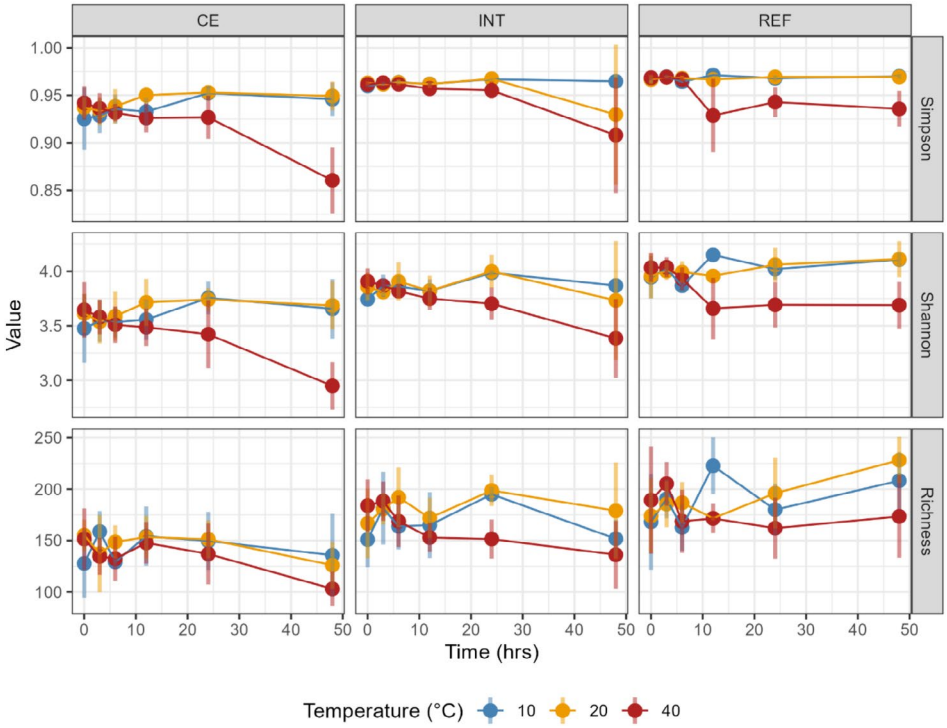

FIGURE 1 | Diversity metrics Shannon, Simpson's, and richness of family-level 16S taxa from sediment incubated at different temperatures prior to preservation. CE, cage edge; INT, intermediate; REF, reference. Data were rarefied to 5000 reads, metrics calculated and then averaged across the grabs at each combination of station, time, and temperature. Standard deviation is shown as error bars.

remaining 10 displayed larger responses at the higher temperature. The observed changes did not occur at the same rates (e.g., log-fold changes of 0.25 to 3.41 for Vibrionaceae, and 0.61 to 1.79 for Lachnospirales) (Table 1).

Assemblage structure was visualized using a non-metric multidimensional scaling (NMDS) plot (Figure 3). The stations clustered into two main groups, Cage Edge on the left (negative NMDS1) and Intermediate/Reference on the right (positive NMDS1). The Intermediate and Reference stations were distinguishable groups, but they were very close to each other on the plot (Figure S2). The station distinction is separated along NMDS axis one, which is the axis with the main differences on it (Figure 3). Axis two of the NMDS shows separation over time, which is influenced by temperature. There was a clear drift along axis two in the 40°C samples, with increasing distance from the T = 0 (unincubated) hours samples with longer incubation times, particularly from 12 h onwards. Very few 20°C samples were affected this way, and no 10°C samples follow the axis two drift. The positions of the identified taxa on the NMDS plot agreed with their positive or negative association with increased temperatures (Figure 3), as they split into two groups on axis two. However, their position within those groups does not correlate to the magnitude of the log fold change produced by ANCOMCB2; for example, Vibrionaceae is closer to the unimpacted cluster than Lachnospirales (Figure 3, Table 1). Oceanospirillaceae and Sedimentibacteraceae appear to associate with high temperatures in Cage Edge samples, while P.Tissierellales, Lachnospirales, Vibrionaceae, Alteromonadaceae, and Bacillaceae were associated with high temperatures in Intermediate and Reference samples (Figure 3).

The NMDS highlighted the importance of station through the clear separation of samples by station, and the PERMANOVA reinforced the importance of it as it contributed the majority (58%) of the variance ($R^2 = 0.58$, Table 2). Grab alone contributed more variance (5.4%) than either time (3.2%) or temperature (5.1%), but when including interactions with other terms, Grab only contributed 10.5% compared to time (13.9%) and temperature (16.5%, Table 2). The interaction between time and temperature, which contributed 4.8% of the variance, is of particular interest (Table 2). The residual variance (13.6%) includes within-Grab variance and variability from the sequencing process, and shows that this is less than the effect of time and temperature, and that including Grab in the residual would make the effect of all biological and laboratory noise larger than the effect of incubation (Table 2).

The IQI predictions generated by the eDNA2IQI pipeline for low-IQI samples (Cage Edge) were changed by incubation, but not for high-IQI samples (Intermediate, Reference) (Figure 4). Predicted values from Cage Edge samples incubated at 40°C increased from as early as three hours and continued to trend upwards with increasing incubation time (Figure 4). Grab variability changed across all explanatory variables; grabs were generally most consistent in Reference samples. The Reference samples did exhibit a point of increased variability between grabs that appeared to occur sooner at higher temperatures—the 10°C samples were most variable at T = 48, the 20°C samples most variable at T = 24, and the 40°C samples were most variable at T = 12 (Figure 4). There was a notable spike in the predicted IQI of the Reference grab two samples that was seen in both 10°C and 20°C 3-h incubations, but not the 40°C.

