


Review

Benthic Foraminiferal Indices and Environmental Quality Assessment of Transitional Waters: A Review of Current Challenges and Future Research Perspectives

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Abstract: Transitional waters straddle the interface between marine and terrestrial biomes and, among others, include fjords, bays, lagoons, and estuaries. These coastal systems are essential for transport and manufacturing industries and suffer extensive anthropogenic exploitation of their ecosystem services for aquaculture and recreational activities. These activities can have negative effects on the local biota, necessitating investigation and regulation. As a result of this, EcoQS (ecological quality status) assessment has garnered great attention as an essential aspect of governmental bodies' legislative decision-making process. Assessing EcoQS in transitional water ecosystems is problematic because these systems experience high natural variability and organic enrichment and often lack information about their pre-human impact, baseline, or "pristine" reference conditions, knowledge of which is essential to many commonly used assessment methods. Here, foraminifera can be used as environmental sentinels, providing ecological data such as diversity and sensitivity, which can be used as the basis for EcoQS assessment indices. Fossil shells of foraminifera can also provide a temporal aspect to ecosystem assessment, making it possible to obtain reference conditions from the study site itself. These foraminifera-based indices have been shown to correlate not only with various environmental stressors but also with the most common macrofaunal-based indices currently employed by bodies such as the Water Framework Directive (WFD). In this review, we firstly discuss the development of various foraminifera-based indices and address the challenge of how best to implement these synergistically to understand and regulate human environmental impact, particularly in transitional waters, which have historically suffered disproportionate levels of human impact or are difficult to assess with standard EcoQS methods. Further, we present some case studies to exemplify key issues and discuss potential solutions for those. Such key issues include, for example, the disparate performance of multiple indices applied to the same site and a proper assignment of EcoQS class boundaries (threshold values) for each index. Disparate aptitudes of indices to specific geomorphologic and hydrological regimes can be leveraged via the development of a site characteristics catalogue, which would enable the identification of the most appropriate index to apply, and the integration of multiple indices resulting in more representative EcoQS assessment in heterogenous transitional environments. In addition, the difficulty in assigning threshold values to systems without analogous unimpacted reference sites (a common issue among many transitional waters) can be overcome by recording EcoQS as an ecological quality ratio (EQR). Lastly, we evaluate the current status and future potential of an emerging field, genetic biomonitoring, focusing on how these new techniques can be used to increase the accuracy of EcoQS assessment in transitional systems by supplementing more established morphology-based methods.

Keywords: transitional waters; foraminifera; anthropogenic stress; pollution; biomonitoring; EcoQS; ecosystem recovery; environmental DNA; reference conditions

1. Introduction

The protection and restoration of continental and marine waters and their essential ecosystems has prompted international regulation and legislature from national governing bodies such as the Clean Water Act (CWA, 33 U.S.C. §1251 et seq. 1972), introduced by the U.S. Environmental Protection Agency; within Europe the Water Framework Directive (WFD, 2000 [1]), and the Marine Strategic Framework Directive (MSFD, 2008 [2]). It involves evaluating the ecological quality status (EcoQS, Figure 1 herein) to assess the health of water systems. Noticeably, transitional water bodies are particularly difficult to assess due to their natural variability and require an in-depth ecological understanding to obtain meaningful results. The term “transitional” was introduced about 20 years ago in the WFD (Water Framework Directive, WFD, 2000 [1]) as a means to complete the continuum between continental and coastal waters. Transitional waters are water bodies influenced by both oceanic and freshwater regimes and include estuaries, deltas, rias, lagoons, and fjords (Table 1). In the WFD, transitional waters are defined as “bodies of surface water in the vicinity of river mouths which are partially saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows” [1]. These waters may also include mesohaline, poly-euhaline, and hyperhaline lagoons [3]. Human-modified transitional waters are classified as “artificial water bodies” [1].

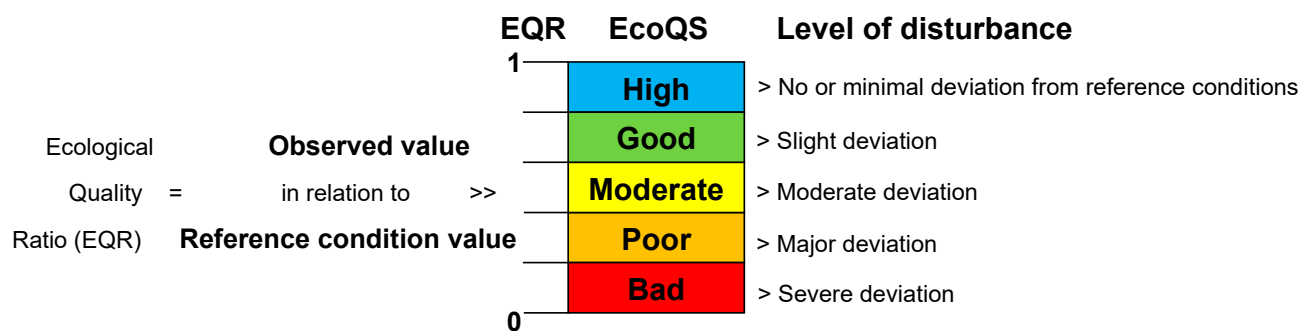


Figure 1. The basic principles of the classification of ecological status based on the EcoQ ratios assigned, assuming equal intervals between the different classes (adapted and re-drawn from [4]).

These ecosystems, located between sea and land, results in a patchwork of highly heterogeneous conditions, which require easily implemented and robust biotic indices. Biotic indices based on the indicator species concept, i.e., on the specific response to organic matter enrichment, for instance, are not fully reliable to assess EcoQS in these water body types (i.e., [5–8]). In fact, the natural features of these ecosystems make it difficult to disentangle natural and human-induced changes. In particular, silt, clay, and organic matter (OM) sedimentary contents are naturally high in transitional waters, promoting tolerant and opportunistic species, while sensitive species naturally decline [9,10]. Furthermore, total organic carbon (TOC) in transitional waters is a mix of labile and refractory OM with important terrestrial inputs [11]. In transitional waters, benthic communities could therefore be naturally similar to those found in anthropogenically disturbed areas [6,10]. Consequently, pristine, naturally disturbed intertidal areas and transitional waters could easily be misclassified in moderate to bad EcoQS (the “estuarine quality paradox”; [9,12,13]), providing misleading estimates of reference conditions and severely complicating the decision-making process [6,8,14,15].

Table 1. Water body types of intertidal areas and transitional waters (modified after [16]).

Water Body Types	Natural Features
Classical estuary	Tidally dominated at the seaward part; salinity notably reduced by freshwater river inputs; riverine dominance landward
Lentic non-tidal lagoon	Limited exchange with the coastal area through a restricted mouth; separated from the sea by sand or shingle banks, bars, coral, etc., shallow area, tidal range < 50 cm
Lentic micro-tidal lagoon	As above but with tidal range > 50 cm
Fjord	Semi-enclosed marine basin, entrance sills separating deeper inner waters from adjacent coastal waters, restricted water circulation/oxygen renewal, sediment sequences removed by glacial erosion
Ria	Drowned river valley, some freshwater inputs; limited exchanges with coastal waters
Delta	Low energy, characteristically shaped, sediment dominated, river mouth area; estuary outflow
Coastal freshwater/brackish water plume	Outflow of estuary or lagoon, notably diluted salinity, and hence differing biota than surrounding coast
Semi-enclosed bay/lagoon	Low energy, notably limited exchange with the open sea waters
Artificial water body	Harbors and docks, constructed dredging pools, and coastal water bodies connected to the sea, created by human activities

The EcoQS is a comparative measure of the current condition of a system, compared to that of a reference system free from the negative impact (Figure 1), e.g., heavy metal pollution. It is therefore essential to obtain reference conditions specific to each site from a relatively “pristine” period (before anthropogenic pollution can be detected in the sediment) and compare these to the conditions thereafter. Here lies the central conundrum in EcoQS assessment, as no marine ecosystems on earth today, let alone those at coastal proximity, can be found in “pristine” condition [5,17,18]. Transitional and coastal systems have hosted a disproportionately high human activity for centuries, and as a result, information regarding their pre-impact reference conditions is often missing. Additionally, even if a relatively “pristine” comparison site can be located, the disparate physical and geochemical setting will immediately make it less useful as an analogue [19–21]. Hence, for most EcoQS assessment indices, a knowledge gap exists in defining the initial “pristine reference conditions” needed to quantify the change of EcoQS. Without this information to provide a baseline for comparison to a more contemporary EcoQS, the effect of anthropogenic stress cannot be properly determined [19,22].

In this context, palaeoecology can be used to bridge the knowledge gap by providing reference conditions from the exact study site prior to any anthropogenic impact. Having high preservation and fossilization potential makes foraminifera reliable palaeoecological indicators, with the potential to provide reference data from the preindustrial period and beyond [19,20,23,24]. For instance, a more robust assessment of EcoQS for transitional waters was obtained with benthic foraminifera using site-specific local reference conditions in the Oslofjord (Norway) [19], in the Boulogne sur Mer harbor (France) [23], and in the Santos estuary (Brazil) [25]. Furthermore, geochemical analyses of dated sediment cores can be used in combination with benthic foraminifera to determine ecological reference conditions in transitional waters [20,26]. In this review paper, we will describe and discuss how several key characteristics of foraminifera-based indices can be used to address this issue. Particularly how they can be used to provide comparable data from the target site, complete with its unique biogeochemical setting, to set a reliable baseline facilitating the calculation of a representative modern-day EcoQS value.

This paper aims: (1) to detail the scientific background of benthic foraminiferal indices, (2) to list the existing indices, (3) to exemplify their implementation in transitional waters, (4) compare and analyse each index, and (5) present ideas to improve their implementation. Considering that EcoQS assessment with benthic foraminifera is still in its infancy, it appears to be timely in order to ensure accurate application of these indices. In this context, the present work specifically focuses on the relevance of benthic foraminiferal indices to assess EcoQS in transitional waters. First, we review the literature to identify current issues in defining accurate reference conditions and other gaps in EcoQS assessment. Secondly, we identify the strengths of benthic foraminifera to actively bridge these gaps. Thirdly, a critical assessment of existing foraminiferal biotic indices is done in order to evaluate their potential of being implemented in transitional waters. Finally, we discuss future directions that the research on foraminiferal indices may need to follow to reach better monitoring of the health of transitional waters, noticeably the strengths and weaknesses of environmental-DNA metabarcoding.

