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Fjords: Estuaries on the Front-line of Climate Change

Assessing Environmental Quality in a Historically Polluted Fjord: A Comparison of Benthic Foraminiferal eDNA and Morphospecies Approaches

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Key Points:

- Natural and anthropogenic variability for example, low oxygen, organic matter and pollutant accumulation, deteriorate Ecological Quality Status
- Morphology-based and molecular assessment methods show congruent responses of benthic foraminifera to environmental stress
- Genetic methods can overestimate Ecological Quality Status in anoxic sites due to dormant propagules or transported DNA presence

Supporting Information:

Supporting Information may be found in the online version of this article.

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Abstract This study is the first assessment of a fjord Ecological Quality Status (EcoQS) by comparing both the traditional morphology-based and emerging metabarcoding techniques in benthic foraminifera. For this, we focus on historically polluted Idefjord on the Swedish Norwegian border, which has experienced high effluent load from pulp and paper mill for almost a century. Based on our results, the morphological data was more sensitive to "naturally stressed" conditions, like course sediments and cascading water inflows at fjord sills. Generally, both data sets report congruous responses in the EcoQS and benthic foraminiferal assemblages to environmental stress factors, showing highest diversity at the coastal reference station and the outer fjord, with a diversity decline in proximity of industrial facilities and at the most oxygen depleted sites in the inner fjord. Genetic methods tend to overestimate EcoQS at highly anoxic sites probably due to a presence of dormant propagules or extraorganismal DNA, emphasizing a need for cross-correlation with morphological methods to validate EcoQS assessment in such conditions.

Plain Language Summary The Ecosystem Quality Status (EcoQS) of fjords can be difficult to assess because of natural variation caused by for example, complex sea floor topography and bottom water oxygen depletion. Fjords have often restricted water exchange, resulting in stagnant bottom waters and pollutant accumulation in basin sediment caused by proximity to industrial facilities. Benthic foraminifera (shell-bearing protists) can be used to provide information about the health of such ecosystems. Environmental DNA (eDNA) extracted from sediment samples can also be used to identify the foraminifera species present and calculate biological indices. Yet, it is still unclear how well the eDNA technique performs in fjord EcoQS assessment and how it compares to the traditional morphology-based approach. In Idefjord (Sweden-Norway), historic dumping of waste from the pulp and paper industry, has led to a build-up of organic matter, chemical pollutants in the sediment and widespread oxygen depletion. Based on our study, the fjord EcoQS determined by both approaches was fairly similar, showing highest diversity at the coastal reference station and the outer fjord, with a decline in diversity in proximity to industrial facilities and in the inner fjord. The genetic method, however, tended to over-estimate the EcoQS at the anoxic stations, likely due to left-over eDNA from transport or resting stages.

1. Introduction

Benthic foraminifera are increasingly used in marine ecological assessment and biomonitoring due to their broad distribution and ability to respond to environmental stressors while serving as geochemical element repositories. Foraminifera have short life spans, typically lasting only a few weeks or months, providing high-resolution environmental data (Murray, 2006). Foraminifera are meiofauna sized components of the biocenose, comprising up to >40%–50% of the eukaryotic biomass in the sediments (Gooday et al., 1992; Moodley et al., 2000). High foraminifera abundance enables statistically significant analyses and calculation of biotic indices from small sample volumes, which have been successfully applied in diverse marine environments for example, (Alve, 1995; Alve et al., 2009; Barras et al., 2014; Bouchet et al., 2012; Dimiza et al., 2016; Jorissen et al., 2018; Silva et al., 2022).

Foraminiferal distribution and assemblage composition is controlled by sensitivity to numerous environmental parameters such as organic matter, oxygen, salinity, nutrient availability, water depth, sediment characteristics,

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pH and carbonate saturation (Murray, 2006). Propagules, or the dormant life stages of foraminifera, can survive within the sediments for up to 2 years before growth commences and could have an important role in recolonization and dispersal of this group (Alve & Goldstein, 2010). Due to sample preparation and misidentification, propagules, small individuals, and soft-shelled clades (mainly Monothalamea or monothalamids) may be excluded from stereoscopic taxonomic morphospecies identification methods.

Recent advancements in high-throughput sequencing (HTS) technologies have led to an increase in environmental DNA (eDNA) studies, which utilize metabarcoding techniques to assess foraminiferal diversity and distribution for biomonitoring purposes (e.g., Brinkmann et al., 2023; Cavaliere et al., 2021; Cordier et al., 2017; Frontalini et al., 2018; Laroche et al., 2016; Pawlowski et al., 2014; Pawlowski et al., 2016; Pochon et al., 2015; Singer et al., 2023). Metabarcoding of eDNA targets specific gene regions amplified from sediment samples, encompassing both intra- and extra-organismal DNA, originating from both living and deceased individuals (Pawlowski et al., 2014). Using foraminifera-specific primers the benthic community can be extracted and amplified, eDNA reads are clustered into Operational Taxonomic Units (OTUs), and an algorithm is used to estimate taxonomic assignments, reducing sample processing time and enable the detection of propagules, as well as small or soft-bodied monothalamids (Keeley et al., 2018; Pawlowski et al., 2014, 2016).

Now, the focus is shifting towards exploring these techniques in the context of monitoring anthropogenic environmental disturbances (Barrenechea et al., 2021; Cao et al., 2022; Frontalini et al., 2020; Laroche et al., 2016; Pochon et al., 2015). By testing and comparing both strategies across a range of environmental gradients, morphological methods can be employed to validate the outcomes of genetic methods and identify potential limitations.

While previous studies have demonstrated the congruence between indices derived from morphospecies and molecular data sets in tropical and subtropical regions (Al-Enezi et al., 2022; Cavaliere et al., 2021), further investigations are required in temperate or boreal transitional waters defined in the appendix by McLusky and Elliott (2007). Fjords are long and narrow coastal inlets, and their slopes are typically steep-sided due to formation by tectonic activities and modified by glacial carving, which has often also formed sills or thresholds within the fjords (Shoemaker, 1986). Fjords have been the location of extensive industrial development and exhibit high natural variability, denoting them as aquatic critical zones (Bianchi et al., 2020). This contributes to the "estuarine quality paradox" (Elliott & Quintino, 2007), which is amplified by sills that hinder bottom water exchange and lead to periodic or permanent occurrences of bottom water hypoxia. In such systems, it is essential to determine which biotic indices accurately reflect the Ecological Quality Status (EcoQS) and which threshold values shall be used for indices based on eDNA techniques.

The development of industries along the Scandinavian coastline commenced in the late 1700s and expanded throughout the 1800s and beyond, with anthroturbation (Zalasiewicz et al., 2014) reaching its peak in the latter half of the 20th century. In 1970, the introduction of the "Clean Water Act" to Norway mandated industrial stakeholders to reduce waste dumping and chemical runoff into the surrounding waterways, closely followed by specific regulation of the Paper and Pulp industry in 1974 (Sæther, 1998). Several studies have utilised foraminiferal assemblages to assess diversity and trace environmental history in Scandinavian fjords based on traditional morphological approach (Hanslik, 2001; Nordberg et al., 2017; Polovodova Asteman et al., 2015, 2021), however, the application of molecular techniques remains unexplored.

This study focuses on the Idefjord located at the Swedish-Norwegian border. The sampling campaign transect covers a several key environmental gradients including salinity, oxygen and total organic carbon (TOC) together with heavy metal contamination, as indicators of industrial impact, allowing for investigation into the in situ response of the biotic community to both natural and anthropogenically induced stressors. The aim is contributing to the application of foraminiferal eDNA techniques for biomonitoring in transitional waters and assess whether eDNA and traditional morphology yield consistent ecological responses to pollution in temperate and boreal fjords.

2. Study Area

The Idefjord or the Ringdalsfjorden-Iddefjorden system is connected to the Skagerrak (North Sea) coast through the Singlefjord and bisected down its length by the Swedish-Norwegian boarder (Figure 1). The outermost section of the fjord, Ringdalsfjorden, extends 6.4 km to a 90° bend where the Norwegian city of Halden is located, and the

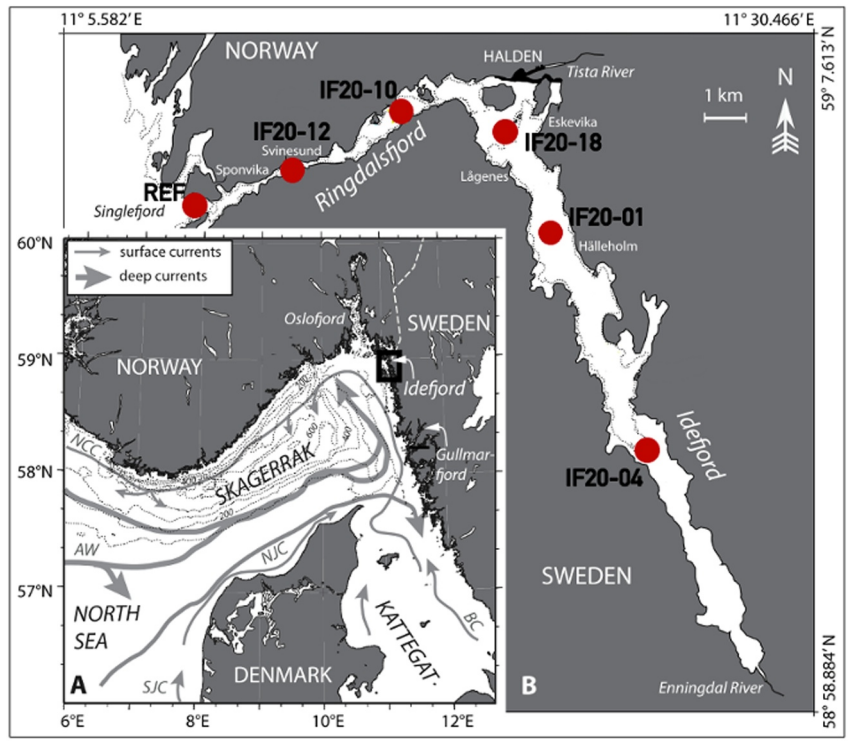

Figure 1. Map of the study area, red dots mark the locations of the sampling stations, adapted from Polovodova Asteman et al. (2015).

remaining inner 18.6 km of the total 25 km -long fjord system is called Iddefjorden in Norwegian. In this paper both fjord sections will be referred to as Idefjord using the anglicized-Swedish terminology.