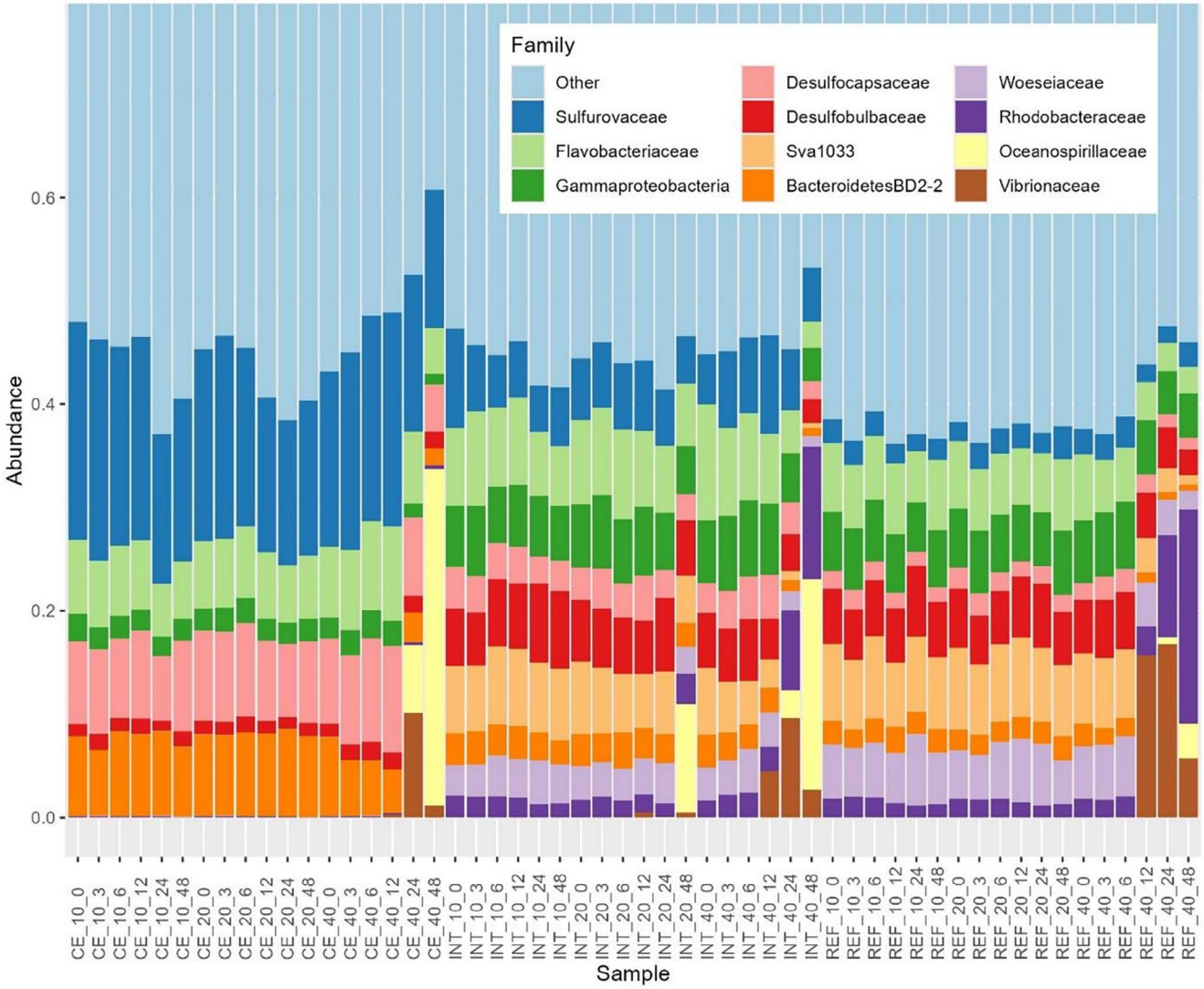

FIGURE 2 | Family level plots of 16S taxa aggregated using 0.06 detection and 0.05 prevalence rates, averaged into groups of temperature (in degrees Celsius) and time (in hours) combinations for sediment incubation. CE, cage edge; INT, intermediate; REF, reference. The plot is aggregated and cropped to emphasize the most prevalent families. The first number in the sample names is the temperature; the second is the subsampling time point.

4 | Discussion

The incorporation of eDNA-based monitoring into research and policy benefits the environment through reduced sampling pressure, and researchers and stakeholders through the faster and cheaper analysis pipeline (Aylagas et al. 2018). It also highlights previously noted issues regarding variability within eDNA processes (Zaiko et al. 2018; Shea et al. 2023). Regulation requires protocols that result in consistent, transparent, and repeatable decision making. This study has investigated the effect of differing conditions prior to sediment sample preservation, specifically the effects of temperature over time. These findings contribute both to the specific use for eDNA in aquaculture monitoring by SEPA and to the broader eDNA community's understanding of causes of inter-sample eDNA variability and sample robustness.

The alpha diversity metrics we used (Simpson, Shannon, and richness) show that samples from different stations were impacted differently by incubation, but samples from all stations respond to some extent after as little as three hours at 40°C. The diversity patterns across all three metrics and stations at 40°C were broadly similar, all indicating that at 40°C both some rare taxa were lost, either completely or just reduced beyond detection, and there was an increase in dominance by some taxa (Nagendra 2002). The extent of the changes in taxa and the time points for largest impact varied by station. Loss of rare taxa could be the result of a general consistent loss of DNA from all taxa, leading to lower Shannon's diversity. Extracellular DNA and DNA in fragile cells (eukaryote) eDNA has been shown to degrade more rapidly in warmer water, with higher degradation rates found at lower temperatures than changes observed here (e.g., 15°C, (Eichmiller et al. 2016; Andruszkiewicz Allan et al. 2021)). Those studies were looking at changes in DNA concentration and the results here do not fully clarify if there is an overall loss of DNA over the incubation periods. The lower Shannon diversity suggests loss at higher temperatures here than in the eukaryote studies (Eichmiller et al. 2016;

TABLE 1 | ANCOMBC2 identified bacteria, with full taxonomy from phylum onwards, and log fold changes (LFC) at 20°C and 40°C compared to 10°C.

Name in text	Phylum	Class	Order	Family	LFC 20	LFC 40
Oceanospirillaceae	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Oceanospirillaceae	0.79	4.61
Thermotaleaceae	Firmicutes	Clostridia	Peptostreptococcales Tissierellales	Thermotaleaceae	3.63	3.5
Vibrionaceae	Proteobacteria	Gammaproteobacteria	Enterobacterales	Vibrionaceae	0.25	3.41
Bacillaceae	Firmicutes	Bacilli	Bacillales	Bacillaceae	0.45	2.74
Exiguobacteraceae	Firmicutes	Bacilli	Exiguobacterales	Exiguobacteraceae	2.53	1.78
Alteromonadaceae	Proteobacteria	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	1.26	1.87
Sedimentibacteraceae	Firmicutes	Clostridia	Peptostreptococcales Tissierellales	Sedimentibacteraceae	0.35	1.87
Lachnospirales	Firmicutes	Clostridia	Lachnospirales	Unknown	0.61	1.79
Chitinivibrionaceae	Fibrobacterota	Chitinivibrionia	Chitinivibrionales	Chitinivibrionaceae	0.03	−1.74
P.Tissierellales	Firmicutes	Clostridia	Peptostreptococcales Tissierellales	Unknown	0.21	1.44
BD78	Proteobacteria	Gammaproteobacteria	BD78	Unknown	−0.03	−1.36
C.Falkowiibacteria	Patescibacteria	ABY1	Candidatus Falkowiibacteria	Unknown	−0.01	−1.28

Note: Red to green gradient scheme background indicates lower to higher LFC (red is reduced, green increased).

