Benthic foraminifera provide a promising tool for ecological quality assessment of marine waters

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A B S T R A C T

This study defines criteria for the use of benthic foraminifera (protists) as a quick and efficient bio-monitoring tool to implement marine legislation. Various sampling and preparation procedures are investigated in an attempt to find the optimal methodology for environmental monitoring using soft-sediment foraminifera with the objective of assessing ecological quality status (EcoQS). Twenty-seven sampling stations in silled basins along the Norwegian Skagerrak coast, NE North Sea, are investigated for environmental parameters and living (stained, including soft-shelled forms) and dead benthic foraminifera. Diversity, expressed as the effective number of species \( \exp \left( H_{\text{b}} \right) \) and community composition are used to evaluate EcoQS using living (stained) benthic foraminifera. Correlation studies show that bottom-water dissolved oxygen concentration at the time of sampling \([O_2]_{\text{b,0}}\) is the main environmental factor controlling variation in diversity. Variables such as grain size, C/N, TOC and TN are less important. Correlation between foraminiferal diversity and \([O_2]_{\text{b,0}}\), as well as correlation between community data and \([O_2]_{\text{b,0}}\), suggest that benthic foraminifera represent an efficient bio-monitoring tool to evaluate EcoQS. A clear pattern from “bad” to “high” EcoQS is established using the strong link between the benthic foraminiferal diversity and the bottom-water oxygen gradient. The study shows that EcoQS can be evaluated quickly and accurately using the following method: sample the top 1 cm of sediment, dry-pick about 250 living (stained) individuals of \(>125 \mu \text{m} \) sized fossilisable (i.e. most of those remaining subsequent to drying) foraminifera from each of three replicates. \( \exp \left( H_{\text{b}} \right) \) based on living benthic foraminifera is a promising tool to assess EcoQS. For fossil assemblages, \( \exp \left( H_{\text{b}} \right) \) has potential for evaluating temporal changes in in situ PaleoEcoQS and for defining reference conditions from pre-impacted times.

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1. Introduction

Since the end of the 19th century, human activities have greatly altered the ecological quality of many coastal areas. In response to concerns about environmental degradation, many nations have enacted legislation such as, for instance, the Clean Water Act (CWA) or Oceans Act in USA, Australia or Canada, the Water Framework Directive (WFD, 2000/60/EC) and the Marine Strategic Framework Directive (MSFD, 2008/56/EC) in Europe, to address anthropogenic pollution. The CWA established the goals of eliminating releases of large amounts of toxic substances into water, eliminating additional water pollution, and ensuring that surface waters would meet standards necessary for human sports and recreation. The WFD established a basis for the protection of ground, continental, transitional, and coastal waters, emphasizing the need to monitor and assess the ecological quality status (EcoQS) of those ecosystems. According to the WFD, European countries must restore the environment to a “good” EcoQS by 2015. The MSFD aims to “achieve good environmental status of the EU’s marine waters by 2020 and to protect the resource base upon which marine-related economic and social activities depend” (www.ee.europa.eu/environment/water/marine.htm). The MSFD highlights the need for the scientific community to increase the scientific knowledge of the elements that define the state of the marine environment. The implementation of these marine legislations worldwide is generating a fruitful debate amongst marine scientists about how to define and implement efficient and reliable bio-assessment tools. In coastal and transitional waters, many biological elements have been considered as assessment tools, including macroalgae, phytoplankton and sea-grasses (Ballesteros et al., 2007), fish (Coates et al., 2007), and benthic macrofauna (see review in Pinto et al., 2009). Monitoring programs in soft-bottom

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coastal and transitional waters have highlighted the difficulty of defining reference conditions (e.g. Bigot et al., 2008; Blanchet et al., 2008; Elliott and Quintino, 2007; Nielsen et al., 2003). According to the WFD, the reference conditions (also called “high” status) are defined as “… the biological, chemical and morphological conditions associated with no or very low human pressure. These reference conditions are type-specific, so they are different for different types of rivers, lakes or coastal waters so as to take into account the broad diversity of ecological regions in Europe. Assessment of quality is based on the extent of deviation from these reference conditions, following the definitions in the Directive” (www.ec.europa.eu/environment/water/marine.htm). It means that a low-diversity assemblage which would be classified as reflecting “poor status” at one site may actually be the reference condition (and should be classified as “high status”) at another site. A recurring problem is that, unfortunately, for most areas, there are no data available from pre-impact times to use as reference conditions, and with which to compare the current EcoQS. Reference conditions are therefore defined using expert judgment. Even though expert judgment may have some advantages, a more objective approach is needed.