The Idefjord reaches an average of 19 m water depth, with a total area of roughly 24.5 km^2, and volume of approximately 404 × 106 m^3 (Hanslik, 2001). The Idefjord system is 25 km long and its bottom topography is characterized by presence of multiple sills restricting bottom water exchange (Polovodova Asteman et al., 2015). Three of the sills are present in the outer fjord (east to west, with 20, 9 and 9.5 m depth), with two shallower sills (23 and 20 m depth) enclosing a smaller basin and last largest basin, which extends to the end of Idefjord and holds approximately 75% of the total water volume in the system (Figure 2).

Two rivers contribute to the fresh-water runoff into the fjord, the Enningdal River in the innermost part and the Tista River, which meets the fjord 8 km from the fjord mouth (Figure 1). The river input and water exchange, with the adjacent Skagerrak, results in water column stratification into three distinct water masses (Johnsson et al., 2007). Cool fresh water from the rivers maintains a thin brackish surface layer of around 2 m thick, with a salinity lower than 10 psu. Extending beneath the outer sills at depths ranging from approximately 2 to 17–22 m, is the intermediate water mass, originating from the adjacent coastal Skagerrak with a salinity range of 15–25. Finally, the deepest water mass found in the basin, below the 17–22 m depth range, is imported water with a salinity of ∼30 (Johnsson et al., 2007).

In the fjord, the estuarine circulation (Stigebrandt, 2012) occurs due the overlaying lower density fresh water flowing out of the system, whilst brackish and saline water is pushed into the fjord due to wave and wind influences from the Skagerrak/Kattegat. Prevailing wind patterns result in the Ekman transport of coastal surface water, driving coastal upwelling within fjords on the west coast of Sweden (Björk & Nordberg, 2003). Upwelling facilitates deep water exchange in fjords, which is often limited due to a presence of sills. Ekman transport also results in deep water exchange when the density of water outside the fjord, above the sill depth, becomes higher than the density below it, facilitating the exchange of deep-water within the basin (Berge, 1994). According to previous studies, such events happen approximately 1–3 times per year in the outer fjord, while they are rare within the inner basin (Josefsson & Nyquist, 1976).

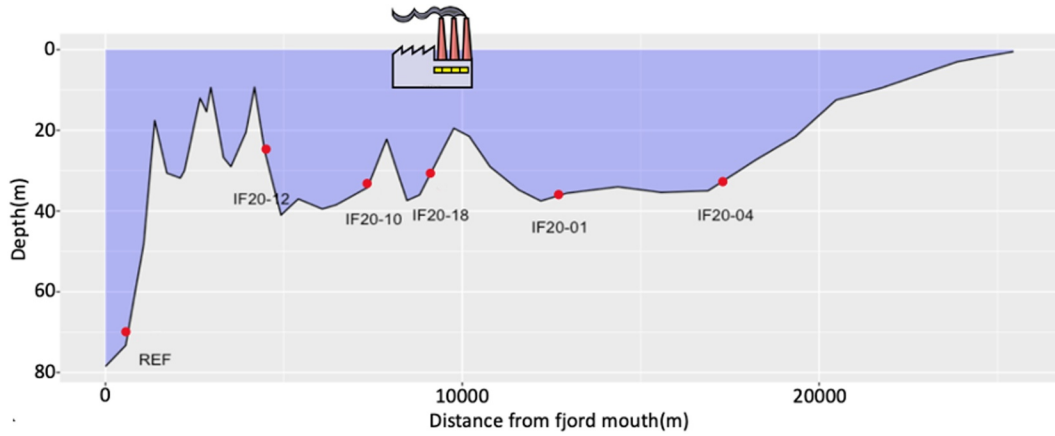

Figure 2. A simplified outline of Idefjord bottom topography, with sampling stations marked by red dots. Position of the pulp and paper mill at the city of Halden, the major pollution source in the fjord, is indicated by a factory symbol.

3. Pollution History

The Idefjord has been recognized as one of the most polluted fjord systems in Scandinavia (Hanslik, 2001; Polovodova Asteman et al., 2015) because of its long history of contamination originating from multiple sources. The primary pathway of pollution into the fjord system has been via the Tista River. The highest levels of pollution detected in the sediments are typically found at the river's outlet, highlighting its significant contribution to the fjord's pollution load (Josefsson & Nyquist, 1976). The pollution included both sewage, discharged from the city of Halden, and waste effluents released by the paper and pulp mill (Josefsson & Nyquist, 1976).

Industrial-scale paper production led to the direct disposal of effluent materials into the Tista River (Berge, 1994; Hanslik, 2001). The contaminant material predominantly consisted of oxygen-consuming organic matter derived from wood pulp, as well as chemicals associated with the bleaching process and sulfate production, in addition to sewage from the city of Halden. The intricate bottom topography of the fjord system with multiple sills (Figure 2), which naturally reduce the frequency of deep-water renewal events, facilitated the accumulation of organic material and pollutants in fjord sediments over time. This led to the development of highly anoxic basins with elevated concentrations of toxic heavy metals, oxygen-consuming organic matter, and limited bioturbation due to lack of macrofaunal organisms in the sediment.

By 1975 the fjord had been regarded dead for decades, which led to legal actions and introduction of wastewater treatment facilities. Studies in early 2000s and 2010s demonstrated that the outer Idefjord was close to recovery, whilst inner fjord still showed environmental degradation (Polovodova Asteman et al., 2015; Polovodova Asteman & Nordberg, 2017).

The sediments of the Idefjord serve as an archive for the environmental changes outlined above. Foraminiferal assemblages based on morphospecies approach combined with sediment geochemistry in sediment cores have demonstrated that fjord environmental changes can be reconstructed in detail (e.g, (Alve, 1991; Dolven et al., 2013; Husum & Hald, 2004; Polovodova Asteman et al., 2015)). However, palaeoecological approaches relying on microfossils can suffer from taphonomic or preservation biases, which can be constrained by looking at the contemporary distribution of living (stained) foraminiferal assemblages (Murray, 2006; Murray & Alve, 2016). Also, by applying the molecular approaches such as eDNA can aid in recovery assessment as eDNA detects both foraminiferal propagules (dormant stages) and non-fossilizing species, usually neglected by traditional morphospecies approaches.

4. Material and Methods

4.1. Sediment Sampling

Surface (0–3 cm) sediment samples were collected from six stations along the fjord transect (Table 1) in November 2020 and 2022 aboard the R/V Skagerrak using a twin-barrel Gemini corer (Ø 80 mm, tube length 700 mm). In 2020, at each station three cores with undisturbed bottom water—sediment interface were sampled

Table 1
Sampling Stations in the Idefjord Including Geographical Position (Latitude and Longitude, Water Depth) and Sampling Date

Station	Sampling date	Latitude	Longitude	Water depth, m
SF20-REF	10/11/2020	59°05.15'N	11°12.73'E	69
IF20-12	10/11/2020 & 09/11/2022	59°05.78'N	11°15.59'E	26
IF20-10	11/11/2020	59°06.47'N	11°18.61'E	41
IF20-18	10/11/2020 & 09/11/2020	59°06.82'N	11°20.46'E	28.5
IF20-01	10/11/2020	59°05.40'N	11°22.60'E	38
IF20-04	11/11/2020	59°03.06'N	11°24.37'E	35

for morphological and genetic analysis and one for sediment bulk geochemistry analysis, including TOC, TN and heavy metals (arsenic, cadmium, cobalt, chromium, copper, mercury, nickel, and zinc). Geochemical data was averaged over 0–3 cm, to capture the recent ecological state of the stations, considering an estimated sedimentation rate of ∼0.8 cm per year (Polovodova Asteman et al., 2015). Additional samples were obtained in November 2022 for stations IF20-18 and IF20-12 to supplement the lack of heavy metal data in the uppermost sediment intervals. Throughout the sampling process, sterile gloves and spoons were used to minimize cross-contamination risk. Molecular samples were double-bagged, immediately frozen at −20°C, and transported in a cool box filled with dry ice to the University of Geneva for eDNA analysis.

At each sampling station, a CTD probe was used prior to sediment sampling to obtain temperature, conductivity, fluorescence, turbidity, salinity, and dissolved oxygen data.

4.2. Bulk Sediment Geochemistry

For geochemistry analyses, wet sediment was weighed, freeze dried and weighed again. Dry sediment was powdered in an agate mortar and was weighed in silver capsules, treated for 48 hr with fumes of HCl to remove any $CaCO_3$, dried and placed in tin capsules which were then tightly closed. A SerCon Isotope Ratio Mass Spectrometer (IRMS) Elemental Analyzer for solid samples at the infrastructure for isotope determination within Earth System Sciences (ISOGOT) facilities (University of Gothenburg) was used to measure the total organic carbon (TOC) and total nitrogen (TN), from which Carbon to Nitrogen ratio (C/N ratio) was calculated.