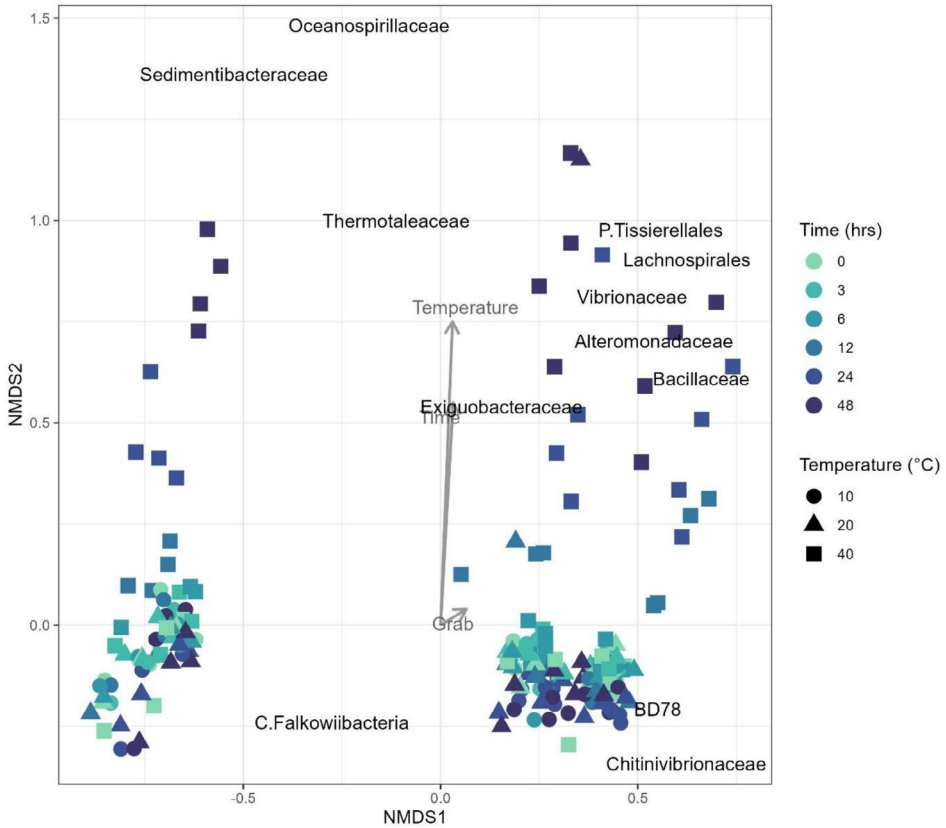

FIGURE 3 | Non-metric multidimensional scaling plot showing distortion of 16S communities created by incubation of sediment at 10°C, 20°C, and 40°C. The NMSD stress is 0.062. Cage Edge is a distinct cluster on the left of the plot, and the Intermediate and Reference stations are closer to each other on the right. A separate plot colored by station (Figure S2) demonstrated that the incubation did not lead to mixing of stations. See Table 1 for full taxonomies.

TABLE 2 | PERMANOVA results showing variance partitioning between variables involved in incubation of sediment and 16S families.

	Df	SumOfSqs	R^2	F	Pr(>F)
Station	2	14.87039	0.584136	294.6605	0.001
Time	1	0.808333	0.031753	32.03463	0.001
Temperature	2	1.310377	0.051474	25.96545	0.001
Grab	9	1.369487	0.053796	6.030382	0.001
Station:Time	2	0.296864	0.011661	5.882432	0.001
Station:Temperature	4	0.406496	0.015968	4.027411	0.002
Time:Temperature	2	1.223889	0.048077	24.25167	0.001
Time:Grab	9	0.354197	0.013913	1.559665	0.055
Temperature:Grab	18	0.493265	0.019376	1.086018	0.303
Station:Time:Temperature	4	0.398300	0.015646	3.946205	0.001
Time:Temperature:Grab	18	0.468558	0.018406	1.031621	0.417
Residual	137	3.456935	0.135795		
Total	208	25.45709	1		

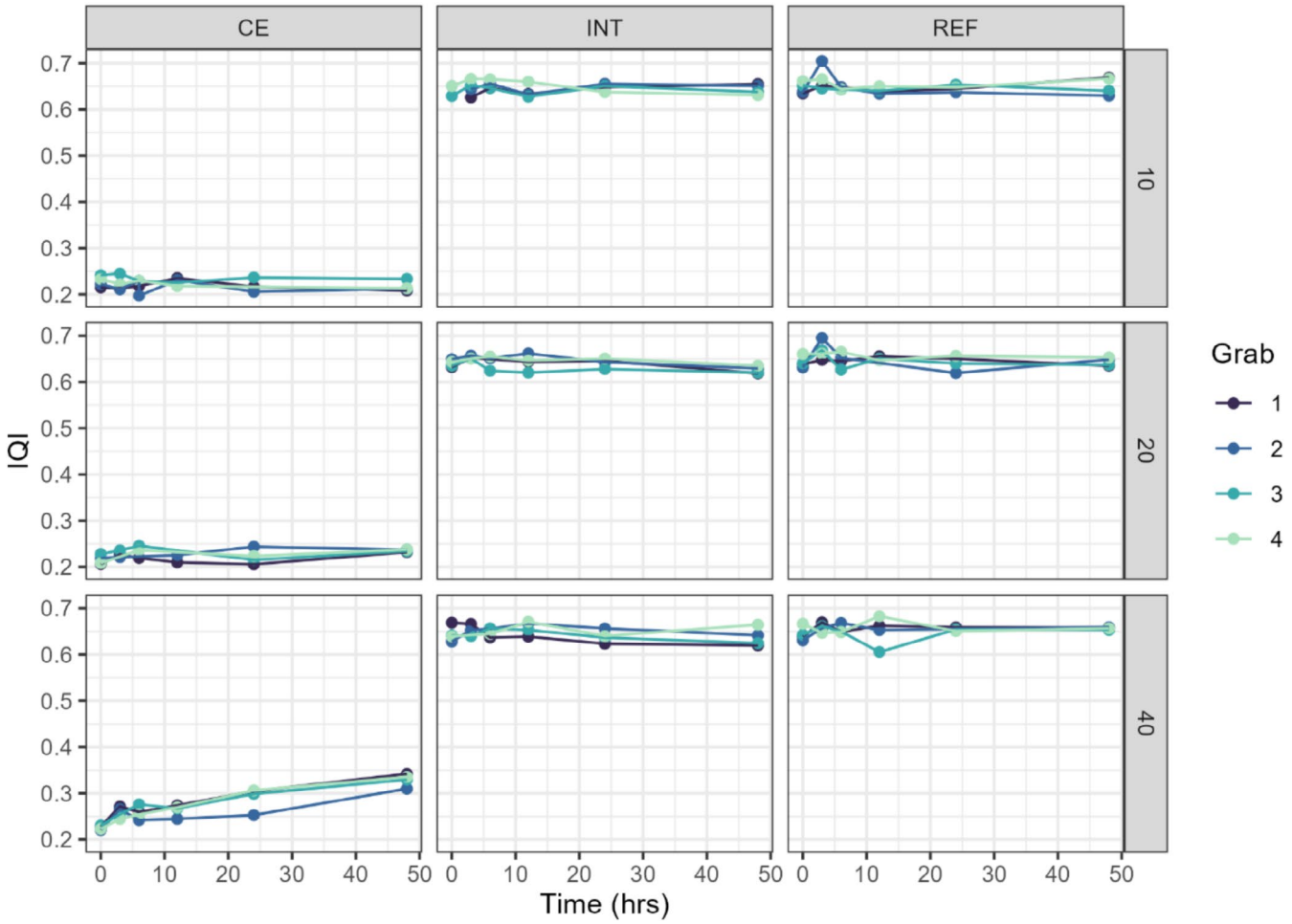

FIGURE 4 | IQI values for each Grab take at each sampling station, predicted using a trained random forest algorithm. CE is Cage Edge, INT is Intermediate, REF is Reference, and the incubation temperatures were 10°C, 20°C, and 40°C.