A component of the meiofauna, benthic foraminifera (protists), has been widely used as bio-indicators of several pollution sources in coastal and transitional waters (e.g. Alve, 1995; Martínez-Colón et al., 2009; Nigam et al., 2006; Scott et al., 2001) such as aquaculture (e.g. Bouchet et al., 2007), oil spills (e.g. Morvan et al., 2004), heavy metals (e.g. Armynot du Châtele et al., 2004), and urban sewage (e.g. Burone et al., 2006). Benthic foraminifera occur in almost all marine environments, and with much higher abundances than macrofauna, which is traditionally used in environmental monitoring. Foraminifera can provide quantitative data with a small (a few cm³) volume of sediment. Considering their shorter generation time, foraminifera have the potential to respond faster than macrofauna to changes in the environmental conditions. *Stainforthia fusiformis*, one of the most abundant species in Northern European fjordic systems, is, for instance, able to reproduce within less than a month in response to food input (Gustafsson and Nordberg, 2001). In the deep sea, benthic foraminifera react within 1–2 months to the deposition of phytodetritus with dramatic changes in the assemblage composition (Gooday, 1988). Benthic foraminiferal assemblages have also been able to recover their composition and abundance within a month after a short-term hypoxic period in intertidal mudflats in S-Western France (Bouchet et al., 2007). Benthic foraminifera leave an easily accessible and abundant fossil record, which allows reconstruction of the characteristics and timing of historical environmental variations (e.g. Alve, 1991a; Debenay and Fernandez, 2009; Hayward et al., 2004). Consequently, it is possible to trace the record of human-induced disturbances over decades or centuries. Indeed, in a pilot study, Alve et al. (2009) suggested that in situ reference conditions can be established using fossil benthic foraminiferal assemblages from dated sediment cores and that they can be used, based on quantitative ecological considerations, to characterise local changes in EcoQS from pre-impact to present times. For example, by comparing the “background” fossil foraminiferal assemblages to the modern living foraminiferal assemblages at the same site, it would be possible to determine if a site is naturally anoxic or has become anoxic with recent human influence. This kind of temporal, in situ monitoring is not possible with soft-bottom sediment macrofauna because they do not leave abundant or, for most species, any fossil records. Benthic foraminifera may thus provide a powerful tool for defining habitat-specific, in situ reference conditions for soft-bottom coastal and transitional waters. At the moment, there is no consensus as to which sampling and laboratory methodologies (e.g. sieving mesh size and use of dead or living assemblages) are optimal for environmental monitoring. This hampers both comparisons between studies and proposals for using foraminifera as a tool for stakeholders. However, currently, an expert group, the FOraMiniferal Bio-MONitoring (FOBIMO)-group (Schönfeld et al., 2011), is working to get consensus on a standardised methodology.

The present study aims to address parts of these problems by focusing on aspects concerning species assemblages and diversity indices which are commonly used for defining EcoQS in environmental monitoring studies. Internationally, there is an increasing demand for quicker and more cost-efficient methods (as compared to the traditional ones) to describe ecological status in marine waters. Consequently, an important objective is to optimise the methods to find the best compromise between effort and accuracy. Key questions for defining the EcoQS using benthic foraminiferal species diversity include the following. What thickness of sediment should be analysed? Should the >63 μm-fraction be used or will the >125 μm-fraction provide a similar result? Is significant information lost if only fossilisable species are considered? Do living and dead assemblages reflect similar EcoQS? How many replicates are required?

The study sites are silled basins along the Norwegian coast of the Skagerrak (NE North Sea) with relatively stable salinity and temperature conditions but otherwise different environmental properties (primarily bottom water dissolved oxygen concentration). To evaluate the potential of benthic foraminifera as a bio-assessment tool, EcoQS of sampling sites are determined using living (stained) benthic foraminifera data compared with bottom water dissolved oxygen concentration. Dissolved oxygen is chosen as it is one of the most important environmental gradients in these systems and because it is frequently impacted by human activity. The study is part of a comprehensive project (PES) which includes both foraminifera and macrofauna collected at the same site at the same time. Macrofauna results and comparisons between foraminifera and macrofauna along the same environmental gradients will be presented in forthcoming papers.

2. Material and methods

2.1. Study area and sampling sites

In August 2008, 27 stations in silled basins along the Norwegian Skagerrak coast, NE North Sea (Fig. 1), were sampled for biological and environmental analyses. Stations were selected to provide an oxygen gradient with stable temperature (5–6 °C; occasionally 8–9 °C at shallow, 40–50 m depth) and salinity (33–34 conditions (see details in Table 1) using information from previous studies (Buhl-Mortensen et al., 2009) and unpublished data from the Norwegian Institute for Water Research (NIVA) and Institute of Marine Research (IMR)). The study basins commonly experience deep-water renewals during winter.

2.2. Field sampling

At each station, sediment samples for biogeochemical, sedimentological, benthic foraminiferal, and macrofaunal analyses, and bottom water samples for dissolved oxygen analyses, were collected. Four sediment cores (8 cm diameter) were collected at each of the 27 stations with a Gemini gravity corer (a modified version of the Niemístö corer (Niemistö, 1974); three replicate cores were used for benthic foraminifera and one for organic carbon and total nitrogen analyses. Once on deck, the bottom water from just above the sediment-water interface in two cores was immediately transferred to Winkler bottles, sealed, and kept dark and cold (−7 °C) for subsequent dissolved oxygen analysis. All sediment cores were sectioned on board and, for the present study, the top 0–1 and 1–2 cm slices were analysed. Sediment samples for total organic carbon and
total nitrogen analyses were frozen immediately after sectioning. Foraminiferal samples were preserved in rose Bengal-stained 70% ethanol (1 g L\(^{-1}\)) to avoid protoplasm degradation and to distinguish living (stained) from dead specimens (discussion in Murray and Bowser, 2000). At each station, one sub-sample of the top 1 cm from a Van Veen grab was collected for grain size analyses.