Heavy metal (As, Cd, Co, Cr, Cu, Hg, Ni, Pb, V, Zn) analysis of the sediment samples was performed by the ALS Scandinavia's laboratory (Luleå, Sweden) (Naturvårdsverket, 2023).

4.3. Calculation of Pollution Indices

To quantify the environmental stress, several pollution indices where calculated. These were Organic Sediment Index (OSI), Contamination Factor (cf.), Modified degree of contamination (mCd) and lastly Pollution Load Index (PLI).

The OSI index is used to distinguish whether sedimentary deposits in lakes, rivers and estuaries are affected by organic pollution. The organic sediment index was calculated to evaluate the organic load, using the following equation:

$$\mathrm{OSI} = [\mathrm{TOC\ wt\%}] \times [\mathrm{ON\ wt\%}]$$

where (TOC) is the total organic carbon and (ON) is organic nitrogen multiplied by 0.95 to account for the naturally occurring background nitrogen (Ballinger & McKee, 1971; Rostan et al., 1987). Organic nitrogen is nitrogen bound to organic matter in the sediment (Naturvårdsverket, 2022). Since Idefjord sediment contains high amounts of TOC (Figure 4a) it is reasonable to assume that most of the nitrogen present in fjord sediments is equivalent to ON. Thus, herein, TN was used instead of ON to calculate OSI due to main pollution source being pulp and paper mill, which accounts for the largest point pollution sources for TN in the Skagerrak (Naturvårdsverket, 2022, 2023).

Table 2
Classification of Environmental Quality Status (EcoQS) Values From the Norwegian Environmental Protection Agency (Miljødirektoratet, 2016), Which Reflect the Ecological Status in Fjords and Coastal Waters

Abiotic variable	Miljødirektoratet (M-608/2016 rev. 30. October 2020)/Molvær et al., 1997				
As (mg/kg)	0–15	15–18	18–71	71–580	>580
Pb (mg/kg)	0–25	25–150	150–1480	1480–2000	2000–2500
Cd (mg/kg)	0–0,2	0,2–2,5	2,5-16	16–157	>157
Cu (mg/kg)	0–20	20–84	-	84–147	>147
Cr (mg/kg)	0–60	60–620	620–6,000	6,000–15500	15,500–25000
Hg (mg/kg)	0–0,05	0,05–0,52	0,52–0,75	0,75–1,45	>1,45
Ni (mg/kg)	0–30	30–42	42–271	271–533	>533
Zn (mg/kg)	0–90	90–139	139–750	750–6,690	>6,690
TOC (%)	<2.0	2.0–2.7	2.7–3.4	3.4–4.1	>4.1
EcoQS	Background	Good	Moderate	Poor	Bad
Class	I	II	III	IV	V

Note. Total organic carbon values are from Molvær et al. (1997).

To summarize the metal contamination, PLI index was calculated (Tomlinson et al., 1980). This index is comprised of the concentration factor (CF) of each metal and the respective background values. It is calculated as follows:

$$Cf = [\text{element}]\, i / [\text{element}]\, \text{background}$$

$$\text{PLI} = \sqrt{Cf1\ Cf2 \ldots Cfn}$$

Where (element) and (element) background are concentrations of metal X and the geochemical background levels (or "pristine conditions"), respectively. Here the background values were based on regional reference values following the Norwegian Environmental Protection Agency (Miljødirektoratet, 2016) (Table 2).

4.4. Analysis of Benthic Foraminiferal Morphospecies

Sediment samples (~50 ml) were stained with rose Bengal—95% ethanol solution (2 g l^{-1}), refrigerated at 4°C and left for a minimum of 2 weeks to ensure adequate staining (Schönfeld et al., 2012). Prior to washing, sediment volume was estimated using the area of the container filled with the sediment. All samples were washed over 1 mm and 63 μm sieves, in line with the Foraminiferal Biomonitoring (FOBIMO) protocol (Schönfeld et al., 2012). During the washing tetrasodium pyrophosphate ($Na_4P_20_7$) was used, if necessary, to disaggregate stubborn clay clumps and fecal pellets.

The sediment residue >63 μm was examined under a stereomicroscope and picked using a 45 squared grid tray, where squares were randomly chosen and picked empty of foraminifera. A minimum of 300 living and 300 dead individuals were wet picked from each sample, ensuring statistically significant sample size and an estimation of foraminiferal densities. Taxonomic classification and identification were done to the lowest possible level using the following literature (Cage et al., 2021; Feyling-Hanssen, 1964; Höglund, 1947; Nordberg et al., 2017; Polovodova Asteman & Schönfeld, 2016; Polovodova Asteman et al., 2021; Qvale & Nigam, 1985). Species numbers per sample were recorded, and relative (%) and absolute abundances (ind. 10 ml^{-1}) for each species were calculated.

4.5. Sedimentary eDNA Analysis

Sedimentary DNA was extracted using a DNeasy® PowerLyzer PowerSoil Kit (QIAGEN), for each of the three biological replicates at ID-Gene Ecodiagnostics Lab (Geneva, Switzerland). Three DNA extraction replicates were performed as per the manufacturer's guidelines, and then stored at −20°C. Extraction controls were added and processed together with other samples (1 control for each 11 extractions). Specific foraminiferal primers

(14F1:5'- AAGGGCACCACAAGAACGC-3' and S15: 5′– CCACCTATCACAYAATCATG–3′.) were used to amplify the 37f hypervariable region of the 18S rRNA gene and generate amplicons ranging from 90 to 190 bp in length (Lejzerowicz et al., 2014). Multiplexing of polymerase chain reaction (PCR) products in sequencing libraries was enabled by tagged primers, 8 nucleotides were attached at the 5'- end (Cavaliere et al., 2021; Esling et al., 2015). Three PCR replicates and one PCR-negative control were amplified per each DNA extract with 1 µl of eDNA in 25 µl of reaction volume using FastStart™ Taq polymerase (Sigma-Aldrich). The 9 combined PCR products for each sediment sample were quantified by high-resolution capillary electrophoresis (QIAxcel System, QIAGEN). The quantified PCR products were pooled in equimolar concentration. The pool was purified using the High Pure PCR Product Purification Kit (Roche) and used for library preparation using the TruSeq DNA PCR-Free Library Prep Kit (Illumina). After quantification using the KAPA Library Quantification Kits (KAPA BIOSYSTEMS), the paired-ed sequencing was conducted on an Illumina MiSeq System using a v2 Miseq reagent kit for 300 cycles.

Strict parameters were applied retaining only high-quality data. The raw sequencing reads were demultiplexed using the demultiplexer tool (module DTD) from SLIM web application (Dufresne et al., 2019), and sequences with mismatched tagged primers or ambiguous bases were filtered out. The subsequent processes, including quality filtering, error learning, merging, chimera removal, and ASV inference, were done using DADA2 (R package (Callahan et al., 2016),) with standard settings included in the dada2 module from SLIM.

Additionally, the sequences were clustered into OTUs at 97% using Vsearch (Rognes et al., 2016). Finally, to remove PCR and sequencing errors or intraindividual variability as recommended by Brandt et al. (2021), we applied a LULU curation (Frøslev et al., 2017) on the clustered sequences using the LULU *R* package using minimum_match = 95, minimum_relative_cooccurence = 0.97 as parameters. We manually checked the sequences, and we kept only those having the end of the conservative region (37f) as in Barrenechea Angeles et al., 2024. The taxonomic annotation was done by comparing the sequences with available sequences in NCBI and using Vsearch at 97% of similarity and Blast (Altschul et al., 1990). Non-foraminiferal sequences were removed and to reduce the number of unassigned sequences, a genetic signature for Globotahalamea and Monothalamea was used to at least identify them at the order level following the genetic signatures as in Barrenechea Angeles et al. (2024).

4.6. Assemblage Diversity and Biotic Indices

Species diversity of morpho- and molecular data was assessed using diversity indices Richness, Shannon-Weiner, Simpson and Exp(H'bc) (Chao & Shen, 2003), calculated on the raw count data set. Rather than filtering via minimum reads threshold, a rarefaction was applied to the genetic data set using *phyloseq* in *R* studio (McMurdie & Holmes, 2013). Rarefaction addresses variability in sequencing depth by subsampling each data set to a common number of reads, mitigating sampling bias and facilitating robust ecological inferences. Samples underwent rarefaction (without replacement) to a depth of 8,080 reads per sample, which matched the minimum read count across all samples. This process resulted in a total of 276 OTUs, with a maximum of 113 OTUs identified in a single sample. Richness, relative abundance, diversity (Shannon and Simpson), Exp(H'bc) were calculated in *R* studio for both morphological and molecular data sets using *entropy* and *vegan* packages (Hausser & Korbinian, 2014; Oksanen et al., 2015). Due to lack of taxonomic resolution in the genetic data set, it was not possible to apply calculation of sensitivity indices to both data sets.