2. Benthic Foraminifera Are Reliable Indicators of Environmental Conditions

Benthic foraminifera have been shown to respond quickly even to rapid and unprecedented environmental changes in transitional waters; by adapting their species composition and population densities, these protists are able to provide valuable ecological proxies [26]. Since their initial use in the early 1960s by Resig and Watkins [27], benthic foraminifera have proved to be a reliable ecological indicator of various types of anthropogenic stress, including desalination discharge [28], oil-spills [29–32], aquaculture [33,34], sewage [35,36], heavy metal pollution [22,29,37–40], and pulp mill effluents (e.g., [41]). At the community and species level, ecological studies have used these protists as a biological quality element (BQE) to decipher the relationship between both natural and anthropogenic environmental variables and the local biota in transitional waters. Foraminiferal assemblages provide additional data (Figure 2) such as changes in population density and species abundance, e.g., [42,43] community compositional shifts (pre-impact sensitive species vs. post-impact opportunists: e.g., [24]) or calcareous vs. agglutinated species: e.g., [44–47], as well as sensitivity and diversity indices [19,48–50], changing depth of habitation in the sediment (observed with living individuals: e.g., [30,51], development of aberrant tests [21,50–55] and pollutant accumulation within tests, e.g., [39,56,57]. In fjords [58,59], lagoons [38,49,60], estuaries, and harbours [20,61,62] alike, foraminiferal proxies have been shown to correlate with environmental changes. For instance, certain opportunistic species such as *Stainforthia fusiformis* [47,58,63], *Spiroplectammina biformis* [24], *Elphidium incertum* [64], *Ammonia aomoriensis* (as *A. beccarii*: [65]), and *Ammonia beccarii* [66] have all been linked to adverse environmental conditions caused by OM enrichment and severely hypoxic bottom waters. Possible features behind the superiority of the opportunists in stressful conditions can be related to the presence of specific intracellular complexes in the living cell (*S. fusiformis*: [67]); ability to denitrify (*S. fusiformis*: [68]), adjust the depth of habitation in the sediment (*E. incertum*: [51]), as well as dormancy inside of a protective cyst (or not) during unfavorable conditions such as anoxia (*E. incertum*: [51,69]), reduced temperature (*Ammonia tepida*: [68]), temperature and chemical exposure (*Amphistegina gibbosa*: [70,71]), or extended darkness [72].

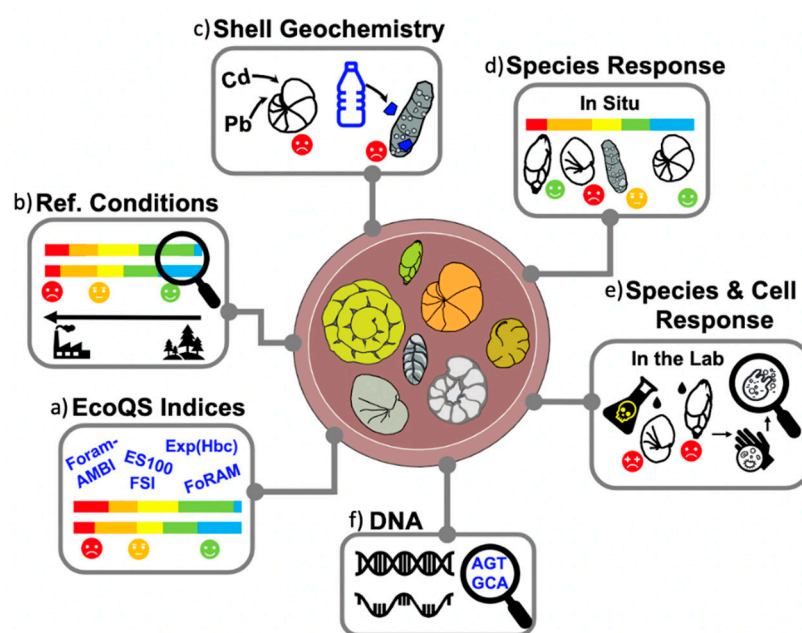


Figure 2. Overview of foraminiferal applications within biomonitoring studies, including (a) EcoQS indices based on species diversity and sensitivity; (b) reconstruction of preindustrial baseline (reference conditions); (c) accumulation of pollutants (e.g., heavy metals and microplastics) within foraminiferal shells; (d) species responses to environmental stress present in situ (e.g., field studies) and (e) simulated in the lab (e.g., culturing experiments); and (f) genomic methods, such as eDNA and sedaDNA.

3. Foraminiferal Biotic Indices

Methods to collect and process benthic foraminifera vary widely in sample acquisition, the sampling gear used, sub-sampling, replication, preservation, preparation, and analysis. In an attempt to address these inconsistencies, Schönfeld et al. [73] proposed using a unified international protocol following a workshop by FOBIMO (Foraminiferal Biomonitoring) group members, which occurred in 2011 in Fribourg, Switzerland. A group of 37 scientists collaborated in the effort to guarantee reproducibility and comparability across studies, with a mind to be adaptable to local conditions [73], inspiring more working groups worldwide to accept foraminifera as a promising BQE to assess EcoQS in contemporary environments. In recent years, however, with the wider acceptance and refinement of the methodology, the momentum of foraminifera index application is gathering rapidly. New foraminiferal indices have been developed, applied, and contrasted between a more extensive range of transitional waters and stressors (Appendix A: Table A1). Below, we provide an overview of foraminiferal indices developed to our knowledge to date based on species diversity and sensitivity and used for EcoQS assessment in transitional waters.

3.1. Foraminiferal Diversity Indices

Using benthic foraminiferal diversity indices, together with associated geochemical parameters, to specifically address human environmental impact was first implemented by Alve et al. [21] investigating changes of ecological status back in time (referred to as paleo-EcoQS) [21]. Although the concept of using foraminiferal diversity had been applied to anthropogenic stress biomonitoring studies earlier [33,35], Alve's pioneering study was based on sediment archives from Oslofjord, Norway, where the authors used foraminiferal data to obtain the Hurlbert's ES (100) and Shannon–Wiener H' (\log_2) indices, (Table 2), which are the benthic macrofauna diversity indices used by the Norwegian Environmental Protection Agency to assess EcoQS. The work of Alve et al. [21] demonstrated that foraminiferal indices reliably reflect the history of pollution in the region and can be used to obtain the preindustrial reference conditions from “beyond time intervals covered by

observational biological time-series". Since the first use of benthic foraminiferal-based diversity indices, the methodology has been adjusted by Bouchet et al. [74], who used Hill's number (N1) $Exp(H'_{bc})$ (1973) and developed novel quantitative threshold values for the EcoQS categories (Table 3) [74].

Table 2. Threshold values for determining EcoQS classes according to Hlog2 and ES100 [75], $Exp(H'_{bc})$ [49,74], TSI-Med [50], FSI [76], Foram-AMBI [77], and NQIf [75].

EcoQS and Associated Color Code	Bad	Poor	Moderate	Good	High
TSI-med	<1	1–2	2–3	3–4	>4
FSI	>5.5	4.3–5.5	3.3–4.3	1.2–3.3	<1.2
Foram-AMBI	>5	3.2–5	1.8–3.2	0.9–1.8	<0.9
NQIf	<0.13	0.13–0.31	0.31–0.45	0.45–0.54	>0.54

Table 3. Threshold values for determining EcoQS classes according to Hlog2 and ES100 [75] and $Exp(H'_{bc})$ [49,74].

EcoQS and Associated Color Code	Bad	Poor	Moderate	Good	High
H'log ₂	<1.2	1.2–1.8	1.8–2.4	2.4–3.4	>3.4
ES100	<9	9–11	11–13	13–18	>18
$Exp(H'_{bc})$ (>125, living, Norwegian fjords)	<2.5	2.5–5	5–7.5	7.5–10	>10
$Exp(H'_{bc})$ (>63, living, Norwegian fjords)	<5	5–10	10–15	15–20	>20
$Exp(H'_{bc})$ (>63, living, Italian transitional waters)	<3	3–7	7–11	11–15	>15

The foraminifera-based $Exp(H'_{bc})$ method has, since then, been successfully tested in a variety of transitional waters, i.e., fjords [74], lagoons [78], harbors [61], bays [78], and estuaries [25,79,80], in both subtidal [49] and intertidal conditions [78]. Furthermore, the index was successfully implemented in different biogeographical regions, i.e., Scandinavia [19], European Atlantic coasts [23,78], the Mediterranean Sea [49], and in South America in Brazil [25]. All these studies have demonstrated several advantages of using benthic foraminifera-based diversity indices. Index $Exp(H'_{bc})$ has shown a statistical relationship observed between the foraminiferal distribution and environmental parameters, such as organic matter enrichment in the context of aquaculture [71,81], sewage outfall [82], or metal pollutants such as copper (Cu), zinc (Zn), and lead (Pb) [48]. It is, however, important to note that recording such correlations does not necessitate a causal link without considering other key parameters and synergistic effects known to influence benthic foraminifera (e.g., organic carbon flux, salinity, sediment pore-water hypoxia/anoxia), which usually require lab culturing and controlled experimentation [21]. Despite this, studies using the $Exp(H'_{bc})$ index have been shown to be applicable to different stressors and in disparate biogeographic regions, presenting the potential for wider application, which can be supplemented by more detailed lab-based experimentation. The studies have also found some shortfalls, e.g., the accuracy of the results is dependent on the sampling effort (number of replicates), high seasonal variability may affect EcoQS assessment, and that the method needs to be better adjusted to the naturally low baseline diversity in transitional waters [21,74,83].

3.2. Foraminiferal Sensitivity Indices

Sensitivity indices, strictly speaking, are based on a theoretical succession of species of each sensitivity category along a certain perturbation gradient (Figure 3). Based on ecological preferences, the taxa are assigned to various sensitivity categories, which are given a numerical ranking, varying from 1–5 or even higher depending on the target specificity. The species abundance in each category changes with the amount of OM, other pollution, or stress within the environment; this delineates the biotic state of the site.

This approach has some limitations, as it oversimplifies species response to environmental parameters if only considering TOC. Specifically, it is questionable if this method to assign species according to their response to TOC gradients is also applicable in transitional waters, where TOC is reflecting both labile and refractory OM [11]. Noticeably, it was, however, not possible to assign typical salt marsh species from the English Channel, the European Atlantic coast, and the Mediterranean Sea [84] due to the presence of labile and refractory OM that hampers TOC characterization [85,86].

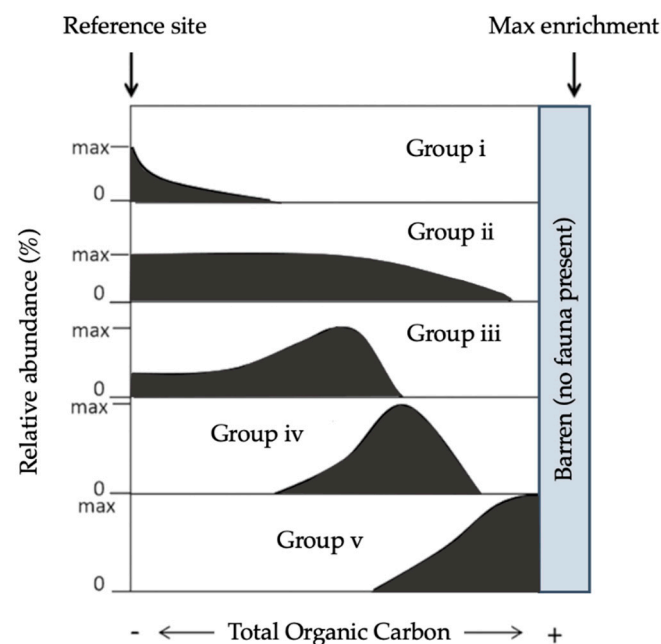


Figure 3. Theoretical relative abundance patterns for benthic foraminifera species along the main stress gradients (total organic carbon), grouped into the five ecological categories adapted from [77,87].