2.3. Laboratory analyses

Bottom-water dissolved oxygen concentrations were analysed using Winkler titration. Sediments were freeze-dried prior to sedimentological and geochemical analyses. For grain size analyses, the dried sediment was weighed, soaked in tap water, and washed on a 63 μm Endecote-sieve; the >63 μm fraction was dried and weighed and the <63 μm-fraction was calculated based on the dry weights. Total organic carbon and total nitrogen were analysed using a CHN analyser (Carlo Erba Elemental Analyzer 1106).

Foraminiferal samples were washed through 500 and 63 μm mesh sieves, and the 63–500 μm fraction was split using a modified Elmgren wet splitter (Elmgren, 1973). One eighth of each sample was re-sieved and all live (stained) foraminifera in the 63–125 and 125–500 μm fractions were identified to species level and counted in the wet state. The number of individuals >500 μm relative to smaller ones was trivial (<0.1%) so including them would not influence the results. The samples were analysed wet rather than dry as this allows preservation of all species (including fragile organic-walled and loosely cemented agglutinated foraminifera). It also makes it easier to discriminate stained from unstained specimens. In this study, the wet-picked, >63 μm, living (stained) foraminifera in the surface 0–2 cm sediment, is called the “complete living assemblage”. Dead assemblage data were obtained by merging the counted 0–1 and 1–2 cm sub-samples and re-washing them on a 63-μm sieve to remove surplus stain. They were then dried at 40 °C and about 300 dead (unstained) tests were dry-picked, mounted on microslides, identified and counted. Distinctions between “fossilizable” and “non-fossilizable” foraminiferal species are based on the species’ presence in dried sediment samples from >10 cm core depth in one or more of 305 samples from the Norwegian Skagerrak coast (Alve, 1991b, 1996, 2000; Alve et al., 2009).

2.4. Data analysis

2.4.1. Diversity indices

The diversity indices Shannon-Wiener index \(H'_s, \log_2\) (Shannon and Weaver, 1963) and Hurlbert index \(E_{S100}\) (Hurlbert, 1971) are used by the Norwegian Pollution Control Authority as metrics to characterise EcoQS along the Norwegian coast. Since \(H'_s\) and \(E_{S100}\) are highly correlated in our data \((r^2 = 0.97, p < 0.001)\), we concentrate on \(H'_s\).

\(H'_s\) is biased when there are unobserved species in the community, a common problem with under-sampling (Chao and Shen, 2003). Chao and Shen (2003) introduce a bias-corrected version of Shannon’s index \((H'_{bc})\), which has little bias (Beck and Schwanghart, 2010), and which we use in this paper. Shannon’s index is an entropy rather than a diversity. The entropy gives the average uncertainty of the identity of an individual picked from the community, not the number of species in the community (e.g. Hayek and Buzas, 1997; Jost, 2006). It can be converted to true diversity, the effective number of species, with the exponential function \((N_1 = \exp(H'_{bc}), \text{ Hill, 1973})\). \(\exp(H'_{bc})\) gives the number of species that would, if each were equally common, produce the same \(H'_{bc}\) as the sample. Given a community sample of three species represented by 100, 50, and 100 individuals, \(H'_{bc} = 1.05\) and \(\exp(H'_{bc}) = 2.87\). Thus 2.87 species of equal abundance gives an \(H'_{bc}\) of 1.05. To determine how many foraminifera need to be counted to get a stable estimate of diversity, we resampled each sample 100 times, with replacement, to different sized subsamples and calculated the coefficient of error of the effective number of species. Correlations between the bias-corrected exponent of Shannon of different fractions of the foraminiferal community, as well as of their microhabitat and the environmental variables, are calculated. All data represent the pooled counts from three replicates per station (rather than the average).

Benthic foraminiferal data are used to evaluate EcoQS at the sampling stations. As no criteria exist for determining EcoQS using benthic foraminifera, it has to be defined. In this study, we set the highest expected \(\exp(H'_{bc})\) to 25 effective species (0–2 cm, >63 μm, wet-picked, living assemblages, Table 2), a value slightly higher than the highest observed value of 22 effective species. Any
Table 1
Characteristics of sampling stations (nd: no data): water depth (m), sill depth (m, Norwegian Hydrographic Service), bottom-water dissolved oxygen (minimum observed value over the 2006–2008 period, min[O₂,], mL O₂ L⁻¹), bottom-water dissolved O₂-concentration at the time of sampling ([O₂], mL O₂ L⁻¹), total organic carbon (TOC, %), total nitrogen (TN, %), C/N ratio, living and dead foraminiferal abundances (0–2 cm, >63 µm, ind. cm⁻³), diversity of living foraminiferal assemblages (H', ES(100) and exp(H'bc) for complete living assemblages and exp(H'bc) for 0–1, >125 µm, fossilisable assemblages) and EcoQS. Colours reflect EcoQS; see Table 2 for location of areas, see Fig. 1.