Andruszkiewicz Allan et al. 2021). As it took higher temperatures to create an observable effect in this study, it is unlikely many fragile cells or extracellular DNA were involved as they would have been impacted at the lower temperatures. Additionally, organic matter is able to protect extracellular DNA (Cai et al. 2006). The samples with the highest organic matter content were the Cage Edge samples, which exhibited the largest change with high temperatures, suggesting organic matter did not have an observable protective effect here. This may instead be related to redox potential, as that is higher at Cage Edge sites where there are likely to be many types of damaging reactive species (Pereira et al. 2004; Shadfar et al. 2023). Any effect from oxygen impacting obligate anaerobes should increase with time and be consistent across all temperatures, but none is observed here, indicating oxygen has not had a notable impact.

Changes in dominance, indicated by the changes in Simpson's diversity, suggest there were unequal impacts on taxa. The major changes seem to correlate with the large relative increases in Oceanospirillaceae and Vibrionaceae. Temperature influences microbial activity, which would affect their abundances as well as impacting degradation (Robador et al. 2009; Adams et al. 2010). As the samples were taken from Scotland, where the annual average water temperature is 11.5°C (Wyness et al. 2026), it is likely that many of the bacteria involved are adapted to cooler temperatures, and as such the temperatures chosen may exceed their tolerances (Zobell and Conn 1940). In a study of arctic (consistently cold) vs. temperate (very seasonal) marine bacteria, sulphate-reducers in temperate regions were more tolerant of higher temperatures, but all activity by arctic bacteria declined by 40°C (Robador et al. 2009). The stability at 20°C and rapid changes at 40°C reported here are similar to the soil storage results seen in Frøslev et al. (2022) and Lauber et al. (2010). Stability at low temperatures has also previously been evidenced (Delavaux et al. 2020). Based on our findings and other studies it appears that short-term storage at 20°C may be appropriate for sediment samples taken in temperate regions.

There were identifiable changes in assemblage structure that were influenced over time depending on temperature. Despite the observed changes in assemblages, samples could still be identified to their source site on the NMDS plot, indicating that the changes did not completely distort the samples. The bacterial communities change across the environmental impact gradient, with taxa adapted to hypoxic/anoxic conditions dominating stations that are experiencing high organic input (Bissett et al. 2006; Choi et al. 2018; Hornick and Buschmann 2018; Rubio-Portillo et al. 2019). It is therefore more likely that the station-dependent resilience is a result of different taxa across the gradient surviving, crashing populations, or even thriving in the high temperatures.

The following inferences are made at the family/order level, and therefore generalize across many species, and are included only to provide some general context. Oceanospirillaceae had the largest log-fold change and appears to contribute to substantial changes, particularly in Cage Edge and Intermediate samples. Oceanospirillaceaee has been associated with spring and summer blooms in the Arctic (Francis et al. 2021; Thiele et al. 2023), suggesting a capacity for opportunistic behavior and adaptation to higher temperatures, and a potential use in climate change monitoring. This may contribute to an explanation for the large changes at 40°C, if opportunistic taxa were able to take over from others. Bacillaceae is considered one of the most robust bacterial families (Mandic-Mulec et al. 2015), with most members being resistant to heat. Vibrionaceae are less resilient as a family, but they have relevance to fish farms as pathogenic members can cause issues (Soto 2022). Exiguobacteraceae appears to be very versatile and contains many extremophiles (Kasana and Pandey 2018; Shen et al. 2024). Alteromonadaceae also contains diverse marine bacteria, often with high optimal growth temperatures which aligns with these findings, however they also typically associated with nutrient-rich environments and this study found them associating with the poorer Intermediate/Reference sites (López-Pérez and Rodriguez-Valera 2014). Thermotaleaceae includes an anaerobic, thermophilic bacterium with an optimal growth temperature of 50°C (Ogg and Patel 2009; Chuvochina et al. 2023). Currently Lachnospirales, P.Tisseriallales, and Sedimentibacteraceae have very little information available, but this work suggests they are tolerant of heat. There is also little information available about the taxa that exhibited negative log-fold changes, however it is worth noting that Chitinivibrionaceae are a small family of highly salt- and alkaline-tolerant bacteria, making them a suspicious finding in these samples (Sorokin et al. 2020).

These samples were collected outside of the normal monitoring period, potentially impacting the expected range of IQI predictions. The IQI predictions were affected by incubation but not distorted beyond the point of recognizable stations. The effect on IQI predictions clearly indicates that taxa the random forest has identified as important in low-IQI samples have been affected, but those from high-IQI samples were not. The higher IQI predictions from Cage Edge (low-IQI) samples that were incubated at 40°C suggest care must be taken regarding compliance assessment samples. The fact that the increase in Cage Edge sample IQI predictions occurred within the first three hours and at 40°C means that any time samples spend above 20°C has the possibility to affect results. There was also some variance noted in the responses of different grabs, which remained lower than the increase created by 40°C incubation. Additionally, the variance partitioning showed that grab contributed to the variance less than the incubation itself. There was also the IQI spike in grab two that appeared to respond to incubation below 40°C, a within-grab effect. Therefore, while grabs are a source of noise and efforts to combine them may reduce noise, they contribute less than a delay does, and combining them should not be prioritized over expedient preservation.

A consideration for this study is that samples were only taken from one fish farm, so whilst other Scottish salmon aquaculture sites are likely to respond in the same way this cannot be guaranteed. The IQI prediction tool is effective, and its training dataset is collated from aquaculture sites around Scotland, supporting the consistency of the bacteria and therefore the potential applicability of this study to broader Scottish aquaculture monitoring. Further, as already stated there are consistencies in the types of bacteria observed across fish farm gradients globally, supporting the applicability of this work to aquaculture beyond Scotland, though there may be variance in the temperature ranges. This work also highlights the fact that bacteria along a gradient may respond to conditions differently, a key consideration for sampling strategy for any comparative bacterial study.

Finally, this study specifically invites further exploration of the 20°C–40°C range to narrow down the point at which samples are affected for cooler temperate climates.

While eDNA is an increasingly popular tool for biomonitoring, it is a relatively new field of study that is still developing standardization. Sediment has been overlooked in studies of the effects of sample treatment on eDNA. As sediment is already being used in fin fish farm monitoring in Scotland, this provided an ideal case study. We observed that temperatures up to 20°C for up to 48 h had very little impact on eDNA2IQI predicted IQI, but that 40°C had a noticeable effect. This effect varied by taxa, though the station could still be determined. Even 10°C incubation can have some impacts depending on the taxa involved, so samples should be handled with caution, depending on the nature of the study. IQI predictions required for Scottish fin fish farm monitoring were noticeably impacted by the 40°C incubation, increasing in Cage Edge samples in under 3 h. Our results do not clarify at what temperature above 20°C this change occurs; therefore, the recommendation is that samples for fish farm compliance assessment should not be stored over 20°C. Sample preservation is therefore an important consideration that is impacted by the study design, even for short periods, and sample collection plans must account for this.

Author Contributions

Conception and study design: Victoria R. Ashley-Wheeler and Thomas A. Wilding. Data acquisition, analysis, and interpretation: Victoria R. Ashley-Wheeler, Adam J. Wyness, Barbara J. Morrissey, and Dasha Svobodova. Manuscript writing: Victoria R. Ashley-Wheeler, Trevor C. Telfer, and Thomas A. Wilding.

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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 in NCBI Sequence Read Archive at https://www.ncbi.nlm.nih.gov/sra, reference number PRJNA1247469. NCBI sequence accession number: PRJNA1247469.