In this section, some examples of sensitivity indices (Table 2) used to evaluate foraminiferal assemblage response to environmental stressors are presented and discussed, and those include indices such as FIEI [88,89], TSI-Med [51], FSI [76], and FoRAM-Index [86,90,91]. Some indices are designed to evaluate the statuses of warm-water coral reefs. We chose to include those indices herein, as some transitional waters such as fjords in Norway and New Zealand are also known to host cold-water coral reef ecosystems [92]. We also discuss how the natural features of transitional waters may affect the outcome of sensitivity-based foraminiferal indices such as Foram-AMBI and comparable methods [79,93].

3.2.1. Foraminiferal Index of Environmental Impact (FIEI)

In response to drill-cutting disposal taking place in tropical east Atlantic outer shelf environments, Mojtahid et al. [88] designed the Foraminiferal Index of Environmental Impact (FIEI). This index is calculated as the cumulative percentage of all pollution-tolerant and/or opportunistic species observed within a system. Here definition of the “opportunistic species” was based on their patterns of distribution throughout the study area; this was combined with the comparison of the contemporary assemblage, in the uppermost 0–2 cm, with that preserved 2–3 cm below the surface of the sediment. The lower sediment intervals were used as the pre-impact “baseline conditions” [88,89]. This technique has been shown to be more discriminative than macrofaunal indices applied in the same study area, highlighting the benefits of foraminifera in providing a working ecological baseline for biomonitoring [87]. However, such an approach may suffer from circular thinking, as it

implies testing the index on the same data set used for the classification of species into tolerant and opportunistic groups. The formula for the FIEI index is given below:

$$FIEI = \frac{(N_r + N_0)}{N_{tot}} \times 100 \quad (1)$$

where N_r is the total quantity of pollution-resistant taxa, N_0 is the number of individuals of opportunistic taxa, and N_{tot} is the total number of counted foraminifera.

3.2.2. Tolerant Species Index (TSI-Med)

Later, working at the French Mediterranean coast, Barras et al. [50] concluded that an index based on the relative proportion of stress-tolerant foraminiferal taxa within a system was the most indicative metric of environmental quality" [50]. Several other parameters were tested in conjunction with stress-tolerance proportion: diversity, indicative species groups, and wall structure proportion, but due to highly variable coastal conditions, all of which were ruled out as lacking significant correlation with coastal ecosystem quality [50]. This index was named the Tolerant Species Index for the Mediterranean (TSI-Med or % TSI) and was developed in response to recent changes to the ecological conditions observed in coastal areas, where there has been strategic clustering of industrial development [50]. The formula for the TSI-Med index is given below:

$$TSI - Med = \frac{\%TS_x - \%TS_{ref}}{100 - \%TS_{ref}} \times 100 \quad (2)$$

where $\%TS_x$ is the percentage of tolerant species and $\%TS_{ref}$ is the theoretical percentage of tolerant species.

An advantage of the TSI-Med index is that it also integrates a correction for natural eutrophication phenomena involving the definition of reference conditions based on grain size distribution. Although this process is rigorous, the assignment of species to ecological groups often also involves the use of expert judgment to complement the literature review. Unfortunately, species classification, even by the best professional judgment, may fail [94]. Quantifying species response to organic enrichment should have also been used to strengthen the classification [77,84,87]. In fact, there is unlikely to be any single approach that works, and it should be recommended to combine literature review, best professional judgment, and numerical analysis in order to achieve the most accurate species assignment [95].

3.2.3. Foraminiferal Stress Index (FSI)

At the Saronikos Gulf, one of the most anthropogenically impacted coastal areas of Greece, Dimiza et al. [76] developed the novel index FSI (Foram Stress Index). Here, the benthic foraminiferal assemblage was grouped into two ecological groups according to their tolerance to OM enrichment based on a literature review [76]. Similar to TSI-Med, FSI lacks numerical quantification to support species assignments. The relative percentages of the weighted proportion of each group were used to formulate and define five ecological-status classes (similar to that of the AMBI index, see below). The results were promising. At this locality, FSI produced "Poor", "Moderate", and "Good" EcoQS rankings, which corresponded well with the level of impact. These were found to correlate strongly with the macrofaunal sensitivity index BENTIX [96], which was applied in the same areas. The formula for the FSI index is given below:

$$FSI = (10 \times Sen) + Str \quad (3)$$

where Sen is sensitive taxa, and Str is stress-tolerant taxa.

3.2.4. Foraminiferal AZTI Marine Biotic Index (Foram-AMBI)

Foram-AMBI has been used in several notable studies [84,87,93] and has been suggested as an additional assessment element to be incorporated into WFD and MSFD's coordinated approach [77]. Taken from Grall and Glémarec [97] and Borja et al. [98], this index was adapted from the AMBI index (originally used to classify macrofauna). Noticeably, benthic macrofaunal species are traditionally assigned to five groups of sensitivity to OM [96,98] based on the seminal work of Pearson and Rosenberg (1978). Species are meant to be indicative of the prevailing environmental conditions, i.e., the level of organic carbon enrichment where they are found. The following characteristics were used to assign benthic foraminiferal species to the five ecological groups (EGs: Figure 3) considering their response to TOC [84,87,94,97,98]:

Group V (EGV): "sensitive species" are sensitive to TOC enrichment. Their relative abundance is highest at the lowest TOC values and drops to zero as organic carbon concentration increases.

Group II (EGII): "indifferent species" are indifferent to the initial stages of organic carbon enrichment and never dominate the assemblage. They occur in low relative abundance over a broad range of organic carbon concentrations but are absent at very high concentrations.

Group III (EGIII): "tolerant species" are able to endure excess organic carbon enrichment. They may occur at low TOC; their highest frequencies are stimulated by organic carbon enrichment, but they are absent at very high organic carbon concentrations. This group has been termed "third-order opportunistic species" [94].

Group IV (EGIV): "second-order opportunistic species" show a clear positive response to organic carbon enrichment with maximum abundances between the maxima of EGIII and EGV (Figure 3).

Group V (EGV): "first-order opportunistic species" exhibit a clear positive response to excess organic carbon enrichment with maximum abundances at a higher stress level induced by organic load than species belonging to EGIV. At even higher TOC concentrations, foraminifera are not able to survive.

The abundance of each category of taxa is indicative of the EcoQS of the environment (Figure 3). Here, the Foram-AMBI index is applied to benthic foraminifera; calculated in the same manner as it was previously designed for macrofauna [98]:

$$\text{Foram-AMBI} = 0 \times \text{EG1} + 1.5 \times \text{EG2} + 3 \times \text{EG3} + 4.5 \times \text{EG4} + 6 \times \text{EG5} \quad (4)$$

Foram-AMBI was first used in the Northeast Atlantic, Arctic fjords, continental shelves, and upper slopes by Alve et al. [77]. In this study, the correlation between TOC and the species sensitivity assignment was based on 19 publications and was tested against independent validation data sets (VDS) to avoid circular arguments [77]. The results confirmed that in organically enriched systems, foraminiferal sensitivity, reflected in assemblage changes, correlates well with TOC gradients (Figure 4). Recently, the assignment of species to the five EGs in transitional waters along the English Channel, European Atlantic coast, and in the Mediterranean Sea allowed for further implementation of Foram-AMBI in transitional waters [84]. The results showed similar correlations to those observed in Alve et al. [77]. A significant positive correlation was observed between Foram-AMBI and organic matter in the two validation data sets. In one of the VDS, the results from Foram-AMBI were also compared to the macrofaunal AMBI index at the same sites, yielding a significant correlation ($R^2 = 0.56$, $p < 0.01$) between both indices and adding to the support for benthic foraminifera as a reliable BQE. These applications support the implementation of Foram-AMBI. However, recent studies [49,99] already suggested to re-assign some species (originally assigned by Alve et al. [77]) into different groups. Except for the Mediterranean Sea [87], the best professional judgment was not used to support the outcome of the numerical approach to assign foraminiferal species to ecological groups [77,84]. We suggest here that a combination of expert judgment informed by literature review and numerical methods should be used in order to obtain the best possible species assignments.

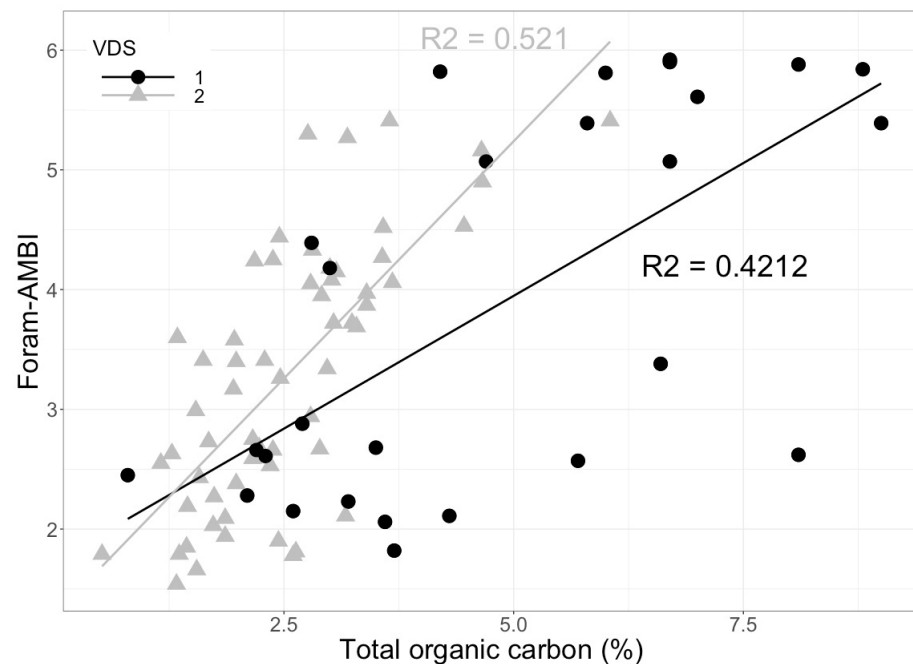


Figure 4. Foram-AMBI index plotted against the total organic carbon (%) in the sediments [94] regression line for validation data set 1 from the Norwegian Skagerrak (VDS 1) is shown by the black line and for validation data set 2 from the Oslofjord (VDS 2) is shown by the grey line (raw data from J. Dolven and E. Alve).