To circumvent the "estuarine quality paradox", instead of using threshold values determined for other localities, the EcoQS boundaries were established using the ecological quality ratio (EQR). The EQR quantifies the ratio between the observed value of a biological parameter, in this case diversity, and its expected value under reference conditions. As local reference conditions, we have chosen a station outside the fjord (SF20-REF), where we expected to find low pollution, highest diversity and well-oxygenated conditions. The EQR is a ratio, where 0 is representing a state of low ecological quality (i.e., bad EcoQS), and 1 is indicating a state of high ecological quality (i.e., high EcoQS). The EQR scale was divided into five equal-size class boundaries, in line with Al-Enezi et al. (2022) and Silva et al. (2022):1–0.8 (high EcoQS), 0.8–0.6 (good EcoQS), 0.6–0.4 (moderate EcoQS), 0.4–0.2 (poor EcoQS), and 0.2–0 (bad EcoQS). This standardised approach allows for comparisons between both sampling strategies.

Agreement or disagreement between the indices calculated from each data set were determined by considering only two EcoQS categories, "Acceptable" (scored as 1) and "Not acceptable" (scored as 0), where the former

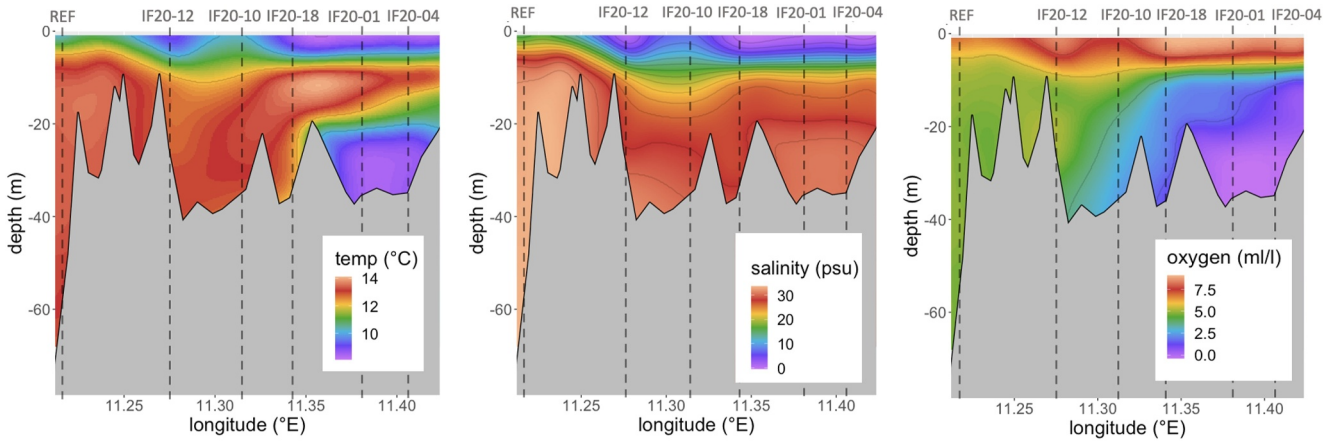

Figure 3. Section plots of Idefjord bathymetry and hydrography based on CTD data, from left to right for temperature, salinity and oxygen. The vertical dashed lined shows the locations of sampled stations.

comprises "high" and "good" and the latter "moderate", "poor" and "bad", in line with WFD restoration goals (Al-Enezi et al., 2022; Blanchet et al., 2008; Bouchet et al., 2012; Cavaliere et al., 2021; Silva et al., 2022). The scores for each data set are summed for each station. To assess the agreement or disagreement on a statistical basis, a non-parametric sign test was also used.

4.7. Statistical Analysis

Only species with a relative abundance of >3% (rescaled back to 100% once low abundance species were removed) were kept in the morphological data set for multivariate statistical analysis.

Multidimensional Scaling (MDS) was employed to visually represent the dissimilarities among foraminiferal assemblages in a reduced-dimensional space and uncover underlying patterns of similarity or dissimilarity among the samples and replicates. MDS were conducted in *R* studio, using *entropy* and *vegan* packages (Hausser & Korbinian, 2014; Oksanen et al., 2015).

A spearman's correlation between the diversity indices was calculated comparing the values along the transect using *dplyr* package in *R* studio (Wickham et al., 2023). A multi regression model was computed between each diversity index and log-transformed pollution indices OSI and PLI. Log-transformation stabilizes variance, normalizes distribution and minimizes the influence of outliers, ensuring that the assumptions of linearity, normality and homoscedasticity are met. Linear regressions were calculated between the morphological and genetic exp(H'bc) and key environmental variables TOC, Salinity, Oxygen and PLI. All analyses were done using ggplot2 and *ggpubr R* studio (Oksanen et al., 2015; Wickham, 2016).

5. Results

5.1. Abiotic Parameters

5.1.1. Fjord Hydrography

The CTD data showed the subdivision of the Idefjord water column into three water masses, which is consistent with previous studies (Johnsson et al., 2007). The overlying cool and freshened water from the riverine input forms a ~5 m-thick surface layer with a temperature of <9°C range and salinity of >5 psu (Figure 3). Below it, Skagerrak coastal water with temperature 13–14°C and salinity of 10–30 psu was found at ~5 to 20–22 m. Finally, below 22 m, the cold basin water with salinity >30 psu and hypoxic conditions (less than 2 ml O_2 l^{-1}, mostly in the inner fjord) was found.

Based on the values proximal to the seafloor and, hence, the most important for benthic communities, the highest basin water salinity was observed at the SF20-REF (33.7 psu) and dropped to 30.1 at IF20-12, then down to 28.7 and 27.6 for IF20-10 and IF20-18, both of which are closest to the pollution source and the city of Halden

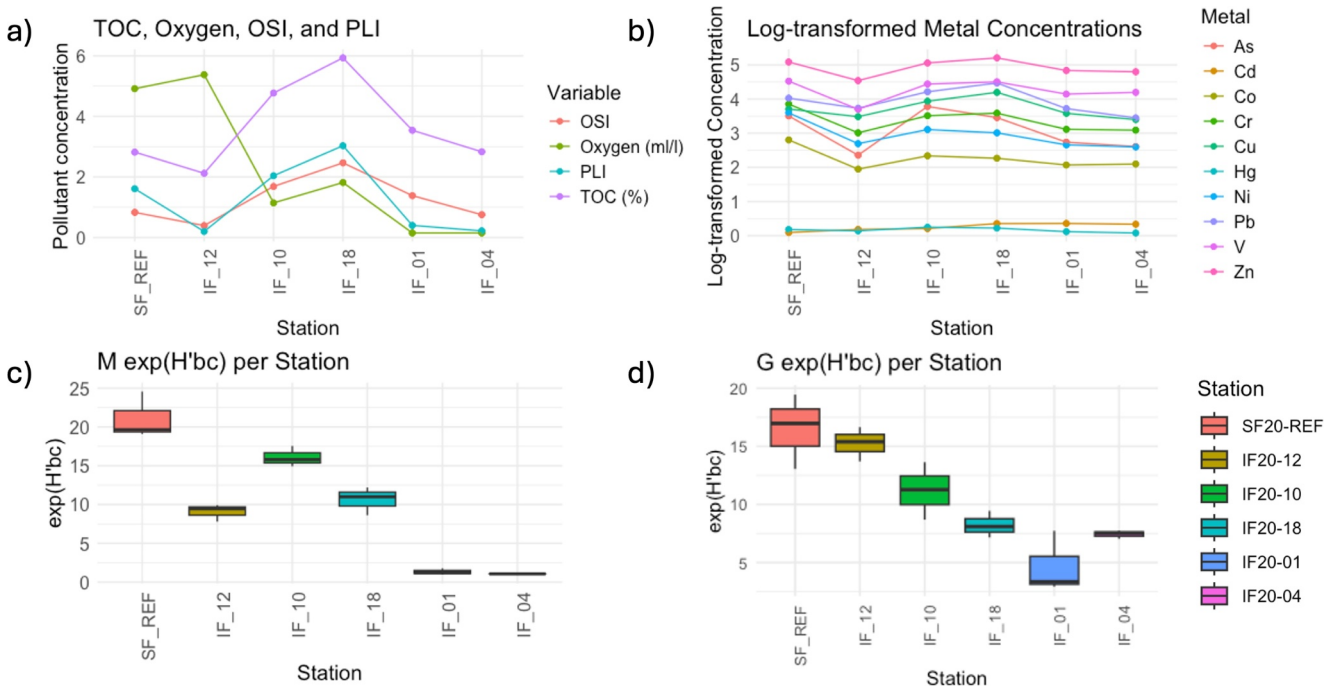

Figure 4. (a) Environmental variables shown at each station along the transect in the Idefjord. Axis denotes log transformed TOC (%), Oxygen (ml/L), OSI, and PLI. (b) Log transformed metal concentrations along the transect. (c) Exp(H'bc) of morphological diversity indices, (d) Exp(H'bc) of Genetic diversity indices.

(Figure 4b). The basin water salinity then increased at the inner fjord stations to 30.7 and 30.6, for IF20-01 and IF20-04, respectively.

There was a clear oxygen gradient in the basin water, from the outer to inner fjord (Figure 4a). The highest oxygen concentrations were observed at the sampling stations in closest proximity to the open sea, 4.9 and 5.4 ml O_2 l^{-1} for SF20-REF and IF20-12, respectively. Oxygen levels then steeply dropped to 1.1 ml O_2 l^{-1} at IF20-10 and 1.8 ml O_2 l^{-1} at IF20-18, with a further reduction for the innermost stations IF20-01 and IF20-04, which were both close to anoxic at the time of sampling (0.2 ml O_2 l^{-1}).

