Can benthic foraminifera serve as proxies for changes in benthic macrofaunal community structure? Implications for the definition of reference conditions

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Abstract

Benthic macrofauna is one of the most widely used biological groups to assess the ecological status of marine systems. Lately, attention has been paid to similar use of benthic foraminifera. In this study, distribution patterns of benthic foraminiferal and macrofaunal species were investigated simultaneously in 11 fjords in southeastern Norway in order to assess correlations and responses to environmental conditions. Selected fjords allowed to investigate contrasted environmental conditions from low total organic carbon (TOC) content (sediment TOC < 2.7%) in normoxia (bottom-water $[O_2] > 2$ mL $O_2.L^{-1}$) up to high TOC content (> 3.4%) in severe hypoxia (< 0.5 mL $O_2.L^{-1}$). Environmental parameters comprised bottom-water dissolved oxygen, grain size, total organic carbon, total nitrogen (TN), pigments and depth below threshold (DBT). Foraminiferal and macrofaunal community data were significantly correlated (Procrustes analysis $m^2 = 0.66$, $p = 0.001$). Hence, benthic foraminiferal distribution patterns mirror those of benthic macrofauna. However, as opposed to the foraminifera, macrofauna was not recorded at the most oxygen-depleted stations and, hence, was more sensitive to severe oxygen depletion. With regard to assigning species to ecological groups for ecological quality status assessment, the results suggest that species, *e.g. Spiroplectammina biformis* (foraminifera), *Scalibregma inflatum* (macrofauna), may exhibit different ecological requirements depending on their habitat. Considering the observed congruent patterns of benthic foraminifera and macrofauna, palaeo-communities of benthic foraminifera could be used as indicators of reference

conditions for benthic macrofaunal community structure. This would however need further developments of algorithms to perform such a translation.

1. Introduction

Most benthic macro-invertebrate species are sedentary, making them dependent on the local environmental conditions. Living at the sediment-water interface, they integrate aquatic as well as sediment conditions. Their communities show dramatic changes in their composition in response to organic matter loads (Pearson & Rosenberg 1978, Bouchet & Sauriau 2008), oxygen depletion (Rosenberg et al. 2002), oil spills (Gray et al. 1990), sewage (Glémarec & Hily 1981), heavy metals (Olsgard & Gray 1995) and physical disturbance (Lavesque et al. 2009). Tolerant species thrive at the early stages of the perturbation, followed by highly tolerant opportunistic species when the perturbation becomes severe (Glémarec & Hily 1981). Hence, macrofauna are routinely used in environmental bio-monitoring studies (e.g. Warwick 1986, Grall & Glémarec 1997, Borja et al. 2003, Bouchet & Sauriau 2008). In response to the implementation of marine legislation such as the Water Framework Directive (WFD), the Clean Water Act, and the Marine Strategy Framework Directive (MSFD), numerous indices based on macrofauna have been established for assessing the ecological quality status (EcoQ, see review in Pinto et al. 2009). Most of the indices developed to assess the EcoQ of marine systems are based on the indicative value of macrofaunal species. They have been classified into 5 ecological groups of sensitivity to disturbances from sensitive to first-order opportunistic species (Glémarec & Hily 1981, Borja 2000). Lately, concerns arose about these indices (see review in Spilmont 2013). One of the main concerns is the definition of reliable reference conditions for benthic macrofaunal communities. This is a crucial point since in the European Water Framework Directive (WFD), reference conditions are mandatory to assess the EcoQ. According to the WFD, the reference conditions (also called “high” status) are defined as “for any surface water body type reference conditions or high ecological status is a state in the present or in the past where there are no, or only very minor, changes to the values of the hydromorphological, physico-chemical, and biological quality elements which would be found in the absence of anthropogenic disturbance” (Common implementation strategy for the Water Framework Directive (2000/60/EC), Guidance Document No.5, transitional and coastal waters – typology, reference conditions and classification systems p. 106). Assessment of environmental quality is based on the extent of deviation from these reference conditions, following the definitions in the WFD (www.ec.europa.eu/environment/water/marine.htm). Most of the coastal areas are modified or have been modified by human activities (Diaz and Rosenberg 2008). Hence, EcoQ assessment within the WFD is facing issues to determine true reference condition (Nielsen et al. 2003, Elliott and Quintino 2007). As for benthic macrofauna, since there is almost no data from pre-impact time, reference conditions have to be determine using today’s communities, which is an issue considering the aforementioned degradation of coastal areas.

There is an increasing interest in the use of benthic foraminifera to characterize the health of marine systems (e.g. Alve 1995, Scott et al. 2001, Mojtahid et al. 2006, Bouchet et al. 2007, Frontalini et al. 2009). Lately, indices to use benthic foraminifera routinely to assess EcoQs have been proposed (Hallock et al. 2003, Alve et al. 2009, Bouchet et al. 2012, Barras et al. 2014, Dimiza et al. 2016). Specifically, benthic foraminiferal species were assigned to ecological groups (EGs) according to their sensitivity/tolerance to increasing organic matter enrichment (Alve et al. 2016, Jorissen et al. 2018), inspired by work on benthic macrofauna to develop the AMBI index (Glémarec & Hily 1981, Borja et al. 2000). It led to the development of the Foram-AMBI to assess the ecological quality status of marine systems. In these studies, the response of species along a gradient of enrichment in organic matter allowed their classification into groups of different sensitivity. Five ecological groups were defined: sensitive (EG1), indifferent (EG2), tolerant (EG3), second-order opportunistic (EG4) and first-order (most) opportunistic (EG5). The AMBI and the Foram-AMBI are computed based on the proportion of the different ecological groups in the species community, allowing assessment of ecological quality status.

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, 1991; Hayward et al., 2004, Dolven et al. 2013, Francescangeli et al. 2016). Consequently, it is possible to trace the record of human-induced disturbance 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. 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. Although the response of macrofauna and foraminifera species to environmental gradients may partly differ (Mojtahid et al. 2008), the few studies comparing the two groups have indicated that their responses to environmental changes are basically similar (Schafer et al. 1975, Schafer et al. 1995, Klitgaard-Kristensen & Buhl-Mortensen 1999, Bouchet 2007, Denoyelle et al. 2010, Dolven et al. 2013, Hess et al. 2013, Wlodarska-Kowalczuk et al. 2013). For instance, benthic foraminiferal and macrofaunal communities showed similar response to oil-based drill mud disposal with tolerant and opportunistic species dominating in the vicinity of the disposal area (Denoyelle et al. 2010). Furthermore, calcareous foraminifera can be reliable indicators for variability in density, diversity and species composition of benthic macrofauna in the Artic at Svalbard (Wlodarska-Koawalczuk et al. 2013). Hence, it is reasonable to suggest that macrofaunal and foraminiferal species with similar sensitivity to environmental changes may show co-occurrence. This would be of great interest when it comes to the definition of reference conditions. It is reasonable to think that fossil benthic foraminifera could be used as proxies to define reference conditions for benthic macrofauna communities. Further baseline studies are however necessary to validate such a hypothesis. It is thus urgent to quantify the level of correlation between the distribution patterns of these two groups in other environments.