References

Adams, H. E., B. C. Crump, and G. W. Kling. 2010. "Temperature Controls on Aquatic Bacterial Production and Community Dynamics in Arctic Lakes and Streams." *Environmental Microbiology* 12, no. 5: 1319–1333. https://doi.org/10.1111/J.1462-2920.2010.02176.X.

Aitchison, J. 1982. "The Statistical Analysis of Compositional Data." *Journal of the Royal Statistical Society: Series B: Methodological* 44, no. 2: 139–177.

Alberdi, A., O. Aizpurua, M. T. P. Gilbert, and K. Bohmann. 2018. "Scrutinizing Key Steps for Reliable Metabarcoding of Environmental Samples." *Methods in Ecology and Evolution* 9, no. 1: 134–147. https://doi.org/10.1111/2041-210X.12849.

Alexander, J. B., M. J. Marnane, J. I. McDonald, et al. 2023. "Comparing Environmental DNA Collection Methods for Sampling Community Composition on Marine Infrastructure." *Estuarine, Coastal and Shelf Science* 283: 108283. https://doi.org/10.1016/j.ecss.2023.108283.

Anderson, M. J. 2001. "A New Method for Non-Parametric Multivariate Analysis of Variance." *Austral Ecology* 26, no. 1: 32–46. https://doi.org/10.1111/J.1442-9993.2001.01070.PP.X.

Andruszkiewicz Allan, E., W. G. Zhang, A. C. Lavery, and A. F. Govindarajan. 2021. "Environmental DNA Shedding and Decay Rates From Diverse Animal Forms and Thermal Regimes." *Environmental DNA* 3, no. 2: 492–514. https://doi.org/10.1002/EDN3.141.

Aranda, C. P., C. Valenzuela, Y. Matamala, F. A. Godoy, and N. Aranda. 2015. "Sulphur-Cycling Bacteria and Ciliated Protozoans in a *Beggiatoaceae* Mat Covering Organically Enriched Sediments Beneath a Salmon Farm in a Southern Chilean Fjord." *Marine Pollution Bulletin* 100, no. 1: 270–278. https://doi.org/10.1016/j.marpolbul.2015.08.040.

Aylagas, E., Á. Borja, I. Muxika, and N. Rodríguez-Ezpeleta. 2018. "Adapting Metabarcoding-Based Benthic Biomonitoring Into Routine Marine Ecological Status Assessment Networks." *Ecological Indicators* 95: 194–202. https://doi.org/10.1016/j.ecolind.2018.07.044.

Aylagas, E., Á. Borja, M. Tangherlini, et al. 2017. "A Bacterial Community-Based Index to Assess the Ecological Status of Estuarine and Coastal Environments." *Marine Pollution Bulletin* 114, no. 2: 679–688. https://doi.org/10.1016/J.MARPOLBUL.2016.10.050.

Baird, D. J., and M. Hajibabaei. 2012. "Biomonitoring 2.0: A New Paradigm in Ecosystem Assessment Made Possible by Next-Generation DNA Sequencing." *Molecular Ecology* 21, no. 8: 2039–2044. https://doi.org/10.1111/J.1365-294X.2012.05519.X.

Bissett, A., J. Bowman, and C. Burke. 2006. "Bacterial Diversity in Organically-Enriched Fish Farm Sediments." *FEMS Microbiology Ecology* 55, no. 1: 48–56. https://doi.org/10.1111/J.1574-6941.2005.00012.X.

Borowik, A., and J. Wyszkowska. 2016. "Soil Moisture as a Factor Affecting the Microbiological and Biochemical Activity of Soil." *Plant, Soil and Environment* 62, no. 6: 250–255. https://doi.org/10.17221/158/2016-PSE.

Bruce, K., R. Blackman, S. J. Bourlat, et al. 2021. *A Practical Guide to DNA-Based Methods for Biodiversity Assessment*. Advanced Books. https://doi.org/10.3897/ab.e68634.

Cai, P., Q. Y. Huang, and X. W. Zhang. 2006. "Interactions of DNA With Clay Minerals and Soil Colloidal Particles and Protection Against Degradation by DNase." *Environmental Science & Technology* 40, no. 9: 2971–2976. https://doi.org/10.1021/ES0522985.

Callahan, B. J., P. J. McMurdie, M. J. Rosen, A. W. Han, A. J. A. Johnson, and S. P. Holmes. 2016. "DADA2: High-Resolution Sample Inference From Illumina Amplicon Data." *Nature Methods* 13, no. 7: 581–583. https://doi.org/10.1038/nmeth.3869.

Chariton, A. A., S. Stephenson, M. J. Morgan, et al. 2015. "Metabarcoding of Benthic Eukaryote Communities Predicts the Ecological Condition of Estuaries." *Environmental Pollution* 203: 165–174. https://doi.org/10.1016/J.ENVPOL.2015.03.047.

Choi, A., H. Cho, B. Kim, and H. C. Kim. 2018. "Effects of Finfish Aquaculture on Biogeochemistry and Bacterial Communities Associated With Sulfur Cycles in Highly Sulfidic Sediments." *Aquaculture Environment Interactions* 10: 413–427. https://doi.org/10.3354/aei00278.

Chuvochina, M., A. J. Mussig, P. A. Chaumeil, et al. 2023. "Proposal of Names for 329 Higher Rank Taxa Defined in the Genome Taxonomy Database Under Two Prokaryotic Codes." *FEMS Microbiology Letters* 370: fnad071. https://doi.org/10.1093/femsle/fnad071.

Clark, I. M., and P. R. Hirsch. 2008. "Survival of Bacterial DNA and Culturable Bacteria in Archived Soils From the Rothamsted Broadbalk Experiment." *Soil Biology and Biochemistry* 40, no. 5: 1090–1102. https://doi.org/10.1016/J.SOILBIO.2007.11.021.

Cordier, T., L. Alonso-Sáez, L. Apothéloz-Perret-Gentil, et al. 2021. "Ecosystems Monitoring Powered by Environmental Genomics: A Review of Current Strategies With an Implementation Roadmap." *Molecular Ecology* 30, no. 13: 2937–2958. https://doi.org/10.1111/MEC.15472.

Cordier, T., P. Esling, F. Lejzerowicz, et al. 2017. "Predicting the Ecological Quality Status of Marine Environments From eDNA Metabarcoding Data Using Supervised Machine Learning." *Environmental Science and Technology* 51, no. 16: 9118–9126. https://doi.org/10.1021/acs.est.7b01518.

Dejean, T., A. Valentini, C. Miquel, P. Taberlet, E. Bellemain, and C. Miaud. 2012. "Improved Detection of an Alien Invasive Species Through Environmental DNA Barcoding: The Example of the American Bullfrog *Lithobates catesbeianus*." *Journal of Applied Ecology* 49, no. 4: 953–959. https://doi.org/10.1111/J.1365-2664.2012.02171.X.

Delavaux, C. S., J. D. Bever, E. M. Karppinen, and L. D. Bainard. 2020. "Keeping It Cool: Soil Sample Cold Pack Storage and DNA Shipment up to 1 Month Does Not Impact Metabarcoding Results." *Ecology and Evolution* 10, no. 11: 4652–4664. https://doi.org/10.1002/ece3.6219.