3.2.5. Foraminifera in Reef Assessment and Monitoring (FoRAM-Index)

The use of foraminifera-based sensitivity indices for EcoQS goes all the way back to 2003 when Hallock et al. [90] developed the FoRAM-Index (Foraminifera in Reef Assessment and Monitoring) to assess the health of tropical coral reef systems. The FoRAM-Index groups foraminifera associated with coral reefs into three categories: large benthic foraminifera, other small heterotrophic taxa, and stress-tolerant foraminifera. The foraminiferal assemblages typically found in reef environments have been shown to be indicative of both the ambient water quality and reef recovery via nursery transplants or coral recruitment. Additionally, the FoRAM-Index was designed to require only limited computing abilities and hinges on uncomplex calculations, making it user-friendly and easy to implement worldwide for researchers of varying expertise [90]. Since its inception, FoRAM has been successfully applied by 23 separate studies [91] and is able to provide resource managers with data to determine the suitability of benthic habitats for communities dominated by photosymbiont-bearing organisms. The formula for the FoRAM-Index is given below:

$$\text{FoRAM Index} = (10.P_s) + (P_o) + (2.P_h) \quad (5)$$

where P_s represents the proportion of symbiont-bearing, P_o stress-tolerant, and P_h other heterotrophic taxa.

3.3. Foraminiferal Multi-Metric Index

Lastly, Alve et al. [75] proposed a new foraminifer-based multimetric index (NQI_f) based on the macrofaunal Norwegian Quality Index (NQI) and tested it in Norwegian fjords on the Skagerrak coast [75]. The study combined paired samples of benthic macrofauna and foraminifera with associated bottom water dissolved oxygen and sediment TOC data. The two BQEs (foraminifera and macrofauna) were intercalibrated via linear regression, and the threshold values for the EcoQS categories were based on those already defined for macrofauna-based indices by the Norwegian governmental guidelines.

This index is composed of the following metrics: (i) a diversity component $\ln S/\ln N$ (InN), where S is the number of taxa and N is the abundance, (ii) a sensitivity component (AMBI), and (iii) a correction factor to down-weight artificially high diversity values in small samples (few individuals, $N/N + 5$). Both the macrofaunal and foraminiferal NQI were found to be significantly correlated with bottom water dissolved oxygen concentration; however, in very low oxygen conditions, only foraminifera could be used for EcoQS assessment because macrofauna were absent [75].

The index is an algorithm, where equal weight is given to diversity (50%) and sensitivity (50%) and is formulated as follows:

$$NQI_f = 0.5 \left(1 - \frac{AMBI_f}{7} \right) + 0.5 \left(\frac{ES100_f}{35} \right) \quad (6)$$

where $AMBI_f$ represents Foram-AMBI (Section 3.2.4) and $ES100_f$ represents foraminiferal diversity expressed as Hurlbert's index.

4. Comparison of EcoQS Indices

4.1. Various Foraminiferal Indices: How Well Do They Perform When Used at the Same Site?

Research shows that foraminifera-based indices have the potential to reveal the ecological status preceding anthropogenic disturbance and are capable of setting informed reference values [26,48,49,91,100–103]; see Section 5.2 below. To produce meaningful actions, however, the results must be comparable and congruous with each other. At the same time, studies aiming for a comparison of these disparate indices and methodologies reveal some discrepancies in the consensus reached. Below, we provide a case study exemplifying this.

The study by El Kateb et al. [48] assessed EcoQS along the Gulf of Gabes (Tunisia) and incorporated the diversity indices such as Shannon Index (H'), Exp (H'_{bc}), sensitivity indices FSI, % TS_{std} (using the FSI- assigned species), % TS_{std} (using Foram-AMBI assigned species), FI' (Modified ForAM-Index), and I_{LS} ("long versus short life span" index), which can be considered in combination [48]. Developed by Mateu-Vicens et al. [104], FI' and I_{LS} rely on foraminifera associated with meadows of the seagrass, present at several locations within the Mediterranean Sea [104]. This study demonstrated that each of these indices assessed EcoQS differently (Figure 5). To understand the reason for this, we must look to the assumptions and criteria considered in each index, as well as the varying geomorphological regimes along the gulf. The indexes that recorded the worst EcoQS values were FI' and I_{LS} epiphytic foraminiferal indices, FSI, and % TS_{std} (FSI-assigned species). Conversely the best EcoQS was reported by Foram-AMBI, % TS_{std} (Foram-AMBI-assigned species), and Shannon Index (H'), while Exp (H'_{bc}) demonstrated intermediate EcoQS values. Begging the question, are the former indices more sensitive to environmental stress than the latter ones? Or perhaps these results reflect the situational aptitude of each index and how compounding environmental variables influence their efficacy? To begin with, FI' and I_{LS} , both being epiphytic foraminiferal indices, reported predominantly "bad" to "poor" EcoQS along the gulf. These indices rely on the presence of sea grass meadows, which have been in decline due to increasing environmental stress. In the absence of such habitats, there are no epiphytic foraminifera, and the area is recorded as "poor", meaning these indices reflect the lowest values indicative of unsuitable growth conditions. El Kateb and colleagues concluded that these indices correctly reflect the ecological status of the area due to seagrass *Posidonia oceanica*'s sensitivity to environmental conditions. Epiphytic indices can, hence, only be used in areas that are known to be suitable for seagrass growth.

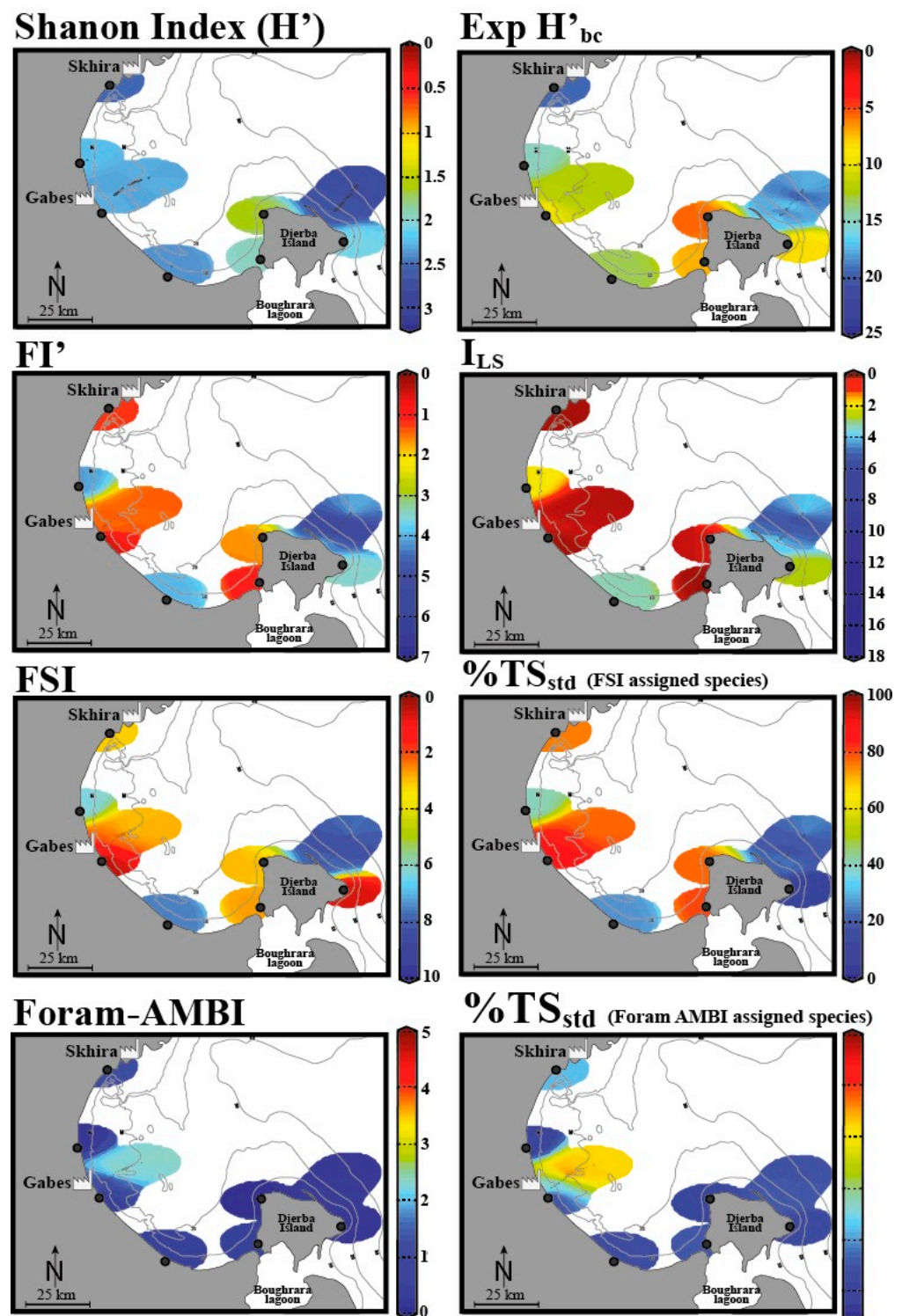


Figure 5. The EcoQS assessment in the Gulf of Gabes (Tunisia) using various foraminifera-based indices (data is spatially interpolated using DIVA software). Adapted from [48]. Colours indicate various EcoQS classes: blue—high, green—good, orange—moderate, yellow—bad, red—poor.

In contrast, Foram-AMBI, % TS_{std} (Foram-AMBI assigned species), and the Shannon Index (H') suggest comparatively unpolluted conditions; to explain this, the authors used two distinct systems sampled from the Gulf region: Gabes, a more polluted area with organic enrichment and fine sediments, and Djerba, with coarser sandy sediments. The near shore silt and mud at Gabes is also reported to be rich in heavy metals (Pb, Li, Zn, Cu) and

phosphorous due to industrial waste discharge. Sites with fine sediments appear to be more suitable for application of ForAMBI, % TS_{std} (ForAMBI-assigned species), and % TS_{std} (FSI-assigned species); as the aforementioned indices are all sensitivity-based, the link here is explained by opportunistic/stress-tolerant species dominating systems. In Djerba, with sandy sediments, however, the contrary was observed; here, the diversity indices Exp (H'_{bc}) and H' as well as the epiphytic indices FI' and I_{LS} exhibit a stronger relationship. El Kateb et al. further addresses ForAMBI alone, pointing out that this index was developed to reflect an increasing OM gradient, relying primarily on sediment mud content, dissolved oxygen, and pollutants, such as metals and polychlorinated biphenyls (PCBs). In the areas of the gulf that do not exhibit these conditions, the index becomes less useful. This highlights that it is essential to understand the components of each index to ensure the results will be representative of the site and that it is advisable to use and compare several indices to reach the soundest conclusion [19].

4.2. Palaeoecological Applications: Toward the Definition of Reference Conditions

In contrast to most benthic macrofauna, the preservation of benthic foraminiferal tests provides information about long-term environmental and biological changes [19,21,23]. Hindcasting approaches (i.e., the use of historical information) are one of the methods recommended by marine legislations to determine reference conditions. Such approaches imply the existence of long-term monitoring or fossil records of taxa used to characterize the EcoQS. Different studies further confirm that fossil benthic foraminifera can enable the reconstruction of in situ local reference conditions in transitional environments with long-term pollution history to determine either contemporary EcoQS or its evolution over time and may contribute to deciphering between natural and anthropogenic stresses. For instance, a more robust assessment of EcoQS for transitional waters was obtained with benthic foraminifera using site-specific local reference conditions in the Oslofjord, Norway [19], in the Boulogne sur Mer harbor, France [23], and in the Santos estuary, Brazil [25]. Furthermore, geochemical analyses of dated sediment cores can be used in combination with benthic foraminifera to determine ecological reference conditions in transitional waters [20,26].