In the present study, living benthic foraminifera and macrofauna were investigated in fjordic systems along the Norwegian Skagerrak coast to assess to which degree their responses to major driving environmental factors correspond. This work is part of a comprehensive project (PES), which includes both foraminifera and macrofauna collected at the same sites at the same time. An understanding of the driving forces of the benthic foraminifera communities, expressed as species diversity, was partly achieved in a previous analysis of the same data set (Bouchet et al. 2012, 2013). The present paper aims (i) to determine the driving environmental factors of both the benthic foraminiferal and macrofaunal community structures, (ii) to identify indicator species of both normal and poor environmental conditions, (iii) to compare species assignment in EG from this study to the existing Foram-AMBI and AMBI species lists, and (iv) to assess the correlation between benthic foraminiferal and macrofaunal communities patterns to identify the potential of benthic foraminifera to serve as a proxy for benthic macrofauna in environmental assessments.

2. Material and methods

2.1 Study area and sampling sites

In August 2008, 27 stations from 11 silled basins along the Norwegian Skagerrak coast, NE North Sea (Figure 1), were sampled for bottom water, sedimentological, biogeochemical and faunal analyses. Stations between 23 and 204 m water depth were selected to provide an oxygen gradient with stable temperature (5-6°C; occasionally 8-9°C at <40 m depth) and salinity (33-34) conditions (Table 1; see Bouchet et al. 2012 for further details) 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 partial to complete deep-water renewals during winter. In addition to measured variables, the depth below threshold (DBT) *i.e.* the difference between the water depth at a station and the sill depth of the fjord, has been characterized for stations. DBT is not a factor *sensu stricto*, but a measure that reflects the degree or duration of water stagnation in the deep areas of the fjord.

2.2 Field sampling

Four sediment cores (8 cm diameter) were collected at each of the 27 stations with a Gemini gravity corer, a modified Niemistö corer (Niemistö 1974). Three replicate cores were used for benthic foraminifera and one for pigments, total organic carbon and total nitrogen analyses. Once on deck, bottom water samples from just above the sediment-water interface in two cores were immediately transferred to Winkler bottles 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 pigments, total organic carbon and total nitrogen analyses were frozen immediately after sectioning, and kept away from the light. 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 (Murray & Bowser 2000). At each station, macrofauna was sampled using a 0.1 m^2 van Veen grab (4 replicates) and a sub-sample of the top 0-1 cm from one grab per station was collected for grain size analyses. The grab sediments were sieved on board through 1 mm screens and fixed in 4% buffered formaldehyde in seawater to preserve macrofauna specimens.

2.3 Biogeochemical analyses

Bottom-water dissolved oxygen concentrations at the time of sampling were analysed using Winkler titration. Classification of stations as normoxic (bottom-water $[O_2] > 2$ mL $O_2.L^{-1}$), hypoxic (0.5 - 2 mL $O_2.L^{-1}$) or severe hypoxic (< 0.5 mL $O_2.L^{-1}$) follows Diaz and Rosenberg (1995). 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 mass. Total organic carbon (TOC) and total nitrogen (TN) were analysed following acid treatment (10% HCl) to remove inorganic C using a CHN analyser (Carlo Erba Elemental Analyzer 1106). Classification of stations as low (<2.7%), medium (2.7-3.4%) and high (>3.4%) TOC content follows that of Molvaer et al. (1997). For pigment analyses, homogenised freeze dried sediment (0.3-0.6 g) was extracted in 5 ml 90% acetone in water. The mixture was sonicated for 30s before overnight extraction. The extract was centrifuged (15 min. at 3500 rpm), and 100 µL of the supernatant was injected on the HPLC for pigment analysis. The analytical system was based on a method described earlier (Wright et al. 1991, Jeffrey et al. 1997). Pigments were identified by comparison of retention time and absorption spectra of authentic standards (DHI Water and Environment, Denmark) and the literature (Jeffrey et al. 1997). Individual pigments were quantified at 436 nm for chlorophylls and pheopigments, and 450 nm for carotenoids. Response factors (RF) for each pigment were obtained by single standard runs. When no standard was available, RF for similar pigments was used with a correction for differences in mass. Pigment concentrations were standardised by the total organic carbon content (Reuss et al. 2010).

2.4 Faunal analyses

Foraminiferal samples were washed through 500 and 63 µm mesh sieves, and the 63-500 µm fraction was split into 8 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. Wet-sample analysis allows preservation of all species, including fragile organic-walled and loosely cemented agglutinated foraminifera. This study is based on the 63-500 µm fraction *i.e.* the sum of 63-125 and 125-500 µm fractions.

In the laboratory, macrofaunal samples were rinsed on 1 mm-mesh sieves and transferred to 70% ethanol. All macrofaunal specimens were counted and identified to species level, or the lowest identifiable taxonomic unit.

Assignments of foraminiferal and macrofaunal species into ecological groups (EG) were made according to Alve et al. (2016) and Borja et al. (2000), respectively. The concept is based on the classification of species (or groups of species) into five EGs (sensitive, indifferent, tolerant, 2nd order opportunist and 1st order opportunist) representing specific sensitivity levels to an increasing gradient of organic matter (Borja et al. 2000). The groups allow the assessment of the ecological quality status of marine systems.

2.5 Numerical analyses

Principal components analysis (PCA) was applied on the set of environmental variables to characterize the main natural gradients and describe relationships between sediments, oxygen and fjord topography. For the species community data, initial detrended correspondence analyses indicated that the gradient is short (1.6 SD) in the foraminiferal data and long (3.3 SD) in the macrofaunal data. Consequently, redundancy analysis (RDA) and canonical correspondence analysis (CCA), respectively, were applied to find which environmental variables were most clearly related to the species communities. Analyses were done on the subset of 15 stations with all chemistry data and at least 30 individuals in the pooled macrofaunal sample (all pooled foraminiferal samples had many more than 30 individuals). Community data were log(x + 1) transformed prior to analysis; taxa occurring at only one station were removed. Environmental variables were selected using forward selection with 999 permutations. Procrustes analysis (Peres-Neto & Jackson 2001) was used to compare unconstrained ordinations of the foraminiferal (PCA) and macrofaunal (correspondence analysis) community data. The m^2 statistics, which is analogous with the r^2 of a correlation, is reported. Co-correspondence analysis (Co-CA, Ter Braak & Schaffers 2004) was used to relate the foraminiferal and the macrofaunal communities in a direct way.

All calculations were performed using the statistical language R version 3.4.1 (R Core Team, 2017). Ordinations were run with the vegan version 2.4-3 (Oksanen et al. 2010). R code is archived at https://github.com/richardjtelford/PES.

3. Results

3.1 Environmental conditions at sampling stations

Depth-below threshold (DBT), bottom-water dissolved oxygen concentration, sediment grain size and sediment organics were rather different among the stations (Table 1). Sandnesfjord, Eidangerfjord, Risørbassenget (R60), Topdalsfjord, Groosefjord, Frierfjord (except F70) and Indre Hvaler have rather shallow DBT below 50 m, whereas the other fjords have DBT up to 169 m. In Kristiansandsfjord there is no sill. High (normoxic) bottom-water oxygen concentrations (>2 mL $O_2.L^{-1}$) characterized stations in the Indre Hvaler basin, Kragerøfjord, Topdalsfjord, the shallower stations in Groosefjord, Frierfjord (F30 and F50), Ærøydypet, Sandnesfjord, Eidangerfjord and Kristiansandsfjord as opposed to stations in Risørbassenget (except R60), the deeper stations in Frierfjord (F70 and F90) and Håøyfjord where hypoxic (0.5-2 mL O_2.L^{-1}) to severe hypoxic (<0.5 mL O_2.L^{-1}) conditions are recorded. Sediments at stations in Risørbassenget (except R160 and R180) and Kristiansandsfjord, as well as station G50 were sandier than the others, with less than 90% of the sediment mass < 63 µm (Table 1). Total organic carbon concentrations were higher than 3.4% in Ærøydypet, Kragerøfjord, Sandnesfjord, Eidangerfjord, Risøbassenget, Topdalsfjord, Frierfjord (F90) and Groosefjord (Table 1).