Dully, V., H. Balliet, L. Frühe, et al. 2021. "Robustness, Sensitivity and Reproducibility of eDNA Metabarcoding as an Environmental Biomonitoring Tool in Coastal Salmon Aquaculture: An Inter-Laboratory Study." *Ecological Indicators* 121: 107049. https://doi.org/10.1016/j.ecolind.2020.107049.

Dully, V., G. Rech, T. A. Wilding, et al. 2021. "Comparing Sediment Preservation Methods for Genomic Biomonitoring of Coastal Marine Ecosystems." *Marine Pollution Bulletin* 173: 113129. https://doi.org/10.1016/j.marpolbul.2021.113129.

Dully, V., T. A. Wilding, T. Mühlhaus, and T. Stoeck. 2021. "Identifying the Minimum Amplicon Sequence Depth to Adequately Predict Classes in eDNA-Based Marine Biomonitoring Using Supervised Machine Learning." *Computational and Structural Biotechnology Journal* 19: 2256–2268. https://doi.org/10.1016/j.csbj.2021.04.005.

Eichmiller, J. J., S. E. Best, and P. W. Sorensen. 2016. "Effects of Temperature and Trophic State on Degradation of Environmental DNA in Lake Water." *Environmental Science and Technology* 50: 1859–1867. https://doi.org/10.1021/acs.est.5b05672.

Francis, B., T. Urich, A. Mikolasch, H. Teeling, and R. Amann. 2021. "North Sea Spring Bloom-Associated Gammaproteobacteria Fill Diverse Heterotrophic Niches." *Environmental Microbiomes* 16, no. 1: 15. https://doi.org/10.1186/s40793-021-00385-y.

Frøslev, T. G., R. Ejrnæs, A. J. Hansen, et al. 2022. "Treated Like Dirt: Robust Forensic and Ecological Inferences From Soil eDNA After Challenging Sample Storage." *Environmental DNA* 5: 1–17. https://doi.org/10.1002/EDN3.367.

Frühe, L., V. Dully, D. Forster, et al. 2021. "Global Trends of Benthic Bacterial Diversity and Community Composition Along Organic Enrichment Gradients of Salmon Farms." *Frontiers in Microbiology* 12: 637811. https://doi.org/10.3389/fmicb.2021.637811.

Gargan, L. M., P. R. Brooks, S. R. Vye, et al. 2022. "The Use of Environmental DNA Metabarcoding and Quantitative PCR for Molecular Detection of Marine Invasive Non-Native Species Associated With Artificial Structures." *Biological Invasions* 24, no. 3: 635–648. https://doi.org/10.1007/S10530-021-02672-8.

Glasl, B., D. G. Bourne, P. R. Frade, T. Thomas, B. Schaffelke, and N. S. Webster. 2019. "Microbial Indicators of Environmental Perturbations in Coral Reef Ecosystems." *Microbiome* 7, no. 1: 94. https://doi.org/10.1186/s40168-019-0705-7.

Gloor, G. B., J. M. Macklaim, V. Pawlowsky-Glahn, and J. J. Egozcue. 2017. "Microbiome Datasets Are Compositional: And This Is Not Optional." *Frontiers in Microbiology* 8: 2224. https://doi.org/10.3389/fmicb.2017.02224.

Gonzalez, S. V., K. A. Dafforn, P. E. Gribben, W. A. O'Connor, and E. L. Johnston. 2023. "Organic Enrichment Reduces Sediment Bacterial and Archaeal Diversity, Composition, and Functional Profile Independent of Bioturbator Activity." *Marine Pollution Bulletin* 196: 115608. https://doi.org/10.1016/j.marpolbul.2023.115608.

Gray, M. A., Z. A. Pratte, and C. A. Kellogg. 2013. "Comparison of DNA Preservation Methods for Environmental Bacterial Community Samples." *FEMS Microbiology Ecology* 83, no. 2: 468–477. https://doi.org/10.1111/1574-6941.12008.

Hamaguchi, M., T. Miyajima, H. Shimabukuro, and M. Hori. 2022. "Development of Quantitative Real-Time PCR for Detecting Environmental DNA Derived From Marine Macrophytes and Its Application to a Field Survey in Hiroshima Bay, Japan." *Water* 14, no. 5: 827. https://doi.org/10.3390/W14050827.

Harris, T. 2012. *Bacterial Biogeochemistry*. Academic Press. https://doi.org/10.1016/C2010-0-67238-5.

Harrison, J. B., J. M. Sunday, and S. M. Rogers. 2019. "Predicting the Fate of eDNA in the Environment and Implications for Studying Biodiversity." *Proceedings of the Royal Society B: Biological Sciences* 286, no. 1915: 20191409. https://doi.org/10.1098/rspb.2019.1409.

Harrison, J. P., P.-M. Chronopoulou, I. S. Salonen, T. Jilbert, and K. A. Koho. 2021. "16S and 18S rRNA Gene Metabarcoding Provide Congruent Information on the Responses of Sediment Communities to Eutrophication." *Frontiers in Marine Science* 8: 862. https://doi.org/10.3389/FMARS.2021.708716.

He, X., S. R. Gilmore, T. F. Sutherland, et al. 2021. "Biotic Signals Associated With Benthic Impacts of Salmon Farms From eDNA Metabarcoding of Sediments." In *Molecular Ecology*, 3158–3174. John Wiley and Sons Inc. https://doi.org/10.1111/mec.15814.

Hornick, K. M., and A. H. Buschmann. 2018. "Insights Into the Diversity and Metabolic Function of Bacterial Communities in Sediments From Chilean Salmon Aquaculture Sites." *Annals of Microbiology* 68, no. 2: 63–77. https://doi.org/10.1007/s13213-017-1317-8.

Imlay, J. A. 2002. "How Oxygen Damages Microbes: Oxygen Tolerance and Obligate Anaerobiosis." In *Advances in Microbial Physiology*, 111–153. Academic Press. https://doi.org/10.1016/S0065-2911(02)46003-1.

Iturbe-Espinoza, P., B. W. Brandt, M. Braster, M. Bonte, D. M. Brown, and R. J. M. van Spanning. 2021. "Effects of DNA Preservation Solution and DNA Extraction Methods on Microbial Community Profiling of Soil." *Folia Microbiologica* 66, no. 4: 597–606. https://doi.org/10.1007/s12223-021-00866-0.

Kasana, R. C., and C. B. Pandey. 2018. "Exiguobacterium: An Overview of a Versatile Genus With Potential in Industry and Agriculture." *Critical Reviews in Biotechnology* 38, no. 1: 141–156. https://doi.org/10.1080/07388551.2017.1312273.

Kaul, A., S. Mandal, O. Davidov, and S. D. Peddada. 2017. "Analysis of Microbiome Data in the Presence of Excess Zeros." *Frontiers in Microbiology* 8: 2114. https://doi.org/10.3389/fmicb.2017.02114.

Khwen, N. N., M. F. AL-Marjani, and S. H. Authman. 2021. "Purification and Characterization of Thermo Stable DNase of *Staphylococcus gallinarum* Isolated From Burns." *Materials Today: Proceedings* 42: 2334–2339. https://doi.org/10.1016/j.matpr.2020.12.324.

Kjær, K. H., M. W. Pedersen, B. De Sanctis, et al. 2022. "A 2-Million-Year-Old Ecosystem in Greenland Uncovered by Environmental DNA." *Nature* 612, no. 7939: 283–291. https://doi.org/10.1038/s41586-022-05453-y.