In the Oslofjord (Norway) [19], the results from applying ES100, H'_{log2} , and Exp H'_{bc} diversity indices were found to be consistent with physical and chemical parameters at the site (metals, TOC, and dissolved oxygen). Furthermore, the temporal pattern and scale of change shown by each of the indices are still the same, and this can be used to define deviation from the reference conditions, regardless of the index used. For instance, in Boulogne sur Mer Harbor, local reference conditions from the pre-impacted period allowed the authors to accurately assess EcoQS based on the ecological quality ratio or EQR [23].

The EQR (Figure 1) is the ratio between the value of the observed biological parameter for a given water body and the expected value under reference conditions [4] and is in line with the WFD definition of EcoQS. The ratio ranges as a numerical value between 0 and 1, with high ecological status represented by values close to one and bad ecological status by values close to zero. The EQR boundaries to define Palaeo-EcoQS were determined using the "Reference Conditions Working Group" recommendations [105]. Type-specific reference conditions (including hydromorphological and physicochemical conditions unique to each water body) are the anchor point of EQR based classifications. Hence, the EQR approach can also be used to resolve issues related to the definition of threshold values (see Section 4.3). Using the foraminiferal fossil record, it is possible to determine in situ local reference conditions. For example, in the Santos estuary (Brazil), the highest value of diversity in ~1902, i.e., Exp (H'_{bc}) of 11.66, corresponds to the pre-impacted period and was defined as the reference value. The EQR was calculated accordingly: $EQR = \text{Exp} (H'_{bc})_{\text{observed value}} / \text{Exp} (H'_{bc})_{\text{reference value}}$ [25]. In that study, the foraminiferal fossil record allowed evaluation of Palaeo-EcoQS based on in situ local reference conditions in accordance with the environmental history of the region.

4.3. Palaeoecological Applications: Defining Correct Threshold Values

EcoQS value uncertainty, due to lack of intercalibration between threshold values, presents some issues for using living foraminiferal assemblages in transitional environments. This can also be observed in Palaeo-EcoQS assessment, which may be exacerbated by preservation bias. By applying the Exp ($H'bc$) diversity index and threshold values from two separate publications, Bouchet et al. [74] and Bouchet et al. [49], to the foraminiferal assemblage data from Idefjord, the EcoQS of the fjord can be categorized very differently (Table A2) [49,74]. Using threshold values from Bouchet et al. [49], the resulting fjord EcoQS is “Moderate” to “Bad”, with a slight reprise back to “Moderate” observed in the early 2000s. The low natural diversity of the system presents as only “Moderate” EcoQS even back to the 1800s, before the time when the anthropogenic activity began to significantly impact the system, highlighting that indices must be adjusted when applied to transitional waters [24]. Conversely, when the “dry picking” threshold values from Bouchet et al. [74] are applied, the fjord shows mainly “Good” EcoQS values until 1890, where the EcoQS decreases to “Moderate” and “Bad”.

The chosen threshold values from Bouchet et al. [74] were based on foraminiferal assemblages from dry picking the $>125\ \mu\text{m}$ sediment fraction, while thresholds from Bouchet et al. [49] are also “dry picking” but “ $63\ \mu\text{m}$ fraction” boundaries modified after Bouchet et al. [74] based on using the PERSE method (Procedure to Establish a Reference State for Ecosystems [106]); a multivariate non-parametric approach to calculate the relative reference states against which EcoQS fluctuations can be detected and quantified. Table A2 makes it clear that the reconstruction “EcoQS 2” is more realistic based on known pollution history in Idefjord [24] by applying lower diversity thresholds originally obtained for Nordic fjord waters (Bouchet et al. [74]). In contrast, the Exp ($H'bc$) diversity values (Bouchet et al. [49]) adjusted for the Mediterranean, due to higher species diversity, obviously underestimate changes of EcoQS in Nordic fjord waters. This is an example of issues related to latitudinal diversity gradients and the effect of these on the characterization of transitional water ecosystems across the globe, which makes a strong case against using “universal” threshold values. Among other issues important to consider when setting threshold values or EcoQS boundary classes are sediment size-fraction analyzed for foraminifera, number of replicates, dry versus wet picking method, and sediment characteristics on a site (soft-bottom muddy sediments versus silty and sandy deposits). Hence, future studies should make efforts toward using class boundaries adjusted locally and base these on the above-mentioned characteristics, as well as on local reference conditions. Other possible means to adjust the threshold values can be: indicator equations (a theoretical approach aimed at finding a correspondence between the index equations), reference indicators (a separate indicator used to calibrate other indicators), and indicator distribution laws mathematical properties of the indicator values (such as distribution laws, which are used to obtain the same proportion) [107].

The example presented in Table A2 demonstrates that EcoQS threshold values can have a huge impact on the message displayed by EcoQS assessment index thresholds, which can be exacerbated by preservation bias by natural abiotic and biotic processes (particularly in older sediments), as well as sample preparation methods, such as dry picking vs. wet picking, staining, and sieving. Hence, EcoQS threshold values should be carefully considered upon their application, which makes index calibration and a conversion factor specific for Palaeo-EcoQS assessment in transitional waters a key in obtaining reliable reference conditions.

4.4. Taphonomical Processes and EcoQS Indices

Another key aspect in characterizing contemporary environmental and paleoenvironmental changes accurately is understanding the difference between living and dead benthic foraminiferal assemblages and all the factors that may lead to their formation [60]. Foraminiferal biomonitoring studies based on total assemblages, particularly those aimed at characterizing environments that have undergone recent and extensive habitat modifica-

tion, either intentionally (e.g., through aquaculture and marina construction) or unintentionally (e.g., agriculture leading to deforestation and subsequent erosion), are susceptible to bias interpretations due to taphonomic (post-mortem) processes affecting the tests (see review in [108]). Those processes, including transportation, CaCO_3 dissolution, and test breakage, can occur more readily post-mortem and impact species composition of the total assemblages together with seasonal population shifts (different species having peaks during different times of the year) and influxes of allochthonous taxa to the area. In highly stratified fjords, for example, it has been demonstrated that sediment archives may also be heavily biased toward agglutinated species due to seasonal hypoxia and carbonate dissolution [47,109]. Some studies performed in shallow estuaries showed that seasonal changes of redox conditions can cause almost complete dissolution of calcareous component of the assemblages resulting in total assemblages being strongly dominated by agglutinated foraminifera [47,109–111], local hydrodynamism in estuaries may hamper the preservation of foraminiferal fauna by sediment resuspension and mixing [112] and bioturbation processes by macro-invertebrates may enhance the homogenization of the upper-sediment layers [107]. Hence, it is important to recognize the conditions that lead to the highest preservation of foraminifera tests and understand how best to adjust data from sub-optimal conditions to most accurately reflect the true ecological conditions of a site. For instance, at the Saquarema lagoon system in Brazil significant difference was found between the living and dead assemblage distributions [60]. These differences were attributed to the hydrodynamic conditions and seasonal variation.

To reduce the effect of taphonomic or post-mortem bias, Belart et al. [60] suggest that in homogenous systems, areas with high living-dead assemblage similarity may be the primary target for palaeo-environmental assessment (to inform the baseline reference conditions) [60], whereas in highly heterogeneous areas, such transitional waters, an alternative approach would be to undertake palaeoecological studies at all stations and adjust the recorded total assemblages by conversion factors inferred from a DCA or PCA plot [113]. Here, the trade-off between effort (time, expertise) input and representative data output must be considered on a site-to-site basis as significantly more extensive data treatment and analysis would be involved.

4.5. Comparison of Foraminifera-Based Ecoqs Indices with Traditional Macrofauna-Based Indices

Increasingly, a multi-metric approach, incorporating several biomonitoring strategies, is being adopted across international management operations; for example, the macrofauna-based Norwegian Quality Index (NQi, see Section 3.3), which employs a sensitivity component with the AMBI index in combination with the H' and ES_{100} diversity [114,115]. In fjords of Norway, Alve et al. [75] has shown that applying an adapted version of the macrofaunal NQi to foraminifera (NQI_f) yields a similar indicator efficiency as its macrofaunal equivalent (Figure 6a); eventually concluding that foraminifera should be included among the biological elements defined by the WFD and used for EcoQS assessment [75]. Another example from Norwegian fjords, where the benthic foraminiferal communities were shown to significantly correlate with benthic macrofaunal ones, comes from the study of Bouchet et al. [84] (Figure 6b). Furthermore, in the oyster farming areas of the Pertuis Charentais (France), Foram-AMBI and macrofaunal AMBI were also significantly correlated [84]. All these examples suggest that other benthic foraminiferal indices may correlate significantly with their macrofaunal counterparts, demonstrating that benthic foraminifera-derived indices can be used to complement the results of other BQEs and can be applied where macrofauna are absent or difficult to sample.

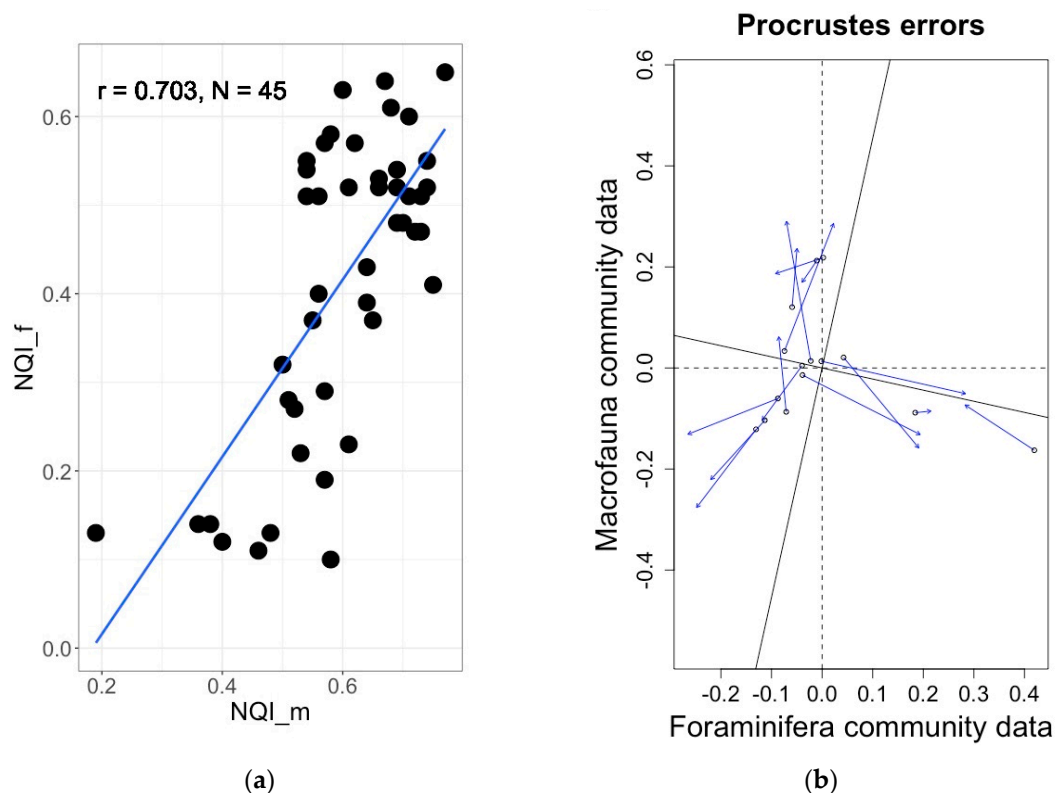


Figure 6. (a) Significant correlation ($R = 0.70$, $p < 0.0001$) between foraminiferal and macrofaunal NQI indices in Norwegian fjords [75]. (b) Significant correlation (procrustes errors analysis $m2 = 0.66$, $p = 0.001$) between foraminiferal and macrofaunal communities in Norwegian fjords [83].