In the PCA of environmental variables, the first two axes explained 75.2% of the variance (Fig. 2). Two main gradients appear; one related to sediment components (particle fine fraction, TOC) and one to oxygen and fjord basin topography (O_2, DBT). The sediment gradient separates stations with very high fine fraction and moderate organic content (lower left: Indre Hvaler IH, Frierfjord F) from stations with lower fine fraction and high organic contents (upper right: Groosefjord G, Risørbassenget R). The oxygen – fjord topography gradient separates stations in deep fjord basins with shallow sill and low oxygen content (lower right: Risør basin R, Håøyfjord 102, deep Frierfjord F) from stations with either shallow water depths or in fjords with deep sills (upper left). The pigments correlate with DBT and characterized the deep basins, particularly the Risør basin. Most pigments were intercorrelated, except allo-xanthin that appeared to be related also to the sediment fine fraction.

3.2 Environmental variables and fauna

In total, 116 foraminiferal taxa and 290 macrofaunal taxa were identified. Benthic foraminifera occurred in all samples, whereas macrofauna were absent from station F90 (replicate 1 and 2), station R120 (replicate 4), station R160 (all replicates) and station R180 (all but replicate 1). Based on RDA and CCA, respectively, benthic foraminifera and macrofauna species were significantly related to the same subset of environmental parameters, viz. TOC and DBT (Figs 3 and 4). Together, the selected parameters explained 43% and 29% of the variation for foraminiferal and macrofaunal species assemblages, respectively.

The distribution of foraminiferal taxa in relation to TOC and oxygen, the latter being related to DBT (Fig. 2), is illustrated in Fig. 5. The species *Micrometula hyalostriata*, *Phainogullmia aurata* and *Spiroplectammina biformis* mainly occur at low TOC (<2.7%) and high bottom-water oxygen concentrations. Highest abundances of *Cassidulina laevigata* and *Bulimina marginata* were observed at stations with low to high TOC (0.8-9%) and high bottom-water oxygen concentrations. *Cylindrogullmia alba* and *Leptohalysis scottii* occur at similar TOC levels but at stations with high to low bottom-water oxygen concentrations. *Liebusella goësi*, *Textularia earlandi* and *Recurvoides trochamminiforme* show highest abundances under TOC concentrations between 3.0 and 4.3% and well-oxygenated conditions. *Stainforthia fusiformis*, *Discorbinella bertheloti, Fissurina* sp. and *Bolivinellina pseudopunctata* thrive under high TOC concentrations (>3.4%) and hypoxic to severe hypoxic conditions.

Compared to the assignments of foraminifera in Alve et al. (2016) four new species (*Micrometula hyalostriata*, *Phainogullmia aurata*, *Cylindrogullmia alba, Leptohalysis scottii*) were assigned and three (*Cassidulina laevigata, Liebusella goësi*, *Bolivinellina pseudopunctata*) were re-assigned based on their abundance relative to sediment TOC in this new data set (Table 2).

The distribution of dominant macrofaunal species on TOC and oxygen is illustrated in Fig. 6. *Pseupopolydora* sp., *Chaetozone setosa*, *Capitella capitata*, *Thyasira* cf. *sarsii* occurred at hypoxic stations with high TOC. *Mediomastus fragilis* was overabundant in Kristiansandfjord that is contaminated from trace elements*. Thyasira equalis* and *Spiophanes kroeyeri* occurred at well-oxygenated stations with medium TOC content. *Amphiura chiajei*, *A. filiformis* and *Scalibregma inflatum* occurred at normoxic stations (Fig. 6B) with low organic matter concentrations (Fig. 6A).

Compared to the assignments of the AMBI list (Borja et al. 2000, last update: June 2017), two species (*Scalibregma inflatum* and *Thyasira* cf. *sarsi*) were re-assigned based on their abundance relative to sediment TOC in this new data set (Table 2).

3.3 Foraminiferal and macrofaunal community relationships

Foraminiferal and macrofaunal community data were significantly correlated (Procrustes analysis $m^2 = 0.66$, $p = 0.001$). Using a co-correspondence analysis on foraminiferal and macrofaunal community, 24% of the variance in the macrofaunal community was explained by the foraminiferal community (Fig. 7).

4. Discussion

In the present study, patterns of distribution of foraminiferal and macrofaunal species are best explained by the variables of TOC and DBT. Along the same lines, deep-water renewal and oxygenation in a fjord depend on DBT as well as on the supply of organic matter (*i.e.*, impacts oxygen consumption). DBT will, however, also represent the influence of other variables, e.g. pigments, different organic components and temperature, that increase or decrease due to the gradually changing conditions with depth. In the following discussion, however, it will be made reference to oxygen rather than of DBT *sensu stricto* that may incorporate several unknown factors. This is further supported by Bouchet et al. (2012) who showed on the same dataset that benthic foraminiferal diversity is correlated to bottom-water dissolved water oxygen. These two variables (oxygen and TOC) are indeed known to be drivers of both diversity and species composition of benthic foraminifera and macrofaunal communities (Alve 1990, Riera et al. 1999, Gustafsson & Nordberg 2000, Rosenberg et al. 2002, Bouchet et al. 2012). In the studied Norwegian fjords, benthic foraminiferal and macrofaunal species responded in the same way to different environmental conditions (Procrustes analysis), except that macrofauna was less tolerant to the oxygen depleted conditions. This is because, unlike macrofauna, some foraminiferal species can perform anaerobic metabolism (e.g., Risgaard-Petersen et al. 2006). Our findings confirmed the results from the few studies assessing and comparing the response of benthic foraminifera and macrofauna to different environmental conditions, mentioned in the introduction.

4.1 Foraminiferal indicator species

In this study, *Micrometula hyalostriata*, *Phainogullmia aurata* and *Spiroplectammina biformis* are sensitive (EG1) to organic enrichment, occurring primarily in normoxic conditions. *Phainogullmia aurata* has been described as a pioneer species showing maximum abundance in the colonization phase on uncontaminated clay and almost disappearing with time as the amount of sediment organic material increased (Hess et al.

2014). The ecology of the soft-walled species *M. hyalostriata* is poorly known. In this study, it was common only at normoxic, low-TOC sites (Fig. 5). *Spiroplectammina biformis* was the second-most common species in the most hypoxic parts of the organic matter enriched Drammensfjord, southern Norway (Alve 1995) but it was not able to survive persistent severe hypoxia below 0.5 mL $O_2.L^{-1}$ (Alve 1991, 1995). It was also reported to tolerate organic enrichment in several eastern Canadian estuaries and embayments and in the North-east Atlantic (Alve et al. 2016, Schafer et al. 1991). In the present study, its highest abundance was at a low-TOC site. It was also sensitive to TOC in the Saguenay fjord and in a study in Canadian fish aquaculture sites (Schafer et al. 1991, 1995).