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<td>3.98</td>
<td>2</td>
<td>2.3</td>
<td>0.2</td>
<td>14.3</td>
<td>57</td>
<td>58</td>
<td>4.1</td>
<td>23</td>
</tr>
<tr>
<td>Indre Hvaler</td>
<td>62</td>
<td></td>
<td>2.42</td>
<td>2.42</td>
<td>2</td>
<td>2.2</td>
<td>0.2</td>
<td>13.2</td>
<td>87</td>
<td>87</td>
<td>3.6</td>
<td>23</td>
</tr>
<tr>
<td>Kristiansandfjord</td>
<td>23</td>
<td></td>
<td>nd</td>
<td>nd</td>
<td>42</td>
<td>0.8</td>
<td>0.1</td>
<td>14.6</td>
<td>30</td>
<td>90</td>
<td>3.6</td>
<td>20</td>
</tr>
<tr>
<td>Kristiansandfjord</td>
<td>31</td>
<td></td>
<td>nd</td>
<td>nd</td>
<td>48</td>
<td>2.7</td>
<td>0.2</td>
<td>14.6</td>
<td>20</td>
<td>75</td>
<td>2.5</td>
<td>12</td>
</tr>
</tbody>
</table>

Assemblages with more than 25 effective species would be set to 25. We use zero effective species to represent an azoic sample. Within this range (0–25 effective species) we follow Rosenberg et al. (2004) and divide this range into the five equal-sized ecological status classes (unacceptable statuses: “bad”, “poor”, “moderate”, acceptable statuses: “good” and “high”) required by the WFD classification (Table 2). The top two, and the bottom three, classes can be combined to give the MSFD classes “good” and “bad”. Accuracy of a less time-consuming method using diversity derived from >125 µm, fossilisable, living assemblages in the surface 0–1 cm of sediment has been tested. Criteria are presented in Table 2.

Table 2
Criteria for determining EcoQS using living benthic foraminifera.

<table>
<thead>
<tr>
<th>Criteria for determining EcoQS using living benthic foraminifera</th>
<th>Bad</th>
<th>Poor</th>
<th>Moderate</th>
<th>Good</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>EcoQS derived from 0-2 cm, &gt;63 µm, living wet-picked assemblages</td>
<td>&lt;5</td>
<td>5-10</td>
<td>10-15</td>
<td>15-20</td>
<td>&gt;20</td>
</tr>
<tr>
<td>EcoQS derived from 0-1 cm, &gt;125 µm, living dry-picked assemblages</td>
<td>&lt;2.5</td>
<td>2.5-5</td>
<td>5.7-5</td>
<td>7.5-10</td>
<td>&gt;10</td>
</tr>
</tbody>
</table>
To estimate the probability of assigning an assemblage to an incorrect EcoQS class, and how this varies with the number of replicates, we use the methods developed by Kelly et al. (2009). We find the relationship between the standard deviation and mean of the effective number of species in the three replicates (Fig. 2). Using this relationship, we estimate the standard error expected for any value of the mean given one, three or six replicates, and therefore the distribution of uncertainty for each mean with the given number of replicates. We can then estimate the probability that any sample will be misclassified. See Kelly et al. (2009) for further details.

2.4.2. Community analysis

Redundancy analysis was used to find the proportion of the variance of the different fractions of the square-root transformed community data explained by the environmental variables. Procrustes analysis (Peres-Neto and Jackson, 2001) was used to compare principal component analyses of the different fractions of the foraminiferal community. We report the $m^2$ statistics, which is analogous with the $r^2$ of a correlation.

All calculations were performed using the statistical language R version 2.11.1 (R Development Core Team, 2010). Ordinations were run with the vegan library version 1.17-2 (Oksanen et al., 2010). Bias-corrected Shannon Index was calculated with the Entropy library version 1.1.5 (Hauser and Strimmer, 2010).

3. Results

The absolute abundance of living (stained) benthic foraminifera (>63 μm; all taxa included) in the surface 0–2 cm of sediment ranged from 6 to 201 ind. cm$^{-3}$ (wet sediment) (Table 1) with an average of $58 \pm 41$ ind. cm$^{-3}$. Of these individuals, 76% occurred in the top 0–1 cm. In total, 116 living (stained) benthic foraminiferal species were recorded of which 88 (or 76%) are potentially fossilizable and these species account for 89% of the living foraminiferal abundance (>63 μm, 0–1 cm). For the dead assemblages (0–2 cm, >63 μm), the absolute abundance ranged from 3 to 514 ind. cm$^{-3}$ (Table 1).

3.1. Living foraminiferal diversity and bottom-water dissolved oxygen

The coefficient of error of $exp(H_{bc})$ is quite high if less than 50 individuals are counted and identified (Fig. 3). There is a progressive decrease in the error until about 250 individuals have been counted, after which the error is low and relatively stable.

Bottom-water oxygen concentration at the time of sampling ([O$_2$]$_{obs}$) is a good predictor (Table 3) of the effective number of species, $exp(H_{bc})$, whereas grain size, TOC, TN and C/N are weaker. Following Diaz and Rosenberg’s (2008) definition for oxygen conditions, anoxia ($0.0$–$0.5$ mL O$_2$ L$^{-1}$) characterizes stations R180, R160, R140, R120 and 102, hypoxia ($0.5$–$2.0$ mL O$_2$ L$^{-1}$) stations F90, R100, F70 and R80 ([O$_2$]$_{obs}$, Table 1). All the other stations have normoxic (>2.0 mL O$_2$ L$^{-1}$) conditions. There is a strong correlation between $exp(H_{bc})$ between the complete living foraminiferal assemblages diversity (0–2 cm, >63 μm) and [O$_2$]$_{obs}$ ($r=0.79$, Table 3). Additionally, [O$_2$]$_{obs}$ explains a large proportion of the variance in the complete living foraminiferal community data.