5. The Genetic Era

Following the successes of the Foram-AMBI and other indices, foraminifera-based indices are now being considered from a novel perspective. Basing environmental quality assessment on DNA (or RNA) present in environmental samples is a promising technique in working toward the development and implementation of both cost-effective, high-impact, and robust methodologies for the long-term monitoring and management of marine ecosystems. An increase in sophistication and accessibility of genetic technologies has fuelled a surge of research into using these as a biomonitoring tool, prompting some to refer to this period of innovation as the “ecogenomic era” [116] or “biomonitoring 2.0” [117]. Below, we focus on two genetic approaches, eDNA and aDNA, increasingly used in biomonitoring with foraminifera as BQE.

5.1. Environmental DNA

Environmental DNA (eDNA) represents a mixture of the genomic DNA, which was present in the living cells and preserved within cellular organelles, or it is extracellular molecules, which reside in fragments of tissue, secretions, and other organic materials [118]. These eDNA fragments can be found externally from their biotic origin and can be extracted from environmental samples without first isolating the target organism. Surface waters provide a wealth of eDNA in the aqueous or sedimentary form [119]. With the use of high-throughput sequencing (HTS) and bioinformatic pipelines, millions of DNA sequences present in environmental samples can be analysed, and as long as the DNA does not degrade and remains uncontaminated, it is possible to target small or elusive taxa present only at low densities, and sample at the diversity of the existing primers [118,119].

Environmental DNA-based technology is mainly applied in one of three ways: through single-species detection, in biodiversity surveys, or for biotic indices. Depending on the study approach, specific technology and methods are necessary to analyse the result-

ing molecular information [120]. Firstly, single-species detection is the most simplistic way to use eDNA for biomonitoring purposes. Based on polymerase chain reaction (PCR) amplification, this approach uses species-specific primers to detect endangered, rare, or alien/invasive species. Though its use in marine sediments is limited, studies on alien or harmful species related to industrial activities show promising results [116].

Secondly, using HTS and targeted PCR amplification, eDNA samples can be indicative of the whole biological community. This technique is referred to as eDNA metabarcoding and has been successfully applied in biodiversity surveys as well as bioindication. This technique is often used to investigate marine biodiversity and can shed light on the biogeography and ecology of planktonic and benthic microbes [121], meio- and macrofauna [122]. Increasingly, however, metabarcoding is being used on benthic foraminifera to assess human impacts on the marine environment, ecosystem quality assessment, and biomonitoring.

Several studies have used these new technologies in conjunction with observation-based taxonomic methodologies to verify the use of genetic data in biomonitoring. For instance, the impact of aquaculture, a multitrophic metabarcoding biotic index for monitoring of organic enrichment associated with fish farms has been proposed by Keeley et al. [123] based on benthic foraminifera, bacteria, and general eukaryotes [123]. Bioindicator taxa were identified by metabarcoding eDNA and eRNA for the taxonomic groups and assigning Eco-Groups to the most abundant operational taxonomic units (OTUs); this information was then used to develop a multitrophic metabarcoding biotic index, which showed a strong relationship with the “enrichment stage” (determined by the chemical and biological properties of the site). These techniques have also been successfully employed with benthic foraminifera to assess the environmental effect caused by offshore fossil fuel drilling and production operations [124,125], in monitoring fish farms [126], and in polluted harbors [125]. These studies pointed toward congruence between conventional morphology- and eDNA-based foraminiferal data. Recently, the first application of foraminifera indices on eDNA data showed a good correlation with environmental parameters and congruent results with indices based on morphological data [127], further supporting the implementation of metabarcoding with benthic foraminifera.

The genetic approach is still a new technique and, as a result, is presently regarded as complementary to traditional methodologies rather than a standalone method. This is due to several issues (i) the high number of unassigned OTUs due to low coverage of sequenced species in the GenBank; (ii) presence of propagules (resting stages) of foraminifera in the sediments, which will not necessarily develop into adults, but will be picked up by the eDNA analysis; (iii) high number of monothalamids present in the OTUs but commonly absent as fossils, making a comparison between morphospecies and eDNA complicated; (iv) biases related to the process of DNA extraction and PCR amplification, e.g., underrepresentation of globothalamids compared to monothalamids as the hard shell makes DNA extraction more difficult; and (v) the number of rRNA gene copies, variation nuclei number or ploidy related to alternation of generation in the foraminiferal life-cycle [126,128]. Greater efforts in barcoding research can be used to close some of these knowledge gaps, as well as a taxonomy-free approach, where biotic indices are computed without the taxonomic assignment of sequences, which was first used on benthic diatoms to assess the ecological status of rivers and streams [129]. This process relies on a much higher percentage of OTUs (up to 95%) compared to morphospecies-based taxonomy; indicator values are assigned to particular species or OTUs based on their occurrences within the metabarcoding data. Other studies have proposed a machine learning approach but are seldom used due to the lack of the extensive training data sets needed [128]. Once the methodology has been fine-tuned, a metabarcoding approach could be used to develop EcoQS indices without taxonomic pitfalls associated with erroneous species identification, offering instead the identification of OTUs.

5.2. Ancient DNA

Ancient DNA (aDNA), in a way similar to eDNA, can be recovered even from species that do not leave behind diagnostic microfossils in the sediment, making it a promising approach for refining the reconstruction of palaeo-environmental conditions persisting over millennia to hundreds of thousands of years back in time [130]. Within marine palaeoecology, the term sedimentary DNA (sedaDNA) is usually synonymous with aDNA, as sedaDNA refers to aDNA that is found within the sediment.

Ancient DNA has been shown to be applicable to foraminifera, with the possibility to reveal the presence of species that are otherwise often excluded from species count due to being less readily fossilized, cryptic, or simply too small to detect. For example, previous palaeoecological surveys have shown that the detection of monothalamous and polythalamous foraminifera is possible, and those make a valuable indicator of changes in EcoQS [126,131]. The molecular inclusion of non-fossilized monothalamids in palaeoecological surveys increases the richness observed within the foraminiferal communities compared with that of the fossil record. Monothalamous species, a group of foraminifera characterized by single-chambered, agglutinated, or organic tests, are soft-shelled and often ignored in foraminiferal studies due to their low preservation potential despite their high abundance and diversity, even making up the majority of the species present in some transitional waters, such as fjords in Scotland [126,132]. Hence, the inclusion of this group could potentially improve the overall accuracy of palaeodiversity estimates influencing the assessment of local reference conditions [126,132].

In order to use genetic data to set reference conditions for EcoQS assessment, it must be recoverable from a time before human impact began to negatively affect the environment. Sedimentary aDNA is required from the preindustrial period, but is this feasible?

Foraminiferal DNA can remain contained within the test and be detectable within the sediment [133]. Various studies have shown that plankton-derived DNA, including foraminifera, can be preserved in marine sediments over tens to hundreds of thousands of years [130,131,134]. It has also been reported that environmental DNA was more concentrated in marine sediments than in aqueous form [135,136]. Kirkpatrick et al. [137] reported recovering fossil diatom DNA from up to 1.4 Ma old sediment core samples, the long-term survival of which was attributed to “protection” provided by being surrounded in the diatom microfossils, suggesting that microfossils increase the preservation potential of DNA over time [137]. This points favorably toward the use of foraminifera microfossils as the target organism of similar aDNA studies in the future.

Due to general lack of research in aDNA area, however, Holman et al. [138] have called for more studies to “advance our understanding of how eDNA deposits and degrades in marine sediments in order to temporally contextualize sediment samples”, opting instead to only process the uppermost sediment, only profiling contemporary species composition [138]. The field of genetic biomonitoring, particularly sedimentary or ancient DNA, holds much potential in the effort to constrain reference conditions within the ecological assessment of transitional waters; however, the lack of experience and understanding of post-depositional genetic degradation and taphonomic preservation bias within the sediments renders this methodology at present, only complementary, rather than a viable standalone approach.

6. Foraminiferal Indices: Strengths, Weaknesses and Way Forward

Foraminifera have been shown to be a consistently indicative basis for biomonitoring indices, applied within a wide range of transitional environments. Foraminifera-based indices offer an alternative or complementary methodology to their more widely accepted macrofaunal analogue, particularly for EcoQS assessment in transitional waters, where a unique mosaic of heterogeneous habitats converge due to influence from terrestrial, freshwater, and oceanic regimes. The ability to provide reliable ecological information across disparate and often highly variable systems makes foraminifera an ideal BQE for EcoQS assessment in transitional waters. Delineating anthropogenic disturbance versus natural variability is

possible due to foraminiferal community composition being comparable directly between sediment layers deposited during contemporary and preindustrial times. Such “preindustrial to modern assemblage composition” can be tracked in sediment archives and directly compared against geochemical changes recorded in the surrounding sediment, which are also able to record ecosystem reprisal and recovery, quantifying the ecological benefits of waste management and industrial regulation. Palaeo-EcoQS assessment can also be instrumental in the determination of pristine reference conditions within the naturally stressed systems of transitional waters. An accurate estimate of EcoQS prevailing long before the anthropogenic economic development and observation-based biomonitoring studies began is necessary to understand the level of human impact and inform counteractive action to ensure proper restoration practices.

6.1. Issues and Resolutions

6.1.1. Differences in Assessed EcoQS Using Different Foraminiferal Indices

In the interest of integrating foraminifera as an official BQE for biomonitoring approved by legislative bodies such as WFD and MSFD, their correlation with macrofaunal indices (the current status quo) has been extensively investigated and shown to be significant. In this pursuit, however, assessment of transitional waters presents an issue; although cross-examination based on both BQEs often shows a similar response to environmental stressors, the various indices based on foraminifera are not always consistent in their EcoQS assessment for the same site. As evidenced by the case studies presented in Section 4, this issue results again from the patchwork nature and naturally stressed conditions found within transitional environments, as well as the individual strengths and weaknesses of the indices within distinct bio-geomorphologic settings. For instance, Foram-AMBI has been developed for fine-grained sediments and is most accurate in OM enriched environments but over-estimates ecological quality in coarse-grained sediments replete with abundant dominant species [48]. Additionally, the threshold values for the EcoQS classes are not easily defined and are usually based on precedent, which can result in the same index, using thresholds applied previously in two different studies, recording different ecological quality values for the same site. To combat these two issues, we firstly suggest an integrated index approach, the rationale in support being that an index appropriate for one environment is not necessarily applicable to the next. To put this into practice, various biotic, abiotic, and geomorphologic features of the site, as well as sampling and sample preparation methods, should be considered as part of the data treatment. In addition, more comparative studies, in which multiple indices are applied to the same system, are needed to provide data on the aptitude of each index and allude to the site characteristics that exhibit the strongest relationship with each index (e.g., Figure 5). Then the most apt index can be chosen and applied to each site to provide the most accurate estimation of ecological health. Although this is more labour-intensive in the present, as the characteristics criteria have yet to be developed and will require some extra diagnostic effort and expert judgment, with an easily accessible checklist (or flow chart) of site characteristics, it would be possible to improve all future biomonitoring assessments within transitional waters and beyond.