Cassidulina laevigata and *Cylindrogullmia alba* show the same distribution pattern along the TOC gradient as for species from the indifferent EG2. *Cassidulina laevigata* is a typical species of the Skagerrak-Kattegat fauna (*sensu* Nordberg et al. 2000), which used to flourish in several Scandinavian fjords until it was replaced by species associated with frequent coastal hypoxia (e.g., Alve 1991, Polovodova Asteman & Nordberg 2013, Polovodova Asteman et al. 2015). In the present study, *C. laevigata* is never abundant, it only occurs at stations with normoxic conditions and mainly, but not solely, where TOC is low to moderate (Fig. 5). Hence, *C. laevigata* may belong in the indifferent group EG2, although it has been classified as a sensitive species (Alve et al.2016). The present results show that *C. alba* can survive under a wide range of TOC concentrations.

Bulimina marginata, *Recurvoides trochamminiforme*, *Liebusella goësi* and *Textularia earlandi* are all tolerant species (EG3) to organic enrichment in the studied fjords, occurring in normoxic conditions. *Bulimina marginata* is tolerant to oxygen-depleted conditions (Alve 1991, Barmawidjaja et al. 1992). However, it is not able to survive persistent severe hypoxia below 0.5 mL $O_2.L^{-1}$ (Filipsson & Nordberg 2004). In this study, it occurred over a broad range of TOC concentrations, but only under normoxic conditions. As for *R. trochamminiforme*, Alve et al. (2016) mentioned it as a tolerant species. *Liebusella goësi* is known not to survive severe hypoxia (Nordberg et al. 2000, Filipsson & Nordberg 2004). Tolerance to TOC enrichment under appropriate oxygen concentrations has already been observed for *T. earlandi* (Schafer et al 1995). It is however sensitive to oxygen depletion (Gustafsson & Nordberg 2000, Polovodova Asteman et al. 2015). Its abundance decreased at dissolved-oxygen concentrations below 1.0 ml $O_2.L^{-1}$ (Gustafsson & Nordberg 2000).

Bolivinellina pseudopunctata, Stainforthia fusiformis, *Leptohalysis scottii* and *Fissurina* sp. are assigned in the opportunistics EG4/5, thriving well in sediment with high TOC content based on this study results, occurring at hypoxic to severe hypoxic stations. The two first species are known to be able to survive at high TOC and hypoxic to severe hypoxic conditions (Alve 1994, Gustafsson & Nordberg 2000, Alve 2003). These species outcompeted the typical Skagerrak-Kattegat species throughout the degradation of the environmental conditions in the area during the 1900s (Gustafsson & Nordberg 2001, Alve et al. 2009, Dolven et al. 2013). *Bolivinellina pseudopunctata* is an opportunistic species able to tolerate low oxygen conditions (Gustafsson & Nordberg 2001), although it has been reported to disappear after periodic severe hypoxia or anoxia (Gustafsson & Nordberg 2000). The observation of this species in the most organic rich stations supports that it is an opportunistic species, which is not in agreement with the classification of Alve et al. (2016) in the indifferent EG2. Our results suggest that *L. scottii* is able to survive under hypoxic conditions as opposed to what has been reported in Havstensfjord in Sweden (Gustafsson & Nordberg 2000). Furthermore, it occurs in this study in fjords with a wide range of TOC concentrations from 2 to 9%. It is a species known to tolerate environmental disturbances such as paper mill and fish farming effluents (Alve & Nagy 1986, Scott et al. 2001, Polovodova Asteman et al. 2015) and it is common in eutrophic environments (Scott et al., 2005; Sabbatini et al., 2012). *Stainforthia fusiformis* is a characteristic species of heavily disturbed environments (see review in Alve 2003). Except that species of *Fissurina* seem to be ectoparasites on *Discorbis vilardeboanus* (d'Orbigny) and *Rosalina bradyi* (Cushman) (see Collen & Newell, 1999, and references therein), little is known about the ecology of *Fissurina* spp. In the present material, *Fissurina* sp always co-occurs with *Discorbinella bertheloti*, perhaps as a parasite?

Discorbinella bertheloti (also found under the name *Cibicides bertheloti*) was found alive (stained) at hypoxic and severe hypoxic stations. With their epifaunal mode of life (Murray 2006), *Cibicides* spp. have been considered as sensitive species not directly linked to sedimentary TOC (Alve et al. 2016). They rather reflect the impact of bottom-near currents (Linke & Lutze 1993). Hence, occurrence of *D. bertheloti* at the most oxygen-depleted stations with high TOC content was probably due to recent transport of living specimens. It is thus difficult to conclude on the indicative value of this species.

4.2 Macrofaunal indicator species

In this study, *Amphiura chiajei*, *A. filiformis*, *Scalibregma inflatum* are sensitive species (EG1) to TOC*,* occurring at normoxic stations. They are known to not tolerate oxygen-depleted conditions (Aschan & Skullerud 1990, Nilsson & Rosenberg 1994, 2000, Rosenberg et al. 2001). The three species have been reported to solely occur at unpolluted sites in the Oslofjord (Mirza & Gray 1981). A high abundance and biomass of *A. filiformis* and *A. chiajei* has been observed at well oxygenated sites in the Skagerrak (Josefson 1990). According to AMBI's ecological groups (EG) of sensitivity to disturbances (Borja et al. 2000), *A. chiajei* and *A. filiformis* are indifferent species (EG2) and *S. inflatum* is a tolerant species (EG3). *Amphiura filiformis* has been reported to be sensitive to severe disturbances around oil platforms (Olsgard & Gray 1995). Note that the tolerance level of *S. inflatum* is rather uncertain since it has been reported to be either sensitive (Mirza & Gray 1981, Rygg 1985b, Nilsson & Rosenberg 1994, 2000, Rosenberg et al. 2001, Pabis & Sobczyk 2015) or tolerant (Rygg 1985a, Aschan & Skullerud 1990, Borja et al. 2000, Levin et al. 2009) to disturbances.

Thyasira equalis and *Spiophanes kroeyeri* dominate at well-oxygenated stations moderately rich in organic matter. They are not present at the hypoxic/severe hypoxic stations, suggesting that neither of the species would tolerate low oxygen concentrations. This contradicts other works on these species (see review in Levin et al. 2009). For instance, *T. equalis* has been reported to survive low-oxygen concentrations (Josefson & Widbom 1988, Nilsson & Rosenberg 2000). It usually occurs in moderately disturbed areas (Pearson 1975, Mirza & Gray 1981, Rygg 1985a), although it has been reported not to tolerate copper pollution (Rygg 1985b). The results of the present study are in accordance with the classification of *T. equalis* in the tolerant EG 3 (Borja et al. 2000). Conversely, *S. kroeyeri* has been mostly reported to disappear in the early stage along a gradient of increasing disturbances *i.e.* oil platform activities, sewage outfall, copper, organic enrichment, oxygen depletion (Mirza & Gray 1981, Rygg 1985b, Aschan & Skullerud 1990, Nilsson & Rosenberg 2000, Mojtahid et al. 2008, Oug et al. 2012). Few studies nevertheless reported the presence of this species in low disturbed sites (Pearson 1975, Gray & Pearson 1982, Rygg 1985a, Moore & Rodger 1991). The classification of *S. kroeyeri* in the tolerant EG3 (Borja et al. 2000) is at least questionable.