3.2. Comparison of foraminiferal sampling and processing methods

The confidence with which samples can be assigned to each status class can be represented as a bell-shaped curve with the maximum at the centre of the relevant status class (Fig. 4A). The tails of these curves overlap, so an observed diversity could potentially belong to several EcoQS classes. As an example, if the diversity was 17.5, the confidence of the condition of the station being “good status” is about 65% (based on a single replicate), and there is an approximately 18% chance that the “true” condition is “high” or “moderate”. If there are 3 replicates, then there is about 90% confidence that the true condition is “good”, and the chance of the true condition being “high” or “moderate” being approximately 7% (Fig. 4B). The risk of misclassification, i.e. of placing a site in any status class other than the true one, decreases with distance from class boundaries, with the lowest risk of misclassification occurring at the centre of a status class. At the class boundaries, it is equally likely that the true EcoQS is in either the lower or the higher status class. The risk of misclassification decreases as the number of replicates increases (Fig. 4C). In the present example, there is a risk of
misclassification of about 35% for a diversity of 17.5, taking a single replicate. The risk drops to approximately 10% with 3 replicates, and is negligible with 6 replicates.

The correlation between living, >63 µm foraminiferal \( \exp(H'_{bc}) \) in the 0–1 cm and \([O_2]_{los}\) is stronger \( (r = 0.79, \text{Table 3}) \) than for that of the 1–2 cm assemblages \( (r = 0.67, \text{Table 3}) \). \( \exp(H'_{bc}) \) values of the assemblages in the different size fractions are also correlated with \([O_2]_{los}\) \( (r = 0.68 \text{ for the } >125 \mu m \text{ and } r = 0.68 \text{ for the } 63–125 \mu m, \text{Table 3}) \). Similar results are achieved using community composition instead of diversity.

The relationship between \( \exp(H'_{bc}) \) and \([O_2]_{los}\) is stronger for the fossilisable assemblages than for the non-fossilisable ones \( (r = 0.79 \text{ and } r = 0.42, \text{respectively, Table 3}) \). Correlation between the >125 µm fraction of live fossilisable assemblages in the 0–1 cm and \([O_2]_{los}\) is slightly weaker than for the complete living assemblages \( (r = 0.69 \text{ and } r = 0.79, \text{respectively, Table 3}) \).

Correlation between \( \exp(H'_{bc}) \) and \([O_2]_{los}\) is stronger for the complete living assemblages than for the dead assemblages \( (r = 0.79 \text{ and } r = 0.54, \text{respectively, Table 3}) \). The diversity in the complete living assemblages is better correlated to the diversity in the living large fossilisable assemblages than to the diversity in the dead assemblages \( (r = 0.86 \text{ and } r = 0.64, \text{respectively, Table 3}) \). Community data from the complete living assemblages and the dead assemblages, and the living fossilisable assemblages and the dead assemblages are also correlated \( (m^2 = 0.59 \text{ and } m^2 = 0.60, \text{respectively, Table 3}) \).

### 3.3. EcoQS based on foraminifera data

\( \exp(H'_{bc}) \) values vary from 1.2 at stations R180 and 6 to 22.0 at station IH30 (complete living assemblage, 0–2 cm, >63 µm, Table 1). Ecological quality status of anoxic stations is “bad” (Fig. 5). At hypoxic sites, stations are all ranked as “bad” EcoQS except station F70 which is “poor”. At normoxic stations, EcoQS vary from “bad” at stations 6, G60, G69 and G50 to high at stations 106 and IH30. Over the last 2 years, stations 6, G60, G69 and G50 experienced periods of severe hypoxia \( \text{Min}[O_2]_{2 \text{years}}, \text{Table 1}) \).

In this study, EcoQS derived from the diversity of the complete living assemblages are “non acceptable” (bad-poor-moderate) at 19 stations, and “acceptable” (good-high) at 8 stations (Table 1). Using the diversity of the 0–1 cm, >125 µm, dry-picked, living
Table 3
Correlation of diversity (\(\exp(F_{\text{sh}})\)) against \([O_2]_{\text{bas}}\) for different live fractions in different sediment layers and for dead foraminifera. Correlation of diversity of the different subsets against diversity in the complete living assemblage. Proportion of the square rooted relative abundances explained by \([O_2]_{\text{bas}}\) in an RDA. Procrustes rotation m² of PCA of complete assemblage against different fractions. Complete assemblage = all live (stained) foraminifera >63 \(\mu\)m in size. All correlations are significant at \(p < 0.05\) (numbers in brackets are correlation or m² of dead foraminifera against fossilisable living foraminifera).