5.1.2. Sediment Geochemistry

The organic matter enrichment in the sediment varied along the fjord transect, with the highest TOC values of 5.93%, 4.77% and 3.54% being observed respectively at IF20-18, IF20-10 and IF20-01, all stations proximal to the Tista River, the city of Halden and the pulp and paper mill (Figures 2 and 4a). These elevated TOC concentrations correspond to bad and poor EcoQS (Table 2). The TOC values for the inner and outer most stations were in the moderate EcoQS category and almost identical, 2.82% at SF20-REF and 2.83% at IF 20–04 (Figure 4a). The sill station IF20-12 recorded the lowest levels of TOC 2.12% in the fjord, and the only station to fall into "acceptable" conditions, with good EcoQS (Table 2).

All metal pollutants displayed a consistent distribution pattern of concentrations along the fjord transect. The highest concentrations for most metals were observed at IF20-10 and IF20-18, except for cadmium, which was highest at IF20-18, IF20-01, and IF20-04 (Figure 4b). Station SF20-REF had good or high EcoQS, aside for Arsenic and Zinc, which were moderate. Station IF20-12 had consistently high or good EcoQS quality, whilst IF20-10 and IF20-18 exhibited moderate EcoQS for Arsenic and Zinc. Finally, stations IF20-01 and IF20-04 also recorded high and good EcoQS for all metal pollutants (Table 2).

5.2. Benthic Foraminifera: Morphological and Molecular Data

In Figure 5a (Morphospecies), the number of ind. 10 ml^{-1} sediment at the reference station was the highest (859.8), within fine grained mud and silt, and at high dissolved oxygen levels. Station SF20-REF was dominated by *Uvigerina* sp., various *Nonionellids* (*Nonionella* sp. T1, *Nonionella turgida, Nonionellina labradorica and*

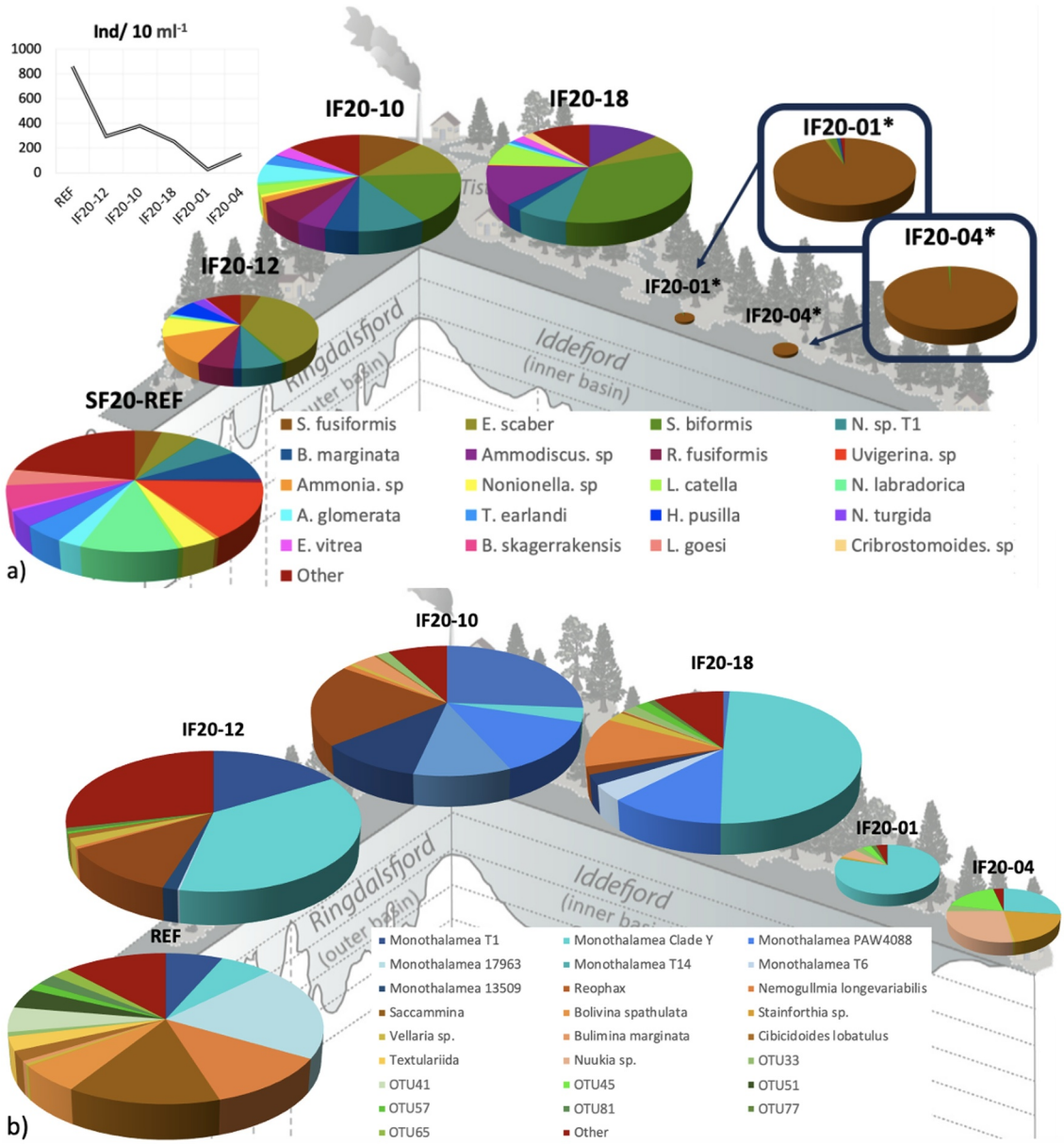

Figure 5. Pie charts illustrating the taxonomic composition of the morphological and genetic data sets at each station along the Idefjord transect. (a) Morphological data set: The pie charts display the dominant and accessory species (relative abundance with at least 3%). All remaining species (<3%) were grouped under "other." Pie chart size is proportional to the number total number of species at each station, including a graph of individuals per 10 cm^{-3} (absolute abundance). (b) Genetic data set: the OTU assignations (over 3.5% of the total data set). OTUs outside of this range classified as "other." The size is proportional to the total OTUs from each station. In Figure 6b Monothalamea are shown in blue hews, Globothalamea in brown and yellow and unnamed OTUs in green. Note. Stations marked with an asterisk (*) For IF20-01*, the composition consisted of 0.6% *Spiroplectammina biformis* and 99.4% *Stainforthia fusiformis*. For IF20-04*, the composition included 27.02% Monothalamea Clade Y, 27.0% *Nuukia* sp., 20.3% *Stainforthia* sp., 17.9% OTU45, 3.8% OTU33, 3.4% other, and 0.48% *Vellaria* sp.

Nonionella spp.) and *Bulimina marginata,* with 28% of the assemblage comprised of "rare" species (less than 3% of the overall assemblage). From station SF20-REF foraminiferal density and diversity decreased, and dominance of species like *Stainforthia fusiformis* and *Spiroplectammina biformis* increased along the transect toward the inner fjord.

For the genetic data set, however SF20-REF, IF20-10 and IF20-18 have similar total number of OTUs (Figure 5b). Both data sets showed a decrease in foraminiferal diversity at IF20-12, compared to IF20-10. The two innermost fjord stations IF20-01 and IF20-04 had the lowest number of unique OTUs but molecular diversity was higher than that of the morphological data set.

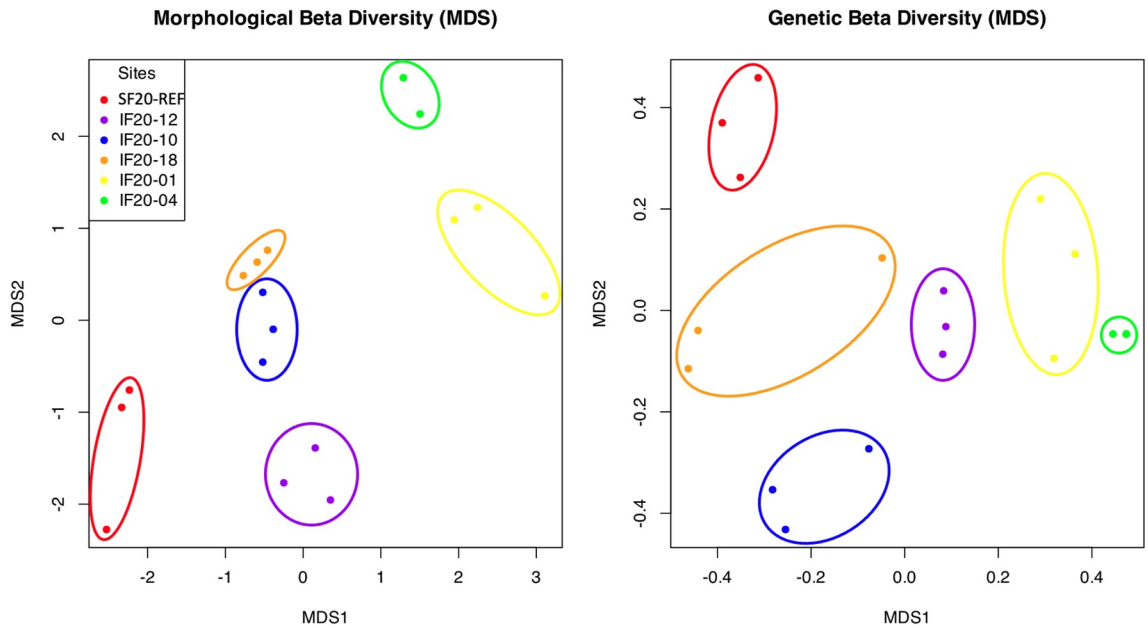

Figure 6. Multidimensional scaling plots of the morphological (left) and genetic (right) data set replicates from each station of the sampling campaign. Replicates from each station are grouped inside the corresponding-colored ellipses.