6.1.2. Threshold Values

Secondly, when defining threshold values, a similar approach can be considered. It is clear that in such variable systems, such as transitional waters, a given EcoQS evaluation is likely only an indication of the actual status; however, to influence legislation, quantification is key. Using threshold values previously applied to a similar system may be sufficient in some cases; however, as suggested in Section 4.2, a more precise assessment can be achieved by obtaining an EQR value. An exciting benefit of foraminifera-based indices, in situ Palaeo-EcoQS, can be used as the baseline for site-specific valuation, which directly compares the preindustrial and modern-day status of only the target locality. Instead of expressing environmental initiatives as a push to improve water bodies to “good” EcoQS

by X year, framing it as achieving an EQR ratio of close to 1, which can also be easily expressed as a percentage allowing for more specific changes to be tracked and targets set.

6.1.3. Intercalibration

Finally, in line with the developments suggested in this review, there has recently been a move toward the intercalibration of benthic foraminiferal indices and macro-faunal indices. This increasingly holistic approach encapsulates more taxa and even entire ecosystems and is being gradually integrated into environmental policy and legislation (e.g., WFD, 2000 [1], MSFD, 2008 [2,124,139,140]). As the application of genetic techniques to marine environments is a recent development, the laboratory and analysis techniques, as well as bioinformatic processing, are not yet fully established or have not yet been standardized [141]. Even less research has been done on the intercalibration of methods across different disciplines with the common goal of achieving a holistic understanding of ecosystem health. In the interest of harmonizing assessment methods for the implementation of the WFD, the EU commissioned the Geographical Intercalibration Work (GIG). The GIG has since inspired several projects, including HARMEX “Harmonisering af Nordiske bundfaunaindeks for økologisk kvalitet i poly- til euhaline områder” (Harmonization of Nordic benthic quality indices in polyhaline and euhaline areas), founded by the Nordic Council of Ministers involving Norway, Denmark, and Sweden [114]. Three Scandinavian multi-metric macrofaunal EcoQS indices (Norwegian NQI and Danish DKI and Swedish BQI) were intercalibrated to assess coastal benthic response to pollution-related stress gradients, using both species diversity and sensitivity. Clear responses to gradients of OM, hypoxia, metals, effluent material, and physical disturbance were observed.

6.2. New Techniques

Metabarcoding has introduced a new potentially valuable tool in ecological assessment studies. Several studies have identified metabarcoding DNA/RNA retrieved from environmental samples as being a useful method for biodiversity assessment and biomonitoring in both past and present, aqueous or sedimentary environments [123,124,142,143]. Studies have instead suggested that the foraminiferal genetic data can provide an additional environmental impact assessment tool where the traditional morphology-based approach is impractical [116]. In addition, the presence of resting stages (propagules) in foraminifera and still relatively low number of sequenced foraminiferal species poses difficulties for the eDNA approach to be used as an independent method. In addition, successfully sequencing the small and often incomplete molecules of DNA/RNA present in sediments is still the biggest barrier to the implementation of this methodology; while it provides some other advantages (see Section 5), the field is still very much in its infancy and will benefit from more years of development and refinement before it can be widely accepted as a standalone alternative. The two methodologies, the morphology-based approach and metabarcoding, are currently regarded as complementary to each other, with disparate scientific trade-offs affecting each, resulting in scenarios where one technique may be more or less appropriate. Here, we advocate for the inclusion of genetic methods into mainstream ecological assessment practices; the additional benefits of this method are clear and require only a small sample volume, which can be collected alongside that used for morphology-based methods. The increased amount of data available about the biological community available at each site will improve assessment accuracy and be available to inform the calibration of indices for future use.

7. Conclusions

Foraminiferal indices and their ability to provide reference conditions beyond observational time series and conventional monitoring have proven their high value for EcoQS assessment even within transitional waters. As evidenced by the range of studies presented here, various aspects of these protists’ living, dead and fossilized assemblages can be used to accurately quantify environmental quality and its temporal changes. Foraminiferal

indices can be applied in a variety of environments to obtain meaningful results that can be used to influence legislation; however, they also require careful adjustment, verification, and intercalibration. Gaining an understanding of the characteristics of each index is key in selecting the most appropriate index for a particular region, waterbody, sediment type, assemblage type, and sampling/sample preparation method. Development of a checklist or flowchart to outline the properties of each index and how these relate to site variables would be highly beneficial, helping to both standardize and simplify the process.

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Data Availability Statement: Synthesized data on available literature on the topic are available in Table A1 in the Appendix A. Data used in Table A2. can be found at Polovodova Asteman et al. [24].

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Abbreviations

aDNA	Ancient DNA
AMBI	AZTI's Marine Biotic Index
BQE	Biological quality element
CWA	Clean Water Act
DCA	Detrended correspondence analysis
eDNA	Environmental DNA
e.g.,	Exempli gratia
EQS	Ecological quality status
EQR	Ecological quality ratio
FI'	Modified FoRAM-Index
FIE	Foraminiferal Index of Environmental Impact
FOBIMO	Foraminiferal Biomonitoring group
Foram-AMBI	Foraminiferal AZTI's Marine Biotic Index
FSI	Foram Stress Index
gAMBI	Genetic AZTI's Marine Biotic Index
GIG	Geographical Intercalibration Work
HARMEX	Harmonisering af Nordiske bundfaunaindeks for økologisk kvalitet i poly- til euhaline områder" (Harmonization of Nordic benthic quality indices in polyhaline and euhaline areas)
HTS	High-throughput sequencing
I _{LS}	"Long Versus Short Life Span" Index
MSFD	Marine Strategic Framework Directive
nEQR	Normalized ecological quality ratio
NQI	Norwegian Quality Index
NQI _f	Norwegian Quality Index (using Foraminifera)
OM	Organic matter
OUT	Operational taxonomic unit
PERSE	Procedure to Establish a Reference State for Ecosystems

PCA	Principal component analysis
PCR	Polymerase chain reaction
PRISMA	The Preferred Reporting Items for Systematic reviews and Meta-Analyses
sedaDNA	Sedimentary DNA
TS	Tolerant species
TSI-Med	Tolerant Species Index for the Mediterranean
TOC	Total organic carbon
VDS	Validation data set
WFD	Water Framework Directive

Appendix A

Appendix A.1. Materials and Methods

To exemplify the implementation of foraminiferal indices within transitional environments, a systematic review was conducted and reported according to PRISMA 2020 guidelines.

The eligibility criteria were as follows: Only original peer-reviewed research papers available in full-text pdf format online and presented first-hand research were used; any reviews and commentaries were omitted. Additionally, any papers in which the search term *foraminifera* or *foraminiferal* appeared only in the references were also not included.

The database used was Clarivate Analytics's Web of Science (WoS), an international research repository. The database covers a large quantity of mostly high-impact, English-language publications and is regarded as the world's leading citation search and analytical information platform [60,144].

The search was not constrained by year and followed a structure similar to that applied in Sousa et al. 2020. The Boolean operators "AND" and "OR" as well as the following search terms were used to collect papers that specifically dealt with the ecological evaluation of transitional waters: ("*biotic assessment*" OR "*biotic index**" OR "*biological indices*" OR "*ecological index**" OR "*ecological indices*" OR "*foraminifera-based index*" OR "*foraminiferal index**" OR "*foraminifera-based indices*") AND ("*ecological quality*" OR "*ecological status*" OR "*ecological quality status*" OR "*ecosystem health*") AND ("*foraminifera**" OR "*foraminiferal*" OR "*foram*" or "*forams*") AND ("*transitional waters*" OR "*naturally stressed environments*" OR "*fjord*" OR "*fjords*" "*estuaries*" OR "*brackish*" OR "*coastal*" OR "*lagoon*" OR "*Delta*" OR "*Ria*"). The addition of quotation marks and asterisks (*) were used on certain search terms such as *foraminifera* to ensure the search terms are returned exactly as written and increase the precision of the search.

The resulting publications were manually filtered and screened by a single author, and once compiled, the table was checked and approved by the remaining authors. Publications found not to focus on foraminifera-based methods were excluded, as well as those concerning the study of waters that do not fall under the definition of transitional such as coral reefs (unless specified to be nearshore).

Information including the (i) the stressor/environmental parameter studied, (ii) the source of the stressor, (iii) the study site or location, (iv) the type of assemblage (living, fossil, or recent but not living), (v) the index used, and (vi) reference (first author and year) was compiled into Table A1.

We later concluded that the table lacked some key studies. This was due to the WoS repository containing papers primarily from high-impact journals only. As a result, the table was supplemented with articles referenced elsewhere in this publication, which included the search criteria in the title or topic.

The information extracted from each paper concerns only the methods used in each study rather than the outcome observed, and hence, is unlikely to be affected by bias as results, conclusions, and efficacy are not discussed.

The tabulation of the information extracted from each publication was kept as simple as possible and repeated where possible in the same order, so it is easier to identify studies that have used the same index.

Appendix A.2. Results

Using these search strategies, a final yield of 41 articles, the oldest being published in 2009 and the latest 2021. After filtering, seven publications were removed due to not meeting the specified criteria, for example, “Is untreated sewage impacting coral reefs of Cay Caulker, Belize” by Emrich et al. 2017 was excluded due to its focus on coral reefs, which do not fall strictly under the definition of transitional waters.

An additional four publications that met the specific search criteria were included from those referenced elsewhere in this paper (these have been marked with an asterisk next to the reference).

The table has been arranged by grouping firstly those that employ diversity indices, then sensitivity, and lastly, test abnormality or accumulation rates.

Table A1. Examples of application of benthic foraminifera as BQEs in environmental studies over the last 20 years across a wide range of environmental stress and in various types of transitional and coastal waters.