<table>
<thead>
<tr>
<th>Subset</th>
<th>Correlation diversity vs ([O_2]_{\text{bas}})</th>
<th>Correlation diversity complete living assemblage vs diversity of subsets</th>
<th>Proportion community variance explained by ([O_2]_{\text{bas}})</th>
<th>Procrustes m² against complete living assemblage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete living assemblage (specimens &gt; 63 (\mu)m, 0–2 cm)</td>
<td>0.79</td>
<td>1.00</td>
<td>0.28</td>
<td>1.00</td>
</tr>
<tr>
<td>Small living (63 (\mu)m &lt; Specimens &lt; 125 (\mu)m, 0–2 cm)</td>
<td>0.68</td>
<td>0.91</td>
<td>0.21</td>
<td>0.81</td>
</tr>
<tr>
<td>Large living (specimens &gt; 125 (\mu)m, 0–2 cm)</td>
<td>0.68</td>
<td>0.87</td>
<td>0.27</td>
<td>0.97</td>
</tr>
<tr>
<td>Shallow living (specimens &gt; 63 (\mu)m, 0–1 cm)</td>
<td>0.77</td>
<td>0.96</td>
<td>0.31</td>
<td>0.98</td>
</tr>
<tr>
<td>Deep living (specimens &gt; 63 (\mu)m, 1–2 cm)</td>
<td>0.67</td>
<td>0.85</td>
<td>0.19</td>
<td>0.76</td>
</tr>
<tr>
<td>Fossilisable (specimens &gt; 63 (\mu)m, 0–2 cm)</td>
<td>0.79</td>
<td>0.98</td>
<td>0.28</td>
<td>0.99</td>
</tr>
<tr>
<td>Not fossilisable living (specimens &gt; 63 (\mu)m, 0–2 cm)</td>
<td>0.42</td>
<td>0.60</td>
<td>0.11</td>
<td>0.45</td>
</tr>
<tr>
<td>Large fossilisable living (specimens &gt; 125 (\mu)m, 0–1 cm)</td>
<td>0.69</td>
<td>0.86</td>
<td>0.29</td>
<td>0.96</td>
</tr>
<tr>
<td>Dead (specimens &gt; 63 (\mu)m, 0–2 cm)</td>
<td>0.54</td>
<td>0.64 (0.69)</td>
<td>0.14</td>
<td>0.59 (0.60)</td>
</tr>
</tbody>
</table>

Fig. 5. \(\exp(F_{\text{sh}})\) of complete living (stained) assemblage (0–2 cm >63 \(\mu\)m, pooled replicates) and corresponding EcoQS against \([O_2]_{\text{bas}}\). Threshold between anoxic, hypoxic and normoxic bottom-water conditions are from Díaz and Rosenberg (2008).

assemblages, EcoQS are “non acceptable” at 15 stations, and “acceptable at 12 stations. Disagreements between the 2 methods occur at stations 200, F70, R60 and KDR.

4. Discussion

By applying the criteria for EcoQS set by the Norwegian Pollution Control Authority to micropalaeontological and geochemical data from near-shore sediment cores, Alve et al. (2009) showed the potential of using fossil (dead) benthic foraminifera to evaluate temporal changes in EcoQS from “reference” (background) to present-day conditions. Correlation between bottom-water oxygen concentration and faunal diversity in the present study takes this a substantial step further. To our knowledge, this is the first work using benthic foraminifera to show quantitative relationships between environmental variables and diversity in coastal areas, and to evaluate EcoQS.

4.1. Methodological considerations

In the literature, there is an obvious lack of standardisation of both sampling and processing methods in foraminiferal ecological studies (see points to consider in Murray, 2006). In scientific studies, the choice of methods depends on the nature of the study area and the ecological questions in focus. However, to promote benthic foraminifera as an efficient environmental monitoring tool to assess EcoQS, there is a need to test and identify methodologies which are optimal both from a scientific and a practical point of view. Key issues addressed in the present study include:

- Replication.
- Thickness of sediment layer analysed.
- Choice of size fraction.
- Analyses of wet (i.e. complete live (stained) assemblage) vs dry samples (i.e. fossilisable species).
- Analyses of live or dead assemblages.

4.1.1. Replication

Patchiness in distribution patterns of benthic foraminifera may occur both on 10-cm and 1-m sample spacing (e.g. Boltovskoy and Lena, 1969; Buzas et al., 2002). It is thus an issue to be considered in environmental studies. Replicate sampling aims to minimize this problem. However, for historical reasons, the use of replicates has not been common practice in benthic foraminiferal studies and in most of those which have included replicates, abundance patterns rather than diversity have been considered.

In this study, the risk of misclassification of the EcoQS is high if data are based on only one sample per station (Fig. 4). The degree of confidence is higher if three replicates per station are taken. Risk of misclassification if six replicates are considered is lower, but the improvement observed from three replicates is not as large as the improvement from one to three replicates. Results presented here provide a basis for sampling methods using benthic foraminifera in environmental monitoring, and suggest that three replicates are sufficient to determine a reliable EcoQS.