The molecular data also showed a community transition along the fjord transect, with Globothalamea being the dominant group at SF20-REF, the Monothalamea proportion increasing at IF20-12, and comprising over two thirds of the total assemblage at IF20-10 and IF20-18 (Figure 5b). Station IF20-01 reported an assemblage almost entirely composed of a single Monothalamea group, despite no morphospecies being detected via picking. Lastly, station IF20-04 was dominated by Globothalamea, however the reliability of this assessment is in question due to the very low total number of reads. Although the taxonomic resolution was generally low and differed from the observed morphological assemblage, *Cibicides lobatulus,* a common foraminiferal species living on fjord sills (Nordberg et al., 2017) was detected at stations IF20-12 and IF20-10.

According to the molecular data set, sill station IF20-12 had the largest proportion of rare species, at 28% of the total assemblage, followed by the reference station SF20-REF at 12% (Figure 5b). Station SF20-REF reported almost equal proportions of Monothalamea (33%) and Globothalamea (38%), with 16% represented by unassigned OTUs, additionally Monothalamea 17,963 dominated the assemblage accounting for 20.62%, but was completely absent at all the other stations. Monothalamea Clade Y made up 6% of the assemblage at SF20-REF, 36% at IF20-12, followed by only 3% at IF20-10, before climbing to 49%, 79% and 27% at IF20-18, IF20-04 and IF20-01, respectively.

Pairwise distance multidimensional scaling (MDS) plots examine the level of similarity of between the sample station replicates and the stations, using the pairwise distances (Figure 6). Although the plot axes were almost an order of magnitude different in scale (the genetic data replicates were more similar due to much higher quantity of data points), the replicates clustered in similar positions (Figure 6). For both data sets the replicates from each station cluster in similar positions on the plot (denoted by ellipses), except IF20-18 in the OTU data set. In both data sets SF20-REF replicates are most dissimilar to the others; most distinctly to IF20-01 and IF20-04, which clustered closely together on the opposite side of the plot.

5.3. Diversity Indices and EcoQS Agreement

Whilst the diversity indices Richness, Simpson, Shannon-Wiener and Exp(H'bc) explore different aspects of the assemblage, here we focus mainly on Exp(H'bc), as a bias-corrected true diversity index which takes into account rare species (Bouchet et al., 2012). Exp(H'bc) applied to the genetic data set demonstrated a similar decrease in diversity to that of the morphological methods, albeit to a lesser degree (Figures 4c and 4d).

Table 3
EcoQS Calculated Using the EQR and Exp(H'bc) Index Values for Each Replicate From the Morphological and Genetic Data Sets, Followed by Their Congruency Scores and Disagreement/Agreement Diagnosis

Site/Replicate	REF 1	REF 2	REF 3	IF-12 1	IF-12 2	IF-12 3	IF-10 1	IF-10 2	IF-10 3	IF-18 1	IF-18 2	IF-18 3	IF-01 1	IF-01 2	IF-01 3	IF-04 1	IF-04 2	IF-04 3
Morpho EQR	1	0.777	0.8	0.385	0.317	0.404	0.608	0.693	0.643	0.497	0.351	0.448	0.05	0.073	0.041	0.044	0.043	0
Genetic EQR	1	0.671	0.873	0.791	0.856	0.705	0.447	0.579	0.701	0.486	0.368	0.416	0.397	0.151	0.172	0.362	0.398	0.387
Scores (M EQR)	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
Scores (G EQR)	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0
Agreement	3/3–Agreement			0/3–Disagreement			1/3–Partial			3/3–Agreement			3/3–Agreement			3/3–Agreement		

Exp(H'bc) at SF20-REF was highest for both the morphological (19.08–24.56) and genetic (13.06–19.45) data sets. In the morphological data, IF20-10 ranked as the second most diverse station (14.93–17.01), while in the genetic data, IF20-12 had the second highest diversity (7.79–9.46). Notably, the genetic diversity decreased from SF20-REF toward the inner fjord, whereas the morphological data also showed higher diversity at IF20-10 and IF20-18 (8.617–12.2) compared to that of IF20-12 (7.79–9.93). The morphological data exhibited more pronounced differences between stations, reflecting their geomorphologic conditions, and the methodology proved more sensitive to low oxygen conditions in the inner fjord, with extremely low Exp(H'bc) of 1–1.79 and 1.05–1.08 observed at IF20-01and IF20-04, respectively. The genetic data also revealed higher Exp(H'bc) at IF20-04 (7.03–7.73) as compared to IF20-01 (2.93–7.73), with IF20-04 displaying similar diversity to IF20-18 (7.15–9.45). Overall, there was a larger range in all diversity indices applied to the genetic data set, particularly prevalent at IF20-04 for Shannon and Simpson. The Shannon index showed similar trends to that of Exp(H'bc), since the latter is derived from the exponentiated former (Figure S1 in Supporting Information S1). Additionally, Spearman rank correlation performed between averaged genetic and morphological indices resulted in significantly positive correlations. Richness showed a strong correlation ($r = 0.71$, $p = 8.6e{-}04$), Shannon exhibited a very strong correlation ($r = 0.77$, $p = 1.7e{-}04$), Simpson and Exp(H'bc) were moderate to strong ($r = 0.6$, $p = 8.5e{-}03$ and $r = 0.65$, $p = 3.2e{-}03$, respectively). These results indicate that both metrics follow a similar distribution along the transect (Table S1 in Supporting Information S1).

A multiple regression analysis evaluated the effects of environmental pollution parameters (OSI and PLI) on genetic and morphological Exp(H'bc) values. For genetic diversity, the model's residuals ranged from −0.51662 to 0.45341, with significant coefficients for the intercept ($p = 7.51e{-}16$), OSI ($p = 3.52e{-}06$), and PLI ($p = 1.39e{-}05$). The model's multiple R-squared was 0.7776, explaining approximately 77.76% of the variance, with an adjusted R-squared of 0.7479. For morphological diversity, residuals ranged from −0.54467 to 0.59084, and the coefficients for the intercept ($p = 1.68e{-}12$), OSI ($p = 1.05e{-}06$), and PLI ($p = 1.11e{-}08$) were significant. This model had a higher multiple R-squared of 0.894, explaining around 89.4% of the variance, with an adjusted R-squared of 0.8799. The pollution indices have slightly more impact on morphological diversity than on genetic diversity.

The EcoQS assessment based on the EQR of Exp(H'bc) index derived from the morphological data set indicated "acceptable" (high or good EcoQS) conditions at two stations, SF20-REF and IF20-10 (Table 3). Although non-parametric sign test results did not indicate a significant deviation from the null hypothesis for the EQR Agreement, 13 out of 18 observations in total were successes (agreements) (Table 3). The estimated probability of success was 0.72, indicating a relatively high level of agreement between the morphological and genetic data sets. The p-value of 0.09 suggests that the probability of an agreement by chance is possible at the arbitrary 0.05 significance level.

The relationship between the morphological and the genetic Exp(H'bc) and individual environmental variables was congruous, particularly with oxygen and salinity (Figure 7). For IF20-12 and IF20-04 stations, morphological Exp(H'bc) is between one and three EcoQS category lower than genetic Exp(H'bc) (Table 3). For all other stations the Exp(H'bc) values for both methodologies overlapped, reporting largely the same EcoQS categories, aside