Pseupopolydora sp., *Chaetozone setosa*, *Capitella capitata* and *Thyasira* cf. *sarsii* occur at stations with hypoxic conditions and high TOC contents. *Mediomastus fragilis* dominates in the heavily polluted with trace metals Kristiansandfjord. It belongs together with *T.* cf. *sarsii* to the tolerant EG3, *C. setosa* and *P.* sp. belong to the second-order opportunistic EG4 and *C. capitata* to the first-order opportunistic EG5 (AMBI-list, Borja et al. 2000). They are all characterizing low-oxygenated environments (Levin et al. 2009). *Mediomastus fragilis* can tolerate pollution such as oil spills, disturbances associated to oil platforms, metals and high level of organic enrichment (Pearson & Rosenberg 1978, Mirza & Gray 1981, Dauvin 2000, Mojtahid et al. 2008, Oug et al. 2012). *Thyasira* cf. *sarsii* is sensitive to low oxygen concentrations (Josefson & Widbom 1988, Nilsson & Rosenberg 2000) but it can colonize sediments polluted by copper, organic matter, and dredging materials from oil platform activities (Pearson 1975, Rygg 1985b, a, Olsgard & Gray 1995). *Capitella capitata* and *P.* spp. are known colonizers of completely anoxic sediments in fjord systems (Rosenberg et al. 2001). They are often the last species to survive these depleted conditions (Pearson & Rosenberg 1978, Mirza & Gray 1981, Oug et al. 2012). *Chaetozone setosa* and *C. capitata* are typical indicators of severe effects around oil platforms of the Norwegian continental shelf (Olsgard & Gray 1995). *Chaetozone setosa* usually thrives in depleted conditions (Rygg 1985a, Olsgard & Hasle 1993, Pabis & Sobczyk 2015). It is a common species in organic enriched areas (Gray et al. 1990).

4.3 Concerns about the indicative value of classified species

The use of the presence of certain species to assess the state of marine systems has a long history. The concept is based on an *a priori* apprehension that some species have narrow ecological requirements. Hence, species are meant to be indicative of the prevailing conditions where they are found. Based on numerous works (e.g., Pearson & Rosenberg

1978, Glémarec & Hily 1981, Hily 1983), lists of benthic macrofaunal species, assigned into groups of sensitivity to disturbances, have been established; the most widely used being the species associated to the AMBI index (Borja et al. 2000). Lately, following the suggestions of the FOBIMO working group, the same approach was used to assign benthic foraminiferal species to EGs of sensitivity/tolerance to conditions along an increasing stress (organic matter enrichment) gradient (Alve et al. 2016, Jorissen et al. 2018). So far, assignment of foraminiferal species has only been done for the North-East Atlantic and Arctic fjords, continental shelves, and slopes and for the Mediterranean (Alve et al. 2016, Jorissen et al. 2018). The use of a single species list classification means that the indicative value of a species is a static concept *i.e.* a species is expected to have a similar sensitivity or tolerance wherever it occurs and regardless of the adaptation ability of this species. However, it is known that some species are plastic enough to adapt to their environment and could change their autecology requirements along environmental gradients (see review in Zettler et al. 2013 and references therein). Hence, a species tolerance spectrum might be wider than a single category, *i.e.* "sensitive species" or "tolerant species". Species even exhibit different responses to disturbance depending on their habitat and the source of disturbances; they behave as sensitive species while they can be tolerant or opportunistic somewhere else or against a different pollution source (Zettler et al. 2013). The biogeographical/ecosystem-type restricted approach chosen to assign foraminiferal species tend to support the ability of species to adapt their sensitivity/tolerance level to their environment.

In this study, we reported discrepancies in the assignment in EG with the study of Alve et al. (2016) for *S. biformis*, *C. laevigata*, *L. goësi* and *B. pseudopunctata*. For *C. laevigata*, our study results suggest a classification in the indifferent EG2 instead of the sensitive EG1 (Alve et al. 2016). These two EGs are rather close conceptually since they include species that cannot tolerate any excess in organic matter. Some biotic indices, *i.e.* Bentix (Simboura & Zenetos 2002), group these two EGs in one EG. This might be something to consider in a further implementation of the Foram-AMBI list. *Spiroplectammina biformis* was assigned to the tolerant EG in the Foram-AMBI list of species from the North-East Atlantic (Alve et al. 2016). In several eastern Canadian estuaries and embayments, *S. biformis* has been reported in moderately polluted local environments (Schafer et al. 1991). This study results however suggest that it is a sensitive species to TOC enrichment. In the Saguenay fjord, *S. biformis* abundances have also been reported to decrease with increasing organic matter load from a pulp mill effluent (Schafer et al. 1991). In a study in four Canadian aquaculture sites, it showed a significant negative correlation to TOC enrichment due to the presence of fish cages (Schafer et al. 1995). These evidences suggest that it is thus not sure whether this species belongs to the sensitive EG1 or the tolerant EG3. Discrepancies in the response of species to TOC enrichment have been found between this study results and previous work for both benthic foraminifera and macrofauna. As for benthic macrofauna, there are eight studies, including this one, reporting a sensitive behavior of the polychaete *S. inflatum* towards disturbances and at least four reporting a tolerant one (see aforementioned references). In the AMBI species list, this species is assigned to the tolerant EG. The assignment of *S. inflatum* within the AMBI list is rather uncertain. The same concerns arose about *T.* cf. *sarsii* which behave like an opportunistic species in this study and is assigned to tolerant EG in the AMBI-list. Occurrencies of *T. equalis, S. kroeyeri* and *S. inflatum* in several Norwegian fjords and coastal areas (Rygg & Norling 2013) showed tolerance levels corresponding to EG3 (tolerant), thus supporting the classification in the AMBI system. These discrepancies may be due to the fact that indices' scores come from large dataset collections covering large geographical regions and subregions and not local scales (Borja et al. 2000, Rosenberg et al. 2004). Hence, they integrate the "experience" from a species behavior over a large span of data or literature data. Local adapation of species ecological requirement may lead to wrong interpetation of the species indicative value (Dauvin et al. 2010, Zettler et al. 2013).

Zettler et al. (2013) reported that the response of macrofaunal species towards organic enrichment would change along a gradient of salinity. They concluded that the interaction between environmental variables should not be neglected when using static indicative value of a species. This study results tend to confirm the latest concerns about the concept of species lists of tolerance/sensitivity. Furthermore, this study shows that these concerns may apply to benthic foraminifera. At a large biogeographical scale, the indicative value may be reliable, but it is rather uncertain at a local geographical scale. This study results definitively support cautions (Dauvin et al. 2010, Zettler et al. 2013) when it comes to the use of indicator species lists. It seems obvious that more studies are needed to constrain these biases. Supplementary approaches should be considered, for instance rank-frequency diagrams (Frontier 1976, Bouchet et al. 2007, Seuront 2013) and diversity indices (Bouchet et al. 2012, Dolven et al. 2013, Francescangeli et al. 2016, Bouchet et al. 2018).

4.4 Benthic foraminifera as indicator of macrofaunal community structure

The assessment and quantification of cross-taxon congruence *i.e.* similar response along an environmental gradient of species belonging to different taxa, in space and time, represents a critical step in the identification of suitable indicator taxa for biodiversity. In this study, Procrustes rotation and co-correspondence analysis (Co-CA) were used to quantify the strength of the congruence between foraminiferal and macrofaunal communities along the studied environmental parameters gradient. Procrustes rotation and Co-CA are powerful and robust methods to evaluate the degree of congruence between two community datasets (Peres-Neto & Jackson 2001, Gioria et al. 2011). Patterns of foraminiferal species distribution closely resembled those of the macrofauna, as is showed by significant correlation between the groups. The strength of the congruence in species composition between benthic foraminifera and macrofauna along the environmental gradient reflects the similarity in their response to TOC enrichment. Interactions can explain congruence in species between these two groups. First, there may be a trophic link between the two groups; benthic macrofauna species being able to feed on benthic foraminifera (Lipps 1983). Secondly, benthic macrofauna bioturbating activities enhance the vertical distribution of foraminifera in deeper sedimentary layers (Bouchet et al. 2009). Last but not least, there is an evident similarity in the patterns of distribution of species of both groups in response to environmental gradients.