Stressor/Environmental Parameters	Source of Stress	Study Site	Assemblage Type	Index Used	Reference
Salinity, oxygen, turbulence, and organic matter inputs	Natural environmental variation	Po coastal plain, Italy	Fossil	Diversity Index Exp (H'bc)	Barbieri et al. 2020 [145]
Organic enrichment	Various sources	Mediterranean Sea Coast, France	Living	Diversity indices, wall structure proportion, and sp. tolerance sensitivity	Barras et al. 2014 [50]
Chemical pollution: polychlorobiphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), barium (Ba), and mercury (Hg)	industries, petrochemical pole	Augusta Harbor, Eastern Sicily, Italy	Living, dead (1960s)	The Foraminiferal Size Index (FSI) and the Lost Species Index (LSI), Sp. richness, diversity, composition, foraminiferal number (FN), and H' index	Bergamin and Romano et al. 2016 [146]
Oxygen depletion, grain size, C/N, TOC, and total nitrogen	Industries and urbanization	Norwegian Skagerrak coastal waters and Fjords, NE North Sea	Living, dead	Exp (H'bc) Diversity Index	Bouchet et al. 2012 [74]
Variety of pollution types	Aquaculture/Sewage	Lagoons and lakes, Italy	Living	Exp (H'bc) Diversity Index	Bouchet et al. 2018b [49]
Metal pollution, organic enrichment, pollution load index, sediment pollution index	Industries, urbanization, natural variation	Marambaia Cove of the Sepetiba Bay, SE Brazil	Living, fossil	Foram Stress Index (FSI) and Exp (H'bc) Diversity Index	Castelo et al. 2021 [76]
Palaeo-environmental hydrological regime	Natural regime variation	Po Delta, Italy	Living, fossil	Epifaunal vs. Infaunal, Shannon–Wiener Index (H'log 2) Index, Fisher evenness and dominance, indicative groups	Dasgupta et al. 2020 [146]
Organic enrichment, metal pollution	Urbanization, industries, shipping activities, domestic pollution	Santos Estuary, Brazil	Living, fossil	Diversity Index Exp(H'bc) based, abundance, diversity, normalized ecological quality ratio (nEQR)	De Jesus et al. 2020 [25]
POP and metal pollution	Industries, shipping activities	Hammerfest harbor, Norway	Living	Exp (H'bc) Diversity Index	Dijkstra et al. 2017 * [61]
Organic enrichment, metal pollution	Urbanization, industries	Oslofjord, Norway	Fossil, dead	Shannon–Wiener Index (H'log 2) and the Hurlbert's Index (ES100), and cluster analyses	Dolven et al. 2013 * [19]

Table A1. Cont.

Stressor/Environmental Parameters	Source of Stress	Study Site	Assemblage Type	Index Used	Reference
Oxygen depletion, salinity fluctuation, temperature, metal pollution	Various Sources	Høgsfjord and Lysefjord, Norway	Living, fossil	Shannon–Wiener Index ($H' \log 2$) and the Hurlbert's Index (ES100)	Duffield et al. 2017 * [147]
Organic enrichment, metal pollution, phosphates	Industries, urbanization	Gulf of Gabes, Tunisia	Living	“Long versus short life span” index (ILS) and the modified FoRAM-Index (FI'), benthic foraminiferal assemblage composition and diversity, e.g., the Tolerant Species Index (TSstd), the Foram Stress Index (FSI), the Diversity Index Exp($H'bc$) and the Newly Developed Foram-AZTI Marine Biotic Index (Foram-AMBI)	El Kateb et al. 2020 [48]
Grain size, metal pollution, organic enrichment	Industries, metallurgic factories, shipping activities, urbanization	Hauts de France, English Channel, France	Living	Diversity Exp ($H'bc$), foraminiferal density (FD), abundance, and indicative groups	Francescangeli et al. 2020 [78]
Oxygen depletion	Urbanization, sewage	Bay of Trieste, Italy	Living	Exp ($H'bc$) Diversity Index	Melis et al. 2019 [80]
organic enrichment, metal pollution	Industries, urbanization	Idefjord, Sweden/Norway	Living, fossil	Fisher Alpha Diversity Index, individual rarefaction ES(100), dominance indices (based on Simpsons Index)	Polovodova Asteman et al. 2015 * [24]
Chemical pollutants, Oxygen depletion, eutrophication, acidification	Various sources	Bellingham Bay, Washington State, USA	Contemporary	The Shannon Index (H'), Diversity Index	Nesbitt et al. 2014 [148]
Oxygen depletion, pH	Industrial effluents	Uppanar Estuary, Tamil Nadu Coast, India	Living	FoRAMFoRAM-Index (FI) ranging, the Foram Stress Index (FSI), Ammonia-Elphidium Index (AEI), indicative groups, abundance, and diversity, species richness	Nagendra and Reddy et al. 2019 [149]
pH, temperature	Urbanization, tourism	Zanzibar Archipelago	Living	The FoRAMFoRAM-Index and species richness patterns, Fisher α ,	Thissen and Langer et al. 2017 [150]
Organic enrichment	Industries, urbanization	Skagerrak coast, Norway	Living, fossil	Foram-AMBI Sensitivity/Tolerance Index with Shannon's diversity ($H' \log 2$)	Alve et al. 2019 [75]
Organic enrichment	Cultural eutrophication (Various anthropogenic sources)	Northeast Atlantic and Arctic fjords	Living, fossil	Foram-AMBI Sensitivity/Tolerance Index with Shannon's diversity ($H' \log 2$)	Alve et al. 2016 [94]
Organic enrichment, potentially toxic elements, oil	Industries, urbanization	Guanabara Bay, Brazil	Living	Ammonia-Elphidium Index, Foram Stress Index	Alves Martins et al. 2020 [151]
Organic enrichment	Oyster Farming	Transitional Waters, English Channel/ European Atlantic coast and the Mediterranean Sea	Living	Foram-AMBI Sensitivity/Tolerance Index	Bouchet et al. 2021 [82]
Dissolved oxygen, organic enrichment, grain size, total nitrogen, pigments, and depth below threshold	Various sources	11 Fjords on the Skagerrak Coast, SE Norway	Living	Cluster/indicative species groups, comparison to macrofaunal changes	Bouchet et al. 2018 [81]

Table A1. Cont.

Stressor/Environmental Parameters	Source of Stress	Study Site	Assemblage Type	Index Used	Reference
Grain size (pollutant proxy)	Industries, urbanization, docks, natural variation	3 Sardinian Coastal areas	Living	Species' richness, foraminiferal density, and the Fisher- α Diversity Index	Buosi et al. 2013 [152]
Organic enrichment, phosphates	Aquaculture (fish farming)	Monastir Bay, Tunisia	Living	AZTI Marine Biotic Index, Foram-AMBI	Damak et al. 2020 [153]
Organic enrichment, metal pollution	Domestic and industrial effluents, aquaculture, tourism	Saronikos Gulf, Greece	Living	Foram Stress Index (FSI), tolerance/sensitivity	Dimiza et al. 2016 [88]
Low oxygen, nutrient enrichment (eutrophication), run-off	Various sources	Society Archipelago, French Polynesia	Contemporary	Species composition, species richness, patterns of distribution, abundance, indicative groups, Fisher α , FoRAM-Index (FI)	Fajemila et al. 2015 [154]
Organic enrichment, metal pollution	Various sources	The Gulf of Manfredonia, southern Adriatic Sea	Living	Foram-AMBI Sensitivity Index and Foram Stress Index (FSI)	Fossile et al. 2021 [155]
Organic enrichment, metal pollution	Industries	Boulogne sur Mer Harbor, France	Fossil	Cluster/indicative species groups	Francescangeli et al. 2016 [22]
increased nutrient and sediment inputs	Urbanization, tourism	Great Barrier Reef, Australia	Living, fossil	Functional groups, Foraminifera in Reef Assessment and Monitoring (FoRAM) Index (FI)	Johnson et al. 2019 [156]
Organic enrichment, metal pollution	Aquaculture (fish farming)	Øksfjord, Norway	Living, fossil	Norwegian Quality Index (fNQI), AZTI's Marine Biotic Index (fAMBI), fHlog2, ES100, absolute abundances and relative abundances of ecological groups (EGs)	Klootwijk et al. 2021 [26]
Metal pollution, oxygen depletion	Urbanization, industries, shipping activities, domestic pollution	Strait of Malacca, Malaysia	Living	Foraminifera Stress Index (FSI)	Minhat et al. 2020 [157]
Organic enrichment, nitrogen, carbonate content, phosphorus, mineralogy, grain size	Urbanization, tourism	Abrolhos, Southwest Atlantic, eastern Brazil	Living	Functional Groups for FoRAMFoRAM-Index (FI)	Oliveira-Silva et al. 2012 [158]
Organic enrichment	Urbanization, sewage	Levantine basin, Mediterranean	Living	TSI-Med and Foram-AMBI	Parent et al. 2021 [99]
Organic enrichment, metal pollution	Trawling, fishing activities, urbanization	Kosterfjord, Sweden	Living, dead	Foram-AMBI and NQIforam Indices	Polovodova Asteman et al. 2021 [45]
Hypoxia, metal pollution	Urbanization, industries	Western Coastal Area of Jakarta Bay	Living, fossil	The Ammonia-Elphidium Index, abnormal morphology	Rositasari et al. 2018 [159]
Metal pollution	Thermal sources and agricultural activities in	Gulf of Edremit, northeastern Aegean Sea, Turkey	Living, fossil	Abnormal morphology, species diversity	Yümün and Önce et al. 2017 [160]
Metal pollution, Organic enrichment, Oxygen depletion	Shipping activities, industries	Horten Inner Harbor, Norway	Living, fossil	Benthic foraminiferal accumulation rate (BFAR)	Hess et al. 2020 [20]

Table A2. Two paleo-EcoQS reconstructions based on diversity index Exp ($H'bc$) applied to the same foraminiferal assemblage data set from site I02-10B in the outer part of the Idefjord [23]. EcoQS threshold values (displayed below) for reconstruction EcoQS 1 are taken from Bouchet et al. [49], and for EcoQS 2 from Bouchet et al. [74], respectively. Years marked with asterisk (*) are interpolated ages based on the average sedimentation rate. EcoQS threshold values (found below) for EcoQS 1 from Bouchet et al. [49], and for EcoQS 2 from Bouchet et al. [74].

Depth	EcoQs ¹		EcoQs ²		Year
0–1	7.71		7.71		2002,0
2–3	4.1		4.1		1998,0
4–5	4.58		4.58		1994,1
6–7	5.5		5.5		1990,1
8–9	4.71		4.71		1986,1
10–11	4.72		4.72		1982,2
12–13	4.96		4.96		1978,2
14–15	6.73		6.73		1974,2
16–17	6.27		6.27		1970,3
18–19	5.71		5.71		1966,3
20–21	4.59		4.59		1962,3
22–23	6.37		6.37		1958,3
24–25	6.12		6.12		1951,2
26–27	6.52		6.52		1944,1
28–29	7.52		7.52		1936,9
30–31	7.45		7.45		1924,4 *
32–33	9.15		9.15		1911,9 *
34–35	9.06		9.06		1899,4 *
36–37	9.59		9.59		1886,9 *
38–39	6.4		6.4		1874,4 *
40–41	8.05		8.05		1861,9 *
42–44	8.51		8.51		1849,4 *
44–45	5.44		5.44		1836,9 *
46–47.7	7.4		7.4		1824,4 *
	High	Good	Moderate	Bad	Poor
EcoQS 1	>15	11–15	7–11	3–7	<3
EcoQS 2	>10	7.5–10	5–7.5	2.5–5	<2.5

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