4.1.2. Thickness of sediment layer analysed

Benthic foraminifera may live several decimeters below the sediment-water interface (see review in Bouchet et al., 2009); how deep they live depends on local geochemical conditions and food availability (e.g. Corliss and Emerson, 1990; Gross, 2000; Jorissen et al., 1995; Linke and Lutze, 1993). To obtain the most representative view of the live (stained) benthic foraminiferal diversity at
4.1.3. Choice of size fraction

The choice of size fraction (e.g., >63, >125, >150 μm) in foraminiferal ecological studies has generated a number of publications. For example, Schröder et al. (1987), in their study of total assemblages (living + dead), showed that focusing on the coarser (>125 μm) rather than the finer (>63 μm) fraction may lead to a loss of indicator species and differences in species abundances. The impact of the choice of size fraction on diversity indices is poorly known. In our study, the diversity and assemblage composition of the >63 μm and the >125 μm fraction assemblages are significantly correlated, and the diversity of both fractions are correlated to bottom water [O\textsubscript{2}]\textsubscript{los}, with the >63 μm fraction giving a slightly better correlation. However, analysing just the coarse fraction is less time consuming, and requires less taxonomic skill than analysing the complete assemblage or the fine fraction. For many species, accurate identification of juveniles to species level may be difficult. This is important, as in addition to its accuracy, a bio-indicator should ideally be fast and easy to apply if it is to be widely adopted. Since the information lost by just using the coarse (>125 μm) fraction is small, this is an adequate choice for calculation of diversity and assemblage indices for monitoring EcoQS.

4.1.4. Analyses of wet vs dry samples

To obtain the most complete picture possible of the foraminiferal assemblages (i.e., including soft- as well as hard-shelled forms) in the study areas, all living foraminiferal samples were wet-counted. However, we did not obtain a substantially better correlation with bottom water [O\textsubscript{2}]\textsubscript{los} by including the fragile forms in the computation of the diversity indices. The fact that the fossilisable fraction of the living assemblages correlates better with the bottom water [O\textsubscript{2}]\textsubscript{los} than the fragile, non-fossilisable component (Table 3) has important implications for the use of benthic foraminifera as palaeoecological status (PalaeoEcoQS) indicators. It implies that, in the present study, the signal contained in the fossilisable assemblage diversity provides an accurate picture of the present-day environmental conditions. This means that even if most of the fragile living forms will not be preserved in the fossil assemblages, the signal given by fossil assemblages would give a good picture of the palaeoenvironmental conditions. This shows that in the present study areas, using the dry-picking method is adequate for reliably defining EcoQS based on benthic foraminiferal diversity. Dry-picking is advantageous because it is quicker. On the other hand, analysing the assemblages in the wet state makes it easier to distinguish between stained and unstained specimens, particularly for some agglutinated and opaque miliolid forms. To some extent, this can be compensated for by wetting or crushing the tests.

4.1.5. Analyses of living or dead assemblages

In the present study, it is not surprising that the dead foraminiferal assemblages do not correlate as well as the living ones with the bottom water [O\textsubscript{2}]\textsubscript{los}. Dead assemblages represent the mixing of tests from a succession of previously living assemblages modified by taphonomic processes (Murray, 2000), i.e., reflecting time-integrated environmental conditions commonly spanning several years. The time-period represented depends on the sedimentation accumulation rate. On the other hand, living assemblages reflect the environmental conditions which have operated at a site during the life-span of the organisms present, i.e., the EcoQS at the time of sampling. Unpublished dating-results of sediment cores from 5 of the investigated sites, show that the dead assemblages in the surface 0–2 cm represent tests (shells) accumulated during time periods of 2 to >10 years. Consequently, the living and dead assemblages reflect two principally different processes and, thereby, different aspects of the environmental conditions at a site. This is why the use of total (live + dead) assemblages, as still recommended by some authors, is difficult to interpret meaningfully and should be avoided (see also discussion in Murray, 2000). Optimal environmental information is gained by analysing and treating the two separately. As illustrated by the better correlation with the bottom water [O\textsubscript{2}]\textsubscript{los}, the living foraminiferal assemblages give a more accurate evaluation of EcoQS at the time of sampling whereas the dead ones reflect the longer time trends in EcoQS. Additionally, in the present study areas, picking living assemblages is not more time consuming as their abundance, relative to dead individuals is quite high (Table 1).

4.2. Evaluate EcoQS using living benthic foraminifera

This is the first study defining criteria to determine EcoQS using living benthic foraminifera for environmental monitoring. At our study sites, [O\textsubscript{2}]\textsubscript{los} reflects a disturbance gradient. Living benthic foraminiferal assemblages diversity (the effective number of species of complete living assemblages) is significantly correlated with [O\textsubscript{2}]\textsubscript{los} (Table 3). Improved EcoQS as reflected by foraminiferal diversity is observed along with increasing oxygen concentrations. Disturbed stations, i.e., here exposed to anoxic conditions, are classified as reflecting “bad” EcoQS based on foraminiferal diversity (Fig. 5). On the other hand, well-oxygenated stations are ranked from “moderate” to “high” EcoQS using the diversity index exp(H<sub>bc</sub>). We obtained a clear pattern from bad to high EcoQS using living benthic foraminifera, and this is in accordance with the bottom-water oxygen gradient ([O\textsubscript{2}]\textsubscript{los}). Indeed, different studies reported drops in benthic foraminiferal diversity in response to a gradient of disturbance (Alve et al., 2009; Elshanaawayi et al., 2011; Mojtahid et al., 2008). Exceptions are reported at stations in Tønsfjord (6) and Groosefjord (G5, G60 and G69) with normoxic conditions at the time of sampling but low diversity, and therefore “bad” EcoQS. Stations 6, G50, G60 and G69 had experienced periods of severe hypoxia over the past 2 years. The minimum value during the recent past at each site captures the extreme conditions which the foraminifera have experienced. They have higher TOC concentrations than stations with similar bottom-water oxygen concentration at the time of sampling. Buhl-Mortensen et al. (2009) reported that fjords in Southern Norway with low historical oxygen level also had in general a high carbon content in the surface sediment. This may indicate that although bottom-water [O\textsubscript{2}]\textsubscript{los} is a good predictor of the foraminiferal diversity (and better than TOC), [O\textsubscript{2}]\textsubscript{los} in the near-bottom water is not the variable that the infaunal foraminifera are actually responding to. Sediment pore-water conditions are more direct drivers of foraminiferal community composition, but will in fjord systems, usually be correlated with the properties of the bottom water.
In this study, we are suggesting that using the 0–1 cm, >125 μm, living dry-picked assemblages would give an accurate evaluation of the EcoQS. Using this suggested method means recording fewer species of the living assemblages, criteria has thus been adjusted (Table 2), as diversity values cannot be as high as for the complete living assemblages. We manage to highlight the gradient of disturbance using this method. Comparing EcoQS derived from the complete living assemblages and from this less-time consuming method, we obtain almost a full agreement in the classification (unacceptable or acceptable), with disagreements at only 4 stations (Table 1).