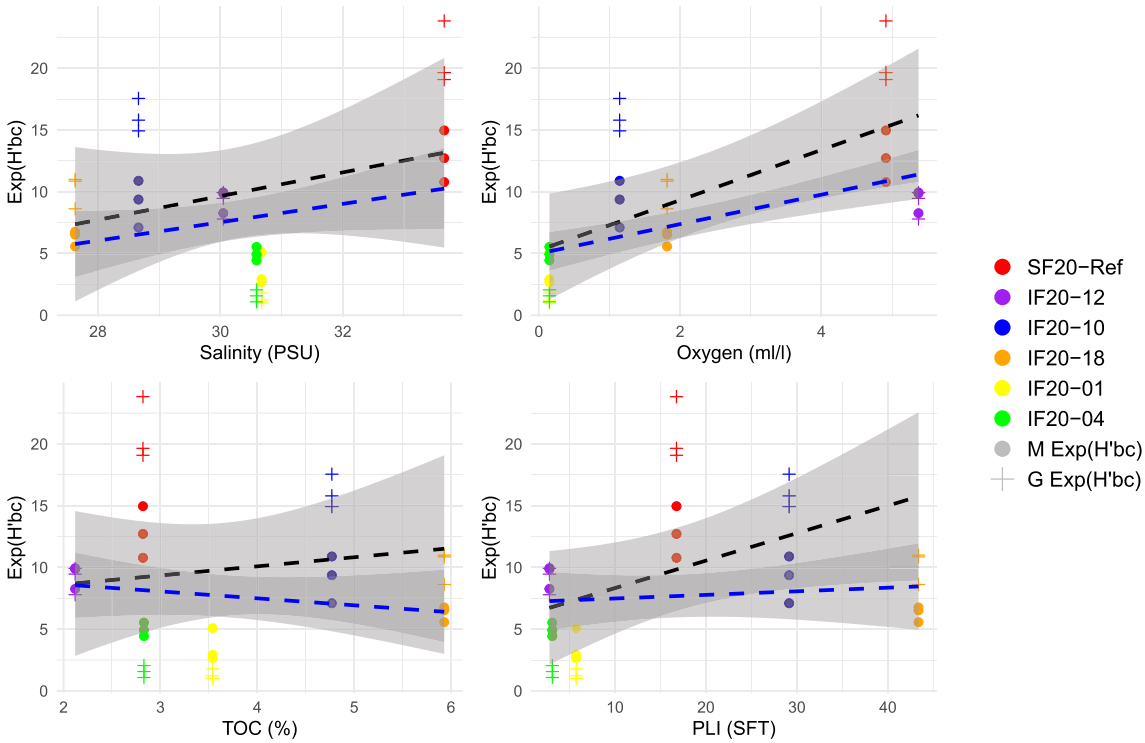

Figure 7. Exp(H'bc) index values for the morphological and genetic data sets replicates derived from complete living assemblage counts and rarefied OTUs against environmental variables (a) Salinity (b) Oxygen (c) PLI(SFT) (d) TOC. Delineation of the thresholds, from left to right, for anoxic (0–0.5 ml/l), hypoxic (0.5–2.0 ml/l) and normoxic (>2 ml/l) conditions are added to the Oxygen plot, after Diaz and Rosenberg (2008). 95% Confidence limits of the linear regression shown in gray. Linear regression dashed lines are shown for G Exp(H'bc) in blue and for M Exp(H'bc) in black.

from IF20-10 which was entirely acceptable conditions for the morphological data set (good) but for genetic two replicates reported only unacceptable moderate quality. Linear regression analyses revealed that salinity had no significant impact on morphological Exp(H'bc) ($p = 0.383$, $R^2 = 0.048$) and a marginally non-significant effect on genetic Exp(H'bc) ($p = 0.109$, $R^2 = 0.153$). In contrast, oxygen showed a significant positive relationship with morphological Exp(H'bc) ($p = 0.001$, $R^2 = 0.501$) and a strong positive relationship with genetic Exp(H'bc) ($p = 7.28 \times 10^{-6}$, $R^2 = 0.726$). Total organic carbon (TOC) had no significant impact on morphological Exp (H'bc) ($p = 0.589$, $R^2 = 0.019$) and a marginally non-significant effect on genetic Exp(H'bc) ($p = 0.085$, $R^2 = 0.174$). Pollution load index (PLI) was significantly positively related to morphological Exp(H'bc) ($p = 0.003$, $R^2 = 0.432$), but had no significant effect on genetic Exp(H'bc) ($p = 0.658$, $R^2 = 0.013$) (Table S1 in Supporting Information S1).

The EcoQS at anoxic stations IF20-01 and IF20-04 was poor or bad (Table 3, Figure 7), whereas in hypoxic conditions both data sets spanned over poor, moderate and good EcoQS. In oxygenated conditions both genetic Exp(H'bc) and morphological Exp(H'bc) had a wider dispersion (poor to high EcoQS). At the highest oxygen concentration (station IF20-12), the morphological data set reported low diversity and poor EcoQS (Figure 2). For salinity the overall positive relationship between morphological Exp(H'bc) and genetic Exp(H'bc) is disrupted by bad and poor EcoQS for station IF20-01 and IF20-04 with 30.67 and 30.59 PSU, respectively.

6. Discussion

Despite both approaches indicated a general decline of foraminiferal diversity along the "coast to the fjord transect", some stations (e.g., IF20-12 and IF20-04) showed deviation from this general trend. Below we discuss potential causes, which may have contributed to differences in EcoQS assessment between the fjord stations and applied assessment methodologies.

6.1. Environmental Setting

Fjords, characterized by their narrow and deep basins, experience restricted water circulation, resulting in the accumulation of dense saline water below sill level (Shoemaker, 1986). Hence, observed high salinity conditions in the Idefjord inner and outer basins (Figure 3) are likely the result of a limited water exchange with the open sea, density stratification and infrequent bottom water renewals (Josefsson & Nyquist, 1976).

The hypoxic conditions of IF20-10 and IF20-18 could be amplified by still elevated TOC despite several decades have passed after decline of pollution. The restricted water circulation was most evident at the innermost stations (IF20-01 and IF20-04), with sediments of a semi-liquid fluffy consistency, clear laminations visible on X-ray images (not shown herein) and black color, confirming the limited bottom water circulation and severe hypoxic to anoxic conditions (Webb et al., 2009). Here the presence of floating organic matter made the sediment-water interface undiscernible, suggesting negative redox conditions and a potentially erroneous TOC measurement. When all free oxygen is fully utilized, sulphides and other partially reduced compounds are formed, creating a "sulphide zone", a process normally associated with high TOC conditions (Pearson, 1980). Common urban pollutants, still found at high concentrations in the surface sediments, including heavy metals (such as mercury, lead, and cadmium) are likely compounded by nutrients (e.g., nitrogen and phosphorus compounds), and organic contaminants (e.g., POPs) derived from domestic and industrial activities. Due to pulp and paper mill effluent, the sediments likely still contain elevated levels of pollutants like PAHs and organic contaminants (Hoffman et al., 2019). Dahlberg et al. (2020) found that wood-fiber banks in the Baltic Sea, a legacy from pulp and paper industry, are coastal hotspots for persistent organic pollutants (POPs), with significantly higher levels of POPs and TOC as compared to other sediment types. The high POPs pore water concentrations in such fiber banks indicate an elevated risk of contaminant dispersal. These substances can persist in sediments, preventing full recovery of conditions within the fjord.

Station IF20-12 had naturally stressed conditions and lower EcoQS than expected due to its location on the sill, and exposure to cascading bottom water inflows; evidenced by high sand content in the sediments and presence of *Cibicides lobatulus* (Hald & Korsun, 1997). Species *C. lobatulus* lives on sills in the fjords and its high presence in fjord basins usually indicate shell transport from the sill (Ferraro et al., 2024; Nordberg et al., 2017). *Cibicides lobatulus* was found at IF20-12 and IF20-10 in both data sets, notably as an important component of the genetic assemblage at IF20-10, suggesting eDNA detects transport from the sill and accumulation in the adjacent basin. The distribution of pollutants (Figure 4) may be influenced by variability of sediment grain-size, as sediments in depositional basins tend to contain a fine-grained fraction that facilitates the accumulation of metal contaminants (Nikulina et al., 2008). Station IF20-12 had the lowest metal contamination concentrations and was found to be highly sandy. Granulometric variability could be a significant factor controlling pollutant accumulation, absolute abundance, assemblage diversity and sedimentary eDNA preservation, by effecting factors like porosity, permeability, nutrient availability, and dissolved oxygen, which influence composition of benthic communities (Freitag & Prosser, 2003). Several clades of foraminifera have been shown to be negatively correlated to a coarser grain size contributing to lower species diversity (Cao et al., 2022), which can explain lower EcoQS classification at station IF20-12.

6.2. Foraminiferal Assemblage Response to Environmental Stressors

A consistently reported effect of contamination on foraminifera, known from sediment archives is a community transition, where pollution-sensitive species are no longer present and replaced by a lower diversity of tolerant species (Alve et al., 2016; Bouchet et al., 2012; Polovodova Asteman et al., 2015). The morphological data set of the SF20-REF station was replete with more sensitive endemic Skagerrak and Kattegat fauna species like *Uvigerina* sp., *Bulimina marginata* and *Brizalina skagerrakensis*, despite the relatively high metal concentrations (Figures 4 and 5), suggesting acclimatization (Romano et al., 2013). The Skagerrak and Kattegat specific taxa declined along the fjord transect (IF20-12, IF20-10, IF20-18), and the relative abundance of opportunists like *Eggerelloides scaber*, *Spiroplectammina biformis*, *Textularia earlandi*, *Stainforthia fusiformis* and non-indigenous species *Nonionella* sp. T1 (Polovodova Asteman & Schönfeld, 2016) increased; benefitting from reduced competition for resources, whilst the oxygen levels remained high enough to support these opportunists.

Previous studies have reported that at high pollution, even opportunistic species are unable to survive, resulting in a "dead zone" (Polovodova Asteman et al., 2015; Scott et al., 2005). In our study, however, stations IF20-01 and IF-04 supported tolerant species, like *Stainforthia fusiformis*, able to recolonize the oxygen depleted zones, which

were reported as barren of any foraminifera in 2000–2001 (Polovodova Asteman et al., 2015), suggesting a reprisal in the ecological conditions and the beginning of a re-colonization process. Polovodova Asteman et al. (2015) analyzed sediments cores from the Idefjord and showed that tolerant species dominance increased in response to elevated pollution and associated reduction in oxygen throughout the 20th century. However, given still high TOC concentrations at the innermost fjord stations (IF20-04 and IF20-01) resulting in poor and bad EcoQS, and natural presence of multiple sills restricting bottom water exchange, development of prolonged seasonal hypoxia or anoxia may interrupt inner fjord recovery and result in hostile conditions even for tolerant taxa, such as *S. fusiformis*, emphasizing a need for ongoing monitoring studies in the fjord.