In this study, benthic foraminiferal and macrofaunal species composition appear to respond to the same environmental factors, in particular bottom-water dissolved oxygen and organic matter content. Changes in the composition of foraminiferal and macrofaunal assemblages are expressed in increased dominance of tolerant/opportunistic species and the progressive disappearance of sensitive species under degraded conditions *i.e.* increasing TOC content and decreasing bottom-water oxygen concentrations. It supports other studies, although there are few, showing similar response of the two groups to adverse environmental conditions. Patterns of foraminiferal species distribution closely resembled those of macrofauna in response to disturbances of a glacier (Wlodarska-Kowalczuk et al. 2013). Similar succession from sensitive to tolerant species has been reported for both groups in response to different pollution sources such as sewage outfalls, industry, oil-based drill mud disposal and aquaculture (Schafer et al. 1975, Schafer et al. 1995, Mojtahid et al. 2008, Denoyelle et al. 2010). However, some studies suggested that benthic foraminifera could be more indicative than benthic macrofauna along a gradient of disturbances (Mojtahid et al. 2008, Denoyelle et al. 2010). In this study, benthic foraminifera exhibited more tolerance to the most depleted conditions (high TOC and anoxic conditions), by maintaining higher densities than did the macrofauna. This confirms previous results showing that benthic macrofauna is more sensitive than benthic foraminifera to depleted oxygen conditions (Josefson & Widbom 1988) and, as previously mentioned, is directly linked to the fact that some foraminiferal species perform anaerobic metabolism (*e.g.*, Risgaard-Petersen et al. 2006). It implies that benthic foraminifera are able to occur along the whole environmental gradient, which is an asset compared to benthic macrofauna in monitoring studies when approaching the "bad" end of the environmental gradient. The strength of the congruence between benthic foraminifera and macrofauna suggests that foraminifera could be reliable indicators of benthic macrofauna community structure. This has interesting implications, in particular with regard to defining reference conditions for areas where the environmental conditions may have changed over time, as suggested by Alve et al. (2009). Defining reference conditions are mandatory according to the WFD and complying to MSFD when it comes to assessing the health of marine systems. Hindcasting is one method that can be used to determine previous conditions. This is hardly possible with benthic macrofauna since historical data are scarce. Mostly the molluscs can fossilise and allow a reconstruction of palaeo-environments (Poirier et al. 2009), meaning that most of the biological signal is lost in the fossil sediment. Conversely, most of the foraminiferal community can fossilise and be preserved in the fossil sediment. It allows reconstruction of palaeo-environments (e.g., Alve 1991, Alve et al. 2009, Dolven et al. 2013, Polovodova Asteman et al. 2015, Francescangeli et al. 2016). Using fossil benthic foraminifera, it is possible to determine objective and reliable reference conditions. Hence, fossil benthic foraminifera could be used as proxies to assess the deviation from reference conditions for benthic macrofauna communities. However, this requires an algorithm for translating foraminiferal community data into macrofaunal community data (specified macrofaunal species and their abundances). More conceivably, the ecological quality status has to be estimated directly from the foraminiferal data. This requires an environmental classification system using foraminiferal indices. The development of Foram-AMBI (Alve et al. 2016, Jorissen et al. 2018) is one step towards such a system.

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Figure Captions

Figure 1: Map of the sampling stations

Figure 2: Principal components analysis (PCA) of environmental variables: biplot of stations and variables for axes 1 and 2 (75 % of variation). Stations with one or more missing values have been omitted from analysis.

Figure 3: Redundancy analysis (RDA) of foraminiferal species assemblages: triplot of species (crosses), stations (dots) and significant environmental variables (vectors). Most dominant species are indicated by abbreviated names. Stations with missing data for environmental variables or strongly impoverished fauna have been omitted from analysis.

Figure 4: Canonical correspondence analysis (CCA) of macrofaunal species assemblages: triplot of species (crosses), stations (dots) and significant environmental variables (vectors). Most dominant species are indicated by abbreviated names. Stations with missing data for environmental variables or strongly impoverished fauna have been omitted from analysis.

Figure 5: Abundances (ind.m^{-2}) of the main foraminiferal species at stations along (A) the TOC-gradient (%) and (B) the bottom-water dissolved oxygen concentration-gradient (mL $O_2.L^{-1}$). Definition of low (<2.7%), medium (2.7-3.4%) and high (>3.4%) level of TOC follows Molvaer et al. (1997). Definition of normoxia (bottom-water $[O_2]$ > 2 mL $O_2.L^{-1}$), hypoxia (0.5 - 2 mL $O_2.L^{-1}$), severe hypoxia (< 0.5 mL $O_2.L^{-1}$) follows Diaz & Rosenberg (1995). Please note: different scales on the x-axes.

Figure 6: Abundances (ind.m^{-2}) of the main macrofaunal species at stations along (A) the TOC-gradient (%) and (B) the bottom-water dissolved oxygen concentration-gradient (mL O_2.L^{-1}). Definition of low (<2.7%), medium (2.7-3.4%) and high (>3.4%) level of TOC follows Molvaer et al. (1997). Definition of normoxia (bottom-water $[O_2]$ > 2 mL O_2.L^{-1}), hypoxia (0.5 - 2 mL O_2.L^{-1}) and severe hypoxia (< 0.5 mL O_2.L^{-1}) follows Diaz & Rosenberg (1995). Please note: different scales on the x-axes.

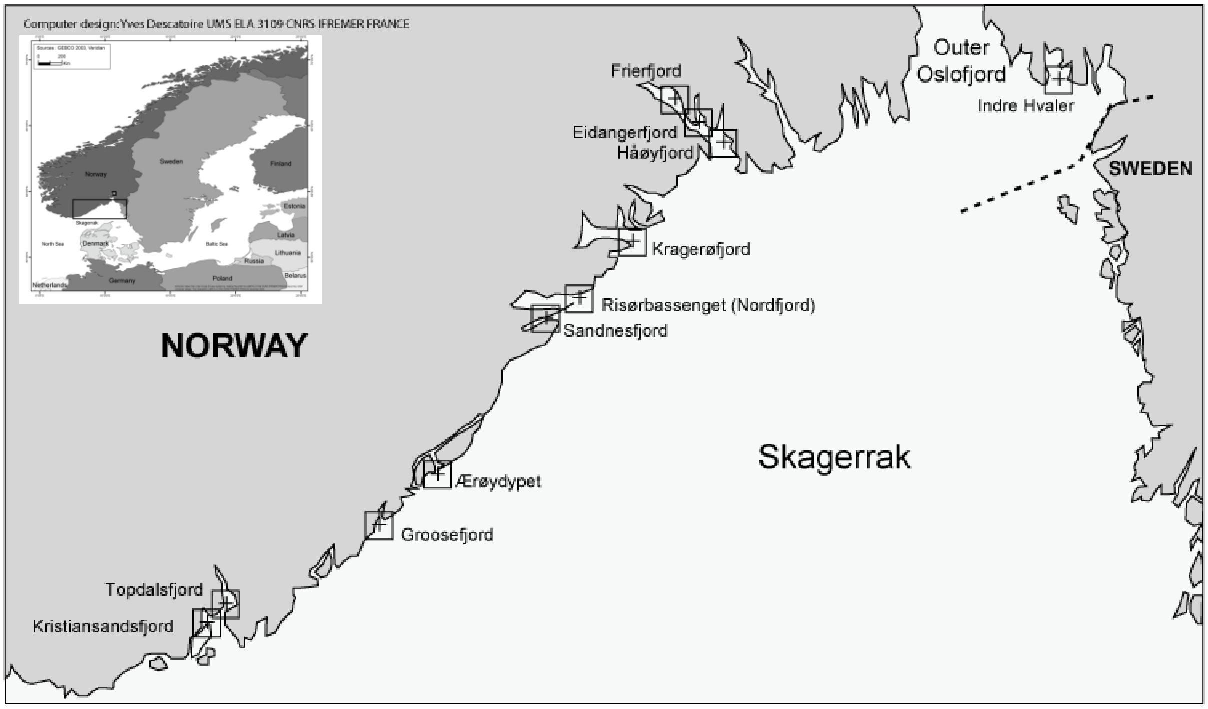
Figure 7: Biplot based on predictive co-correspondence analysis (CoCA) of foraminifera (left) against macrofauna (right) showing 49% and 24% of the total variance in the foraminiferal and macrofaunal data, respectively.