Kramer, B., and J. Thielmann. 2016. "Monitoring the Live to Dead Transition of Bacteria During Thermal Stress by a Multi-Method Approach." *Journal of Microbiological Methods* 123: 24–30. https://doi.org/10.1016/j.mimet.2016.02.009.

Lauber, C. L., N. Zhou, J. I. Gordon, R. Knight, and N. Fierer. 2010. "Effect of Storage Conditions on the Assessment of Bacterial Community Structure in Soil and Human-Associated Samples." *FEMS Microbiology Letters* 307, no. 1: 80–86. https://doi.org/10.1111/J.1574-6968.2010.01965.X.

Lin, H., and S. D. Peddada. 2024. "Multigroup Analysis of Compositions of Microbiomes With Covariate Adjustments and Repeated Measures." *Nature Methods* 21, no. 1: 83–91. https://doi.org/10.1038/s41592-023-02092-7.

López-Pérez, M., and F. Rodriguez-Valera. 2014. "The Family Alteromonadaceae." In *The Prokaryotes*, 69–92. Springer. https://doi.org/10.1007/978-3-642-38922-1_233.

Luna, G. M., A. Dell'Anno, and R. Danovaro. 2006. "DNA Extraction Procedure: A Critical Issue for Bacterial Diversity Assessment in Marine Sediments." *Environmental Microbiology* 8, no. 2: 308–320. https://doi.org/10.1111/j.1462-2920.2005.00896.x.

Luna, G. M., E. Manini, and R. Danovaro. 2002. "Large Fraction of Dead and Inactive Bacteria in Coastal Marine Sediments: Comparison of Protocols for Determination and Ecological Significance." *Applied and Environmental Microbiology* 68, no. 7: 3509–3513. https://doi.org/10.1128/AEM.68.7.3509-3513.2002.

Mandic-Mulec, I., P. Stefanic, and J. D. van Elsas. 2015. "Ecology of Bacillaceae." *Microbiology Spectrum* 3, no. 2: 1–24. https://doi.org/10.1128/microbiolspec.tbs-0017-2013.

Martin, B. C., M. S. Alarcon, D. Gleeson, et al. 2020. "Root Microbiomes as Indicators of Seagrass Health." *FEMS Microbiology Ecology* 96, no. 2: fiz201. https://doi.org/10.1093/femsec/fiz201.

Martin, M. 2011. "Cutadapt Removes Adapter Sequences From High-Throughput Sequencing Reads." *EMBnet.Journal* 17, no. 1: 10–12. https://doi.org/10.14806/EJ.17.1.200.

Mauvisseau, Q., J. Davy-Bowker, M. Bulling, et al. 2019. "Combining ddPCR and Environmental DNA to Improve Detection Capabilities of a Critically Endangered Freshwater Invertebrate." *Scientific Reports* 9, no. 1: 1–9. https://doi.org/10.1038/s41598-019-50571-9.

Mauvisseau, Q., L. R. Harper, M. Sander, R. H. Hanner, H. Kleyer, and K. Deiner. 2022. "The Multiple States of Environmental DNA and What Is Known About Their Persistence in Aquatic Environments." *Environmental Science and Technology* 56, no. 9: 5322–5333. https://doi.org/10.1021/ACS.EST.1C07638.

Mauvisseau, Q., E. Kalogianni, B. Zimmerman, M. Bulling, R. Brys, and M. Sweet. 2020. "eDNA-Based Monitoring: Advancement in Management and Conservation of Critically Endangered Killifish Species." *Environmental DNA* 2, no. 4: 601–613. https://doi.org/10.1002/EDN3.92.

McMurdie, P. J., and S. Holmes. 2012. "Phyloseq: A Bioconductor Package for Handling and Analysis of High-Throughput Phylogenetic Sequence Data." *Pacific Symposium on Biocomputing. Pacific Symposium on Biocomputing*: 235–246.

McMurdie, P. J., and S. Holmes. 2014. "Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible." *PLoS Computational Biology* 10, no. 4: 1003531. https://doi.org/10.1371/journal.pcbi.1003531.

Nagendra, H. 2002. "Opposite Trends in Response for the Shannon and Simpson Indices of Landscape Diversity." *Applied Geography* 22, no. 2: 175–186. https://doi.org/10.1016/S0143-6228(02)00002-4.

Nearing, J. T., G. M. Douglas, M. G. Hayes, et al. 2022. "Microbiome Differential Abundance Methods Produce Different Results Across 38 Datasets." *Nature Communications* 13, no. 1: 342. https://doi.org/10.1038/s41467-022-28034-z.

Ogata, M., R. Masuda, H. Harino, et al. 2021. "Environmental DNA Preserved in Marine Sediment for Detecting Jellyfish Blooms After a Tsunami." *Scientific Reports* 11, no. 1: 16830. https://doi.org/10.1038/s41598-021-94286-2.

Ogg, C. D., and B. K. C. Patel. 2009. "*Thermotalea metallivorans* Gen. Nov., sp. Nov., a Thermophilic, Anaerobic Bacterium From the Great Artesian Basin of Australia Aquifer." *International Journal of Systematic and Evolutionary Microbiology* 59, no. 5: 964–971. https://doi.org/10.1099/ijs.0.004218-0.

Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2024. vegan: Community Ecology Package. https://vegandevs.github.io/vegan/.

Pavlovska, M., I. Prekrasna, I. Parnikoza, and E. Dykyi. 2021. "Soil Sample Preservation Strategy Affects the Microbial Community Structure." *Microbes and Environments* 36, no. 1: 1–7. https://doi.org/10.1264/jsme2.ME20134.

Pearman, J. K., S. A. Wood, M. J. Vandergoes, et al. 2022. "A Bacterial Index to Estimate Lake Trophic Level: National Scale Validation." *Science of the Total Environment* 812: 152385. https://doi.org/10.1016/J.SCITOTENV.2021.152385.

Pearson, T., and R. Rosenberg. 1978. "Macrobenthic Succession in Relation to Organic Enrichment and Pollution of the Marine Environment." *Oceanography and Marine Biology. Annual Review* 16: 229–311.

Pereira, P. M. F., K. D. Black, D. S. McLusky, and T. D. Nickell. 2004. "Recovery of Sediments After Cessation of Marine Fish Farm Production." *Aquaculture* 235, no. 1–4: 315–330. https://doi.org/10.1016/J.AQUACULTURE.2003.12.023.

Phillips, G., A. Anwar, L. Brooks, L. J. Martina, A. C. Miles, and A. Prior. 2014. *Infaunal Quality Index: Water Framework Directive Classification Scheme for Marine Benthic Invertebrates*. Environment Agency. www.environment-agency.gov.uk.

Pomeroy, L. R., and W. J. Wiebe. 2001. "Temperature and Substrates as Interactive Limiting Factors for Marine Heterotrophic Bacteria." *Aquatic Microbial Ecology* 23, no. 2: 187–204. https://doi.org/10.3354/AME023187.

Quast, C., E. Pruesse, P. Yilmaz, et al. 2013. "The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Research* 41, no. D1: D590–D596. https://doi.org/10.1093/NAR/GKS1219.