In the genetic data set there was also a community transition, a shift in the dominance of Globothalamea, which comprised most of the assemblage at SF20-REF but was replaced by Monothalamea at all other stations; aside from IF20-04, which recorded a low total number of distinct OTUs (Figure 5). Previous studies have identified that compositional changes in the genetic sequence abundance data can be a response to chemical stress, particularly organic enrichment, oxygen depletion and sulphide toxicity (Hargrave et al., 2008; Pochon et al., 2015), therefore suggesting that a high proportion of Monothalamea, which are often missed in the conventional foraminifera picking process, could be a good indicator of stressed conditions (Pochon et al., 2015). Thus, the higher presence of Monothalamea picked up by genetic approach at station IF20-01 explains both overestimated diversity and EcoQS at the almost anoxic innermost fjord setting (Table 3; Figure 5).

6.3. eDNA Proxies for Biomonitoring

By circumventing time-consuming processes, which require specific expertise, like separating the specimens from the substrate, sorting individuals and assigning them to species level by morphological identification, sedimentary eDNA metabarcoding can avoid technical and operational hurdles associated with morpho-taxonomic inventories (Bellisario et al., 2021; Lejzerowicz et al., 2015; Pawlowski et al., 2022). Morpho-taxonomic methods are also low throughput, hence limiting the spatio-temporal frequency of surveys (Cahill et al., 2018) and reducing ability to produce timely responses to anthropogenic impact by environmental management agencies (Pawlowski et al., 2022).

Conversely, presence of metal or organic compounds in aquatic sediments can impede DNA extraction by binding to the sediment matrix and inhibiting key enzymes involved in cell lysis, as well as hindering PCR efficiency by interfering with the amplification enzymes (Fortin et al., 2004; Pawlowski et al., 2022). This may compromise accuracy and reliability of genetic analyses, risking low diversity metabarcoding outputs being misinterpreted as genuine loss of benthic community diversity but being a consequence of inefficient amplification. This risk is particularly pronounced in sediments containing high levels of inhibitors, like industrial pollution, which also negatively affects benthic communities (Krolicka et al., 2020; Lanzén et al., 2016). In this study however, the morpho-taxonomic analysis provides supportive evidence for the low diversity genetic assemblage in the inner basin stations. Additionally, a total of 27 PCR amplifications per replicate were conducted (3 replicates per layer, each layer extracted in triplicates which were then PCR amplified), far higher than the 8 PCR replicates usually employed to overcome lower PCR yield (Ficetola et al., 2015), hence the stations with low genetic diversity estimates are likely representative of the actual benthic conditions.

Taxonomic resolution of metabarcoding is highly dependent on the availability of reference libraries with databased sequences, hence stronger results may be obtained by targeting a broader spectrum of taxa, for example, all eukaryotes (Cordier et al., 2018; Keeley et al., 2018). As more foraminifera species are sequenced and added to online data bases like the National Center for Biotechnology Information (NCBI) and SRA, the algorithms used in data processing pipelines will be used to estimate to a closer taxonomic level. Whilst such reference libraries are nearly complete for a less diverse planktonic foraminifera group (Morard et al., 2015) barcoding libraries of benthic foraminifera group characterized by much higher diversity are still incomplete (Pawlowski & Holzmann, 2014). Finally, if morphological approach is eventually to be abandoned, future studies must include both barcoding of the species present in the samples as well as metabarcoding, in order to contribute to building regional or local barcoding libraries for benthic foraminiferal eDNA data interpretation.

6.4. Diversity Indices and EcoQS Comparison

Despite minor disparities between genetic and morphological methodologies, the application of diversity indices, including Exp(H'bc), consistently upheld the overall EcoQS assessment (Figures 4c and 4d, Figure S1 in

Supporting Information S1). Spearman analysis and "agreement or disagreement" showed congruous trends (Table 3), in that the ecological condition of the fjord worsens along the transect, in line with the observed oxygen depletion, due to both natural topographic impediments and increase in pollution.

Additionally, the multi-regression model and linear regressions conducted to examine the relationship between the diversity indices, calculated from both data sets and environmental parameters showed a statistically significant level of similarity, particularly in response to oxygen and pollution gradients (Figure 7). Morphological diversity, however, responded positively to PLI, reflecting the association of metal pollution with finer grained sediments, which are also generally preferred by benthic foraminiferal taxa (Nikulina et al., 2008). Notably, the morphological assemblage showed more pronounced response to habitat heterogeneity, such as that of site IF20-12 located on the fjord sill, where the morphological diversity EQR reported poor to moderate EcoQS, whilst the genetic diversity remained at good or moderate EcoQS (depending on the filter applied). This again, emphasizes bias of transported and propagule DNA, which would be characteristic of the metabarcoding approach. Previous studies have reported similar bias, when eDNA of shallow water species (such as *Ammonia* spp.) was recovered from the deep (>100 m) fjord basin sediments, suggesting propagule transport from the slopes (Brinkmann et al., 2023; Morin et al., 2023).

Our study aligns with findings from recent research comparing eDNA and morphological methods for EcoQS assessment using benthic foraminifera. These studies consistently highlight the complementary strengths of both approaches. For instance, Damasceno et al. (2024) applied both methods along with geochemical data in Sepetiba Bay, Brazil, finding a decline in foraminiferal diversity and taxa relative abundance in response to environmental stressors using both genetic and morphological methods, with congruence among various EcoQS indices for 63% of the stations surveyed. Similarly, Cavaliere et al. (2021) evaluated the EcoQS in the Bagnoli area, Italy, and observed significant differences in foraminiferal diversity and assemblage composition between polluted and less polluted areas, with molecular indices effectively identifying poor-to-bad EcoQS in heavily polluted sites.

Al-Enezi et al. (2022) investigated Kuwait Bay, finding that both approaches reflected the environmental stress gradient, with the lowest EcoQS values in the innermost parts of the bay, whilst Frontalini et al. (2020) assessed the impact of gas platforms in the Adriatic Sea, showing congruent results between the two methods, with lower foraminiferal diversity near the platforms and the potential of certain OTUs as new bioindicators. Finally, Brinkmann et al. (2023) explored foraminiferal biodiversity in a Swedish fjord system and found that eDNA-based methods revealed a wider diversity than traditional morphological observations, emphasizing the need for larger sediment samples for reliable diversity representation.

In Idefjord, morphological and genetic approaches showed disparities in EcoQS assessments at certain stations, with eDNA indicating higher EcoQS due to the detection of transported/dormant propagule DNA and species missed by conventional methods. This suggests that despite methodological differences, both approaches provide valuable insights into the different characteristics of the sedimentary environment, benthic foraminiferal communities and overall ecological status of the fjord. Whilst morphological methods are more sensitive to habitat heterogeneity, they do not consider complete diversity of the surface sediment community, induced by for example, presence of soft-shelled taxa. The genetic methods instead provide a much quicker and efficient tool to obtain a complete picture of foraminiferal diversity and clearly will become a common method in future monitoring studies. To get there, however, we all need to work toward an improvement of barcoding reference libraries for benthic foraminifera. On the other hand, morphological approach (facilitated by automated identification with machine learning) perhaps can still be used to compensate for the "propagule DNA bias", which in some cases reports presence of eDNA from exotic species, never described as living in the region (Brinkmann et al., 2023; Broman et al., 2021).

7. Conclusions

Our study shows that based on foraminiferal and pollutant distribution in surface sediments of Idefjord, contemporary EcoQS assessment results in high and good classes at the coastal and the outer fjord stations (SF20-REF, IF20-10 and IF20-18), respectively, despite the latter two being in proximity of industrial facilities. Station IF22-12 located at fjord sill exhibits intermediate EcoQS and naturally stressed conditions, likely due to coarse sediments and cascading water inflows. The two innermost stations (IF20-04 and IF20-01) showed poor and bad EcoQS based on metal and TOC levels together with severe hypoxic to anoxic conditions in the inner fjord basin. Foraminiferal diversity indices based on morphological and genetic approach show some disparities in their

EcoQS assessment for stations IF22-12, IF20-04 and IF20-01, with genetic approach resulting in overall higher EcoQS due to a detection of transported DNA (e.g., propagules) and high presence of Monothalamea, both missed by conventional assessment method. This suggests that despite the methodological differences, both approaches provide valuable insights into the different characteristics of the benthic environmental and overall ecological status of the fjord.

In line with previous studies, the EcoQS of Idefjord appears to be influenced by naturally occurring environmental perturbations, which are worsened by anthropogenic pollution. The inner fjord stations, which were uninhabited by foraminifera two decades ago, are now populated by some pollution tolerant species such as *Stainforthia fusiformis*. This suggests that the conditions in the fjord may be improving, but future biomonitoring efforts will reveal if the situation continues to improve over time.

In future studies the influence of granulometric variability should be included as a potentially significant factor affecting pollutant accumulation, community abundance, diversity, and the preservation of sedimentary eDNA. Furthermore, a special consideration for sites polluted by the paper and pulp industry may be testing for the presence of POPs in the sediments, which affects not only the EcoQS but also DNA preservation, thus impairing the viability of genetic assessment methods.

Glossary of Terms

Anthroturbation	Environmental stress induced by human activities
Biocenose	Encompassing all the living organisms in a biological community, as well as their interactions with each other and their environment
Estuarine Quality Paradox	Estuaries exhibit highly variable physical conditions for example, oxygen, temperature, salinity in both the water column and bed sediment dynamics. The resident biota must be able to cope with high levels of environmental stress. These natural environmental stressors are similar to that which is anthropogenically induced

Data Availability Statement

Individual count data and environmental parameters available at Pangaea, https://doi.pangaea.de/10.1594/PANGAEA.965583. Raw sequence data available at Sequence Read Archive (SRA) under the accession number PRJNA1012459. Script for calculation of Exp(H'bc) in *R* is provided in Supporting Information S1.

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