Table captions

Table 1: Characteristics of sampling stations (nd: no data): water depth, depth below threshold (Norwegian Hydrographic Service), bottom-water dissolved O_2-concentration at the time of sampling ($[O_2]_{tos}$, mL O_2.L^{-1}), sediment grain size (%<63µm), total organic carbon (TOC, %), Total nitrogen (TN, %) and C/N ratio of sampling stations. For location of areas, see Fig. 1.

Table 2: Suggested new assignments into EGs for foraminifera and macrofauna species according to Alve et al (2016) and Borja et al. (2000), respectively (na: not assigned).

Figure1


Computer design: Yves Descatoire UMS ELA 3109 CNRS IFREMER FRANCE
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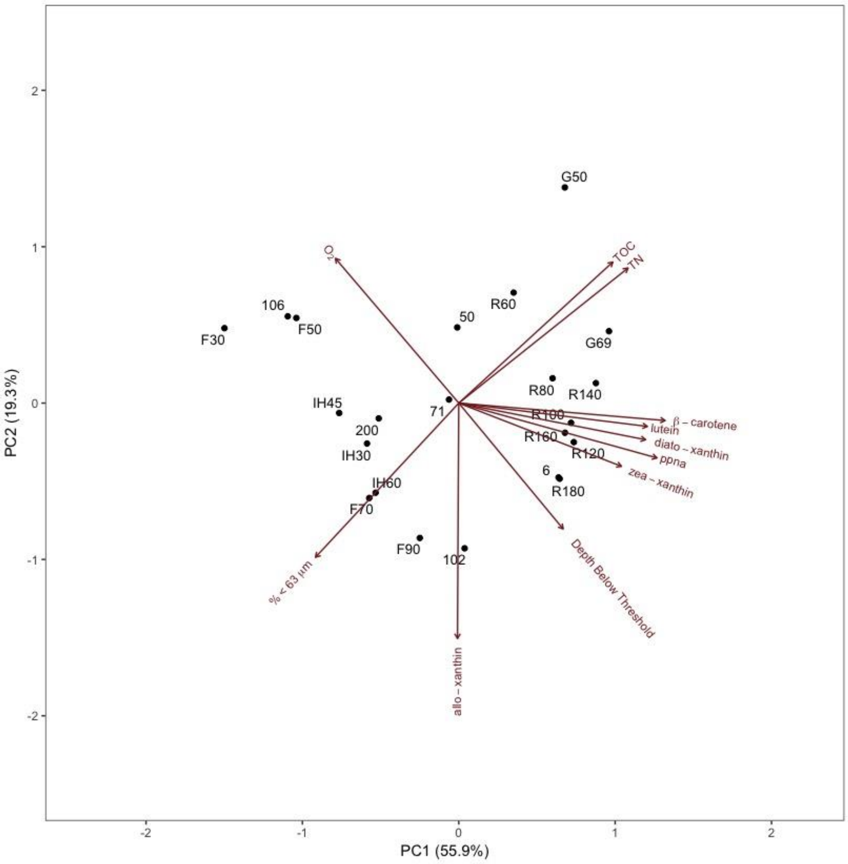
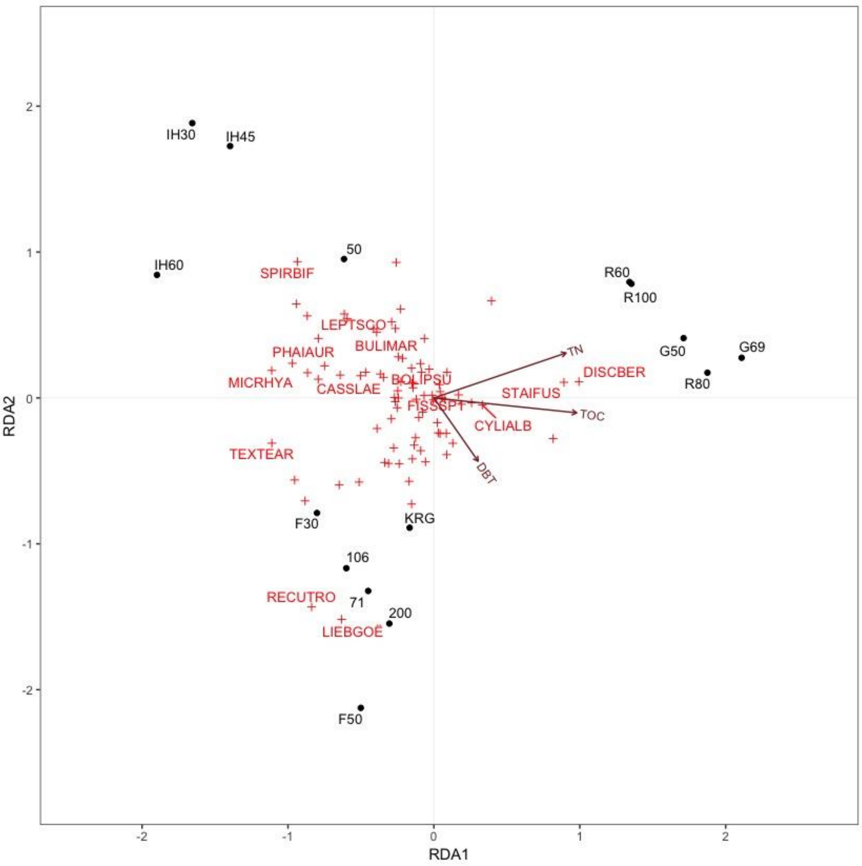
Sweden
Finland
Estonia
Latvia
Lithuania
Russia
Belarus
Poland
Germany
Denmark
Netherlands
North Sea
Baltic Sea
Skagerrak
NORWAY
Frierfjord
Eidangerfjord
Håøyfjord
Kragerøfjord
Risørbassenget (Nordfjord)
Sandnesfjord
Ærøydypet
Groosefjord
Topdalsfjord
Kristiansandsfjord
Outer Oslofjord
Indre Hvaler
SWEDEN
Skagerrak

Figure2



G50
O2
TOC
TN
106
F50
F30
50
R60
G69
R80
R140
IH45
71
R100
β – carotene
lutein
diato – xanthin
ppna
zea – xanthin
200
IH30
R160
R120
6
R180
IH60
F70
F90
102
% < 63 µm
Depth Below Threshold
allo – xanthin
PC2 (19.3%)
PC1 (55.9%)
2
1
0
-1
-2
-2
-1
0
1
2

Figure3



2
1
0
-1
-2
RDA2
-2
-1
0
1
2
RDA1
IH30
IH45
IH60
50
SPIRBIF
R60
R100
G50
G69
R80
LEPTSCO
BULIMAR
PHAIAUR
TN
DISCBER
MICRHYA
CASSLAE
BOLIPSU
STAIFUS
FISSSP1
TOC
CYLIALB
TEXTEAR
DBT
F30
KRG
106
RECUTRO
71
200
LIEBGOE
F50

Figure4

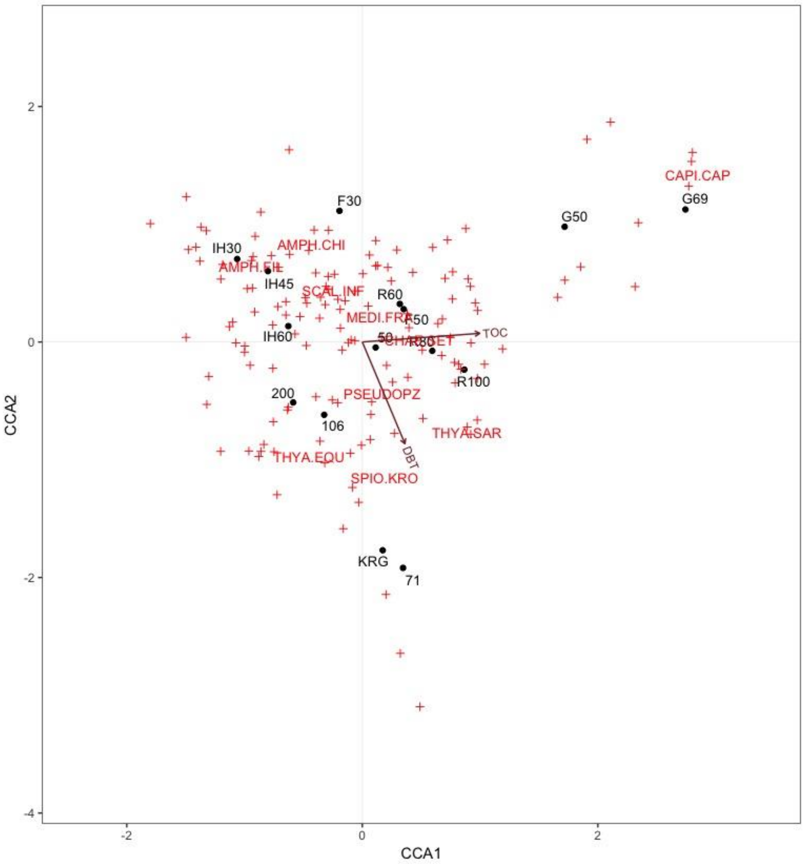

CAPI.CAP
G69
G50
F30
IH30
AMPH.CHI
AMPH.FIL
IH45
SCAL.INF
R60
MEDI.FRA
F50
TOC
IH60
50
CHAR.SET
R80
R100
200
PSEUDOPZ
106
THYA.SAR
THYA.EQU
DBT
SPIO.KRO
KRG
71
CCA1
CCA2
2
0
-2
-4
-2
0
2

Figure5A

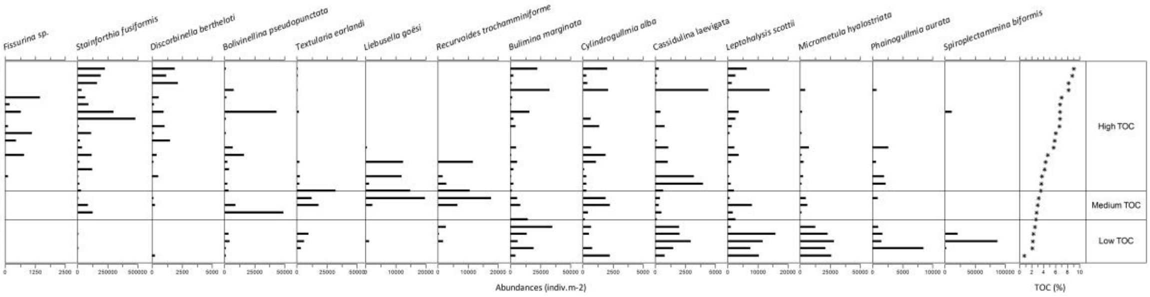


Figure5B

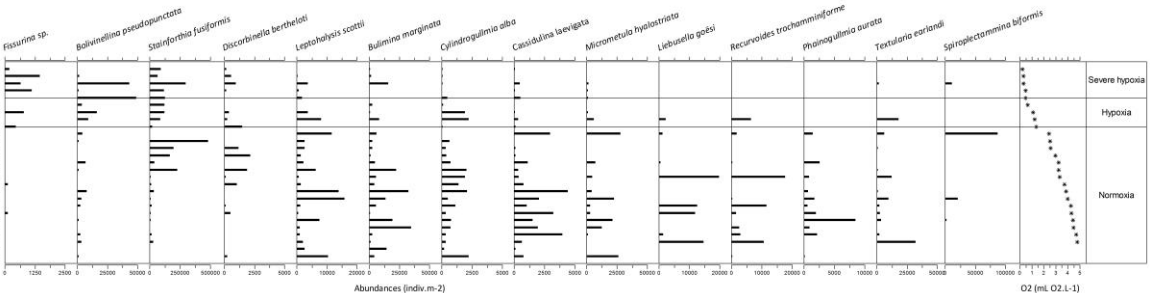


Figure6A

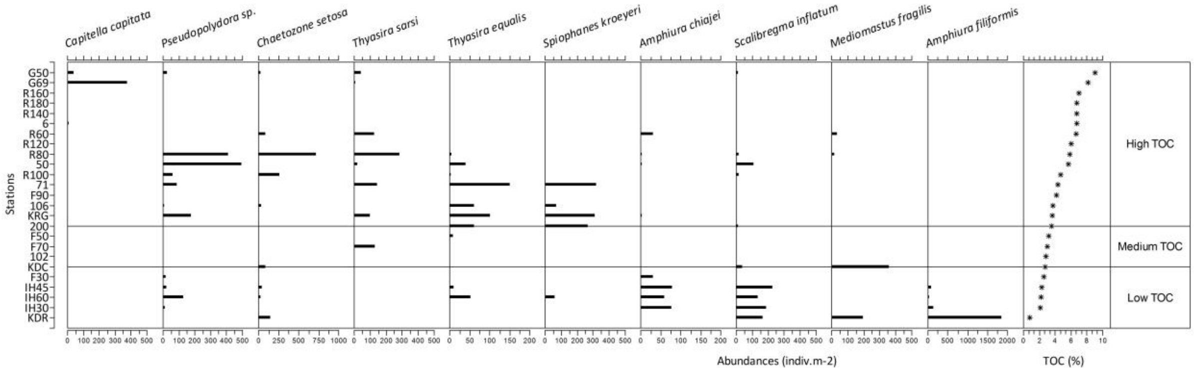


Figure6B