Robador, A., V. Brüchert, and B. B. Jørgensen. 2009. "The Impact of Temperature Change on the Activity and Community Composition of Sulfate-Reducing Bacteria in Arctic Versus Temperate Marine Sediments." *Environmental Microbiology* 11, no. 7: 1692–1703. https://doi.org/10.1111/j.1462-2920.2009.01896.x.

Rosenberg, R., M. Blomqvist, H. C Nilsson, H. Cederwall, and A. Dimming. 2004. "Marine Quality Assessment by Use of Benthic Species-Abundance Distributions: A Proposed New Protocol Within the European Union Water Framework Directive." *Marine Pollution Bulletin* 49, no. 9–10: 728–739. https://doi.org/10.1016/j.marpolbul.2004.05.013.

Rubio-Portillo, E., A. Villamor, V. Fernandez-Gonzalez, J. Antón, and P. Sanchez-Jerez. 2019. "Exploring Changes in Bacterial Communities to Assess the Influence of Fish Farming on Marine Sediments." *Aquaculture* 506: 459–464. https://doi.org/10.1016/j.aquaculture.2019.03.051.

Schloss, P. D. 2024a. "Rarefaction Is Currently the Best Approach to Control for Uneven Sequencing Effort in Amplicon Sequence

Analyses." *mSphere* 9, no. 2: e0035423. https://doi.org/10.1128/msphere.00354-23.

Schloss, P. D. 2024b. "Waste Not, Want Not: Revisiting the Analysis That Called Into Question the Practice of Rarefaction." *mSphere* 9, no. 1: e0035523. https://doi.org/10.1128/msphere.00355-23.

Sellers, G. S., C. di Muri, A. Gómez, and B. Hänfling. 2018. "Mu-DNA: A Modular Universal DNA Extraction Method Adaptable for a Wide Range of Sample Types." *Metabarcoding and Metagenomics* 2: e24556. https://doi.org/10.3897/mbmg.2.24556.

SEPA. 2023. *Marine Finfish Farms Guidance on the Use of DNA in Monitoring Compliance With Seabed Mixing Zone Area Limits*. SEPA. https://www.sepa.org.uk/regulations/how-we-regulate/operator-monitoring/.

Shadfar, S., S. Parakh, M. S. Jamali, and J. D. Atkin. 2023. "Redox Dysregulation as a Driver for DNA Damage and Its Relationship to Neurodegenerative Diseases." *Translational Neurodegeneration* 12: 18. https://doi.org/10.1186/s40035-023-00350-4.

Shea, M. M., J. Kuppermann, M. P. Rogers, D. S. Smith, P. Edwards, and A. B. Boehm. 2023. "Systematic Review of Marine Environmental DNA Metabarcoding Studies: Toward Best Practices for Data Usability and Accessibility." *PeerJ* 11: e14993. https://doi.org/10.7717/peerj.14993.

Shen, L., Y. Liu, L. Chen, et al. 2024. "Genomic Basis of Environmental Adaptation in the Widespread Poly-Extremophilic Exiguobacterium Group." *ISME Journal* 18, no. 1: wrad020. https://doi.org/10.1093/ismejo/wrad020.

Singh, B., and R. T. Marshall. 1966. "Bacterial Deoxyribonuclease Production and Its Possible Influence on Mastitis Detection1." *Journal of Dairy Science* 49, no. 7: 822–825. https://doi.org/10.3168/jds.S0022-0302(66)87953-5.

Sorokin, D. Y., A. V. Mardanov, and N. V. Ravin. 2020. "Chitinivibrionaceae." In *Bergey's Manual of Systematics of Archaea and Bacteria*, 1. John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118960608.fbm00383.

Soto, W. 2022. "Emerging Research Topics in the Vibrionaceae and the Squid–Vibrio Symbiosis." *Microorganisms* 10, no. 10: 1946. https://doi.org/10.3390/microorganisms10101946.

Suzuki, M. T., and S. J. Giovannoni. 1996. "Bias Caused by Template Annealing in the Amplification of Mixtures of 16S rRNA Genes by PCR." *Applied and Environmental Microbiology* 62, no. 2: 625–630. https://doi.org/10.1128/AEM.62.2.625-630.1996.

Taberlet, P., E. Coissac, M. Hajibabaei, and L. H. Rieseberg. 2012. "Environmental DNA." *Molecular Ecology* 21, no. 8: 1789–1793. https://doi.org/10.1111/J.1365-294X.2012.05542.X.

Tatangelo, V., A. Franzetti, I. Gandolfi, G. Bestetti, and R. Ambrosini. 2014. "Effect of Preservation Method on the Assessment of Bacterial Community Structure in Soil and Water Samples." *FEMS Microbiology Letters* 356, no. 1: 32–38. https://doi.org/10.1111/1574-6968.12475.

Thiele, S., A. Vader, S. Thomson, et al. 2023. "The Summer Bacterial and Archaeal Community Composition of the Northern Barents Sea." *Progress in Oceanography* 215: 103054. https://doi.org/10.1016/j.pocean.2023.103054.

Wilding, T. A., T. Stoeck, B. J. Morrissey, S. F. Carvalho, and M. W. Coulson. 2023. "Maximising Signal-To-Noise Ratios in Environmental DNA-Based Monitoring." *Science of the Total Environment* 858: 159735. https://doi.org/10.1016/j.scitotenv.2022.159735.

Wyness, A. J., T. Stoeck, B. J. Morrissey, et al. 2026. "eDNA2IQI: An eDNA/Random Forest-Based Prediction Tool That Accurately Predicts Ecological Status for Regulatory-Compliance Assessment." *Aquaculture* 612: 743126. https://doi.org/10.1016/j.aquaculture.2025.743126.

Yilmaz, P., L. W. Parfrey, P. Yarza, et al. 2014. "The SILVA and "All-Species Living Tree Project (LTP)" Taxonomic Frameworks." *Nucleic Acids Research* 42, no. D1: D643–D648. https://doi.org/10.1093/NAR/GKT1209.

Zaiko, A., X. Pochon, E. Garcia-Vazquez, S. Olenin, and S. A. Wood. 2018. "Advantages and Limitations of Environmental DNA/RNA Tools for Marine Biosecurity: Management and Surveillance of Non-Indigenous Species." *Frontiers in Marine Science* 5, no. SEP: 322. https://doi.org/10.3389/FMARS.2018.00322/BIBTEX.

Zhang, B., M. Brock, C. Arana, et al. 2021. "Impact of Bead-Beating Intensity on the Genus- and Species-Level Characterization of the Gut Microbiome Using Amplicon and Complete 16S rRNA Gene Sequencing." *Frontiers in Cellular and Infection Microbiology* 11: 678522. https://doi.org/10.3389/fcimb.2021.678522.

Zobell, C. E., and J. E. Conn. 1940. "Studies on the Thermal Sensitivity of Marine Bacteria." *Journal of Bacteriology* 40, no. 2: 223–238. https://doi.org/10.1128/jb.40.2.223-238.1940.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Plot of IQI predictions including rejected samples, circled in gray. There are five samples contained within four circles. **Figure S2:** NMDS of sediment incubation rarefied to 5000 reads, colored by station. **Table S1:** Full list of ANCOMBC2 identified bacteria, with full taxonomy from phylum onwards, and log fold changes (LFC) at 20°C and 40°C, compared to 10°C.