Consequently, diversity-based indices derived from benthic living foraminifera seem to be an adequate method to highlight a gradient of disturbances, and to determine EcoQS. However, it needs to be adjusted and further tested against natural or human-induced disturbances other than oxygen and in different environments.

4.3. Determine reference conditions using fossil benthic foraminifera

Since the assessment of ecological quality is based on the extent of deviation from reference conditions (WFD, 2000/60/EC), it is crucial to know the “true” reference conditions to obtain a valid classification. Currently, environmental monitoring studies use samples from similar and supposedly non-impacted environments as reference conditions or use expert judgment to define it (e.g. Bigot et al., 2008; Borja and Tunberg, 2011; Borja et al., 2012; Bouchet and Sauriau, 2008; Murikaga et al., 2007). Use of historical data, as suggested by the WFD, would provide an objective way of defining reference conditions but such data are rarely available. Alternatively, Alve et al. (2009) suggested that benthic foraminifera have a good potential to be used to determine reference conditions using the fossil record, provided possible impacts of taphonomic processes are considered. To what extent the signal contained in the living assemblages is preserved in the dead ones remains uncertain. In this study, diversity of living fossilisable and dead assemblages are correlated. This indicates that in areas with minimal tidal influence (minimal transport of tests) and sediment accumulation rates of one or a few mm year<sup>−1</sup> (common in S Norwegian fjords), the diversity of dead assemblages can be used as a proxy for the diversity of past (late summer) living communities. This correlation suggests that the environmental signal in the living foraminifera is well preserved in the fossil record. Hence, analyses of fossil benthic foraminifera allow for reconstruction of PalaeoEcoQS back to pre-impacted times (i.e. reference conditions). The detection of anthropogenic signals against background environmental noise is an issue in environmental monitoring studies (Elliott and Quintino, 2007), and requires long-term data, e.g. from palaeoecological work. It is very difficult to distinguish between a faunal assemblage with a low diversity due to a simply naturally stressed environment and one with low diversity induced by an anthropogenic stress. Looking at the PalaeoEcoQS reconstruction, it would be possible to determine whether or not the site has always been stressful for the benthic fauna.

The preservation of benthic foraminifera in the fossil record is a great asset compared to the non-preservation of benthic macrofauna, the traditional monitoring tool. The present results suggest that diversity-based indices derived from living benthic foraminifera represent a promising tool to assess EcoQS not only under present-day conditions but also in the past. It is also mentioned in the WFD that reference conditions are type-specific, so they are different for different types of coastal waters so as to take into account the broad diversity of ecological regions in Europe. It might actually be more appropriate to use “site-specific” boundaries between EcoQS classes instead of “type-specific” (Krause-Jensen et al., 2005) to decrease the risk of misinterpreting EcoQS. Criteria established in this study would then need to be adjusted at each site for a more accurate use of benthic foraminifera diversity as a bio-monitoring tool.

5. Conclusion

In this study, we show that consistent evaluation of ecological quality status (EcoQS) using the effective number of species derived from living (stained) benthic foraminifera can be obtained by sampling the 0–1 cm of sediment, pick about 250 living individuals of fossilizable species (i.e. dry-picked) from the >125 μm-fraction of each of three replicates per station. Sampling and processing benthic foraminiferal samples using this method provide a quick and efficient method to evaluate EcoQS. Correlation between living foraminiferal diversity and [O<sub>2</sub>], as well as correlation between complete community data (here surface 0–2 cm, >63 μm, living (stained) foraminifera) and [O<sub>2</sub>], suggest that benthic foraminifera can be an efficient bio-monitoring tool to evaluate EcoQS. At the moment, researchers are having problems finding and corroborating “true” reference conditions in present-day ecosystems. Since foraminifera are preserved in the fossil record, it would be possible to use this biological element to evaluate PalaeoEcoQS and estimate “true” reference conditions. The use of benthic foraminifera as a bio-monitoring tool would be a benefit for environmental monitoring programs worldwide. It nevertheless needs to be further assessed and tested in other ecosystems and against different types of pollution pressures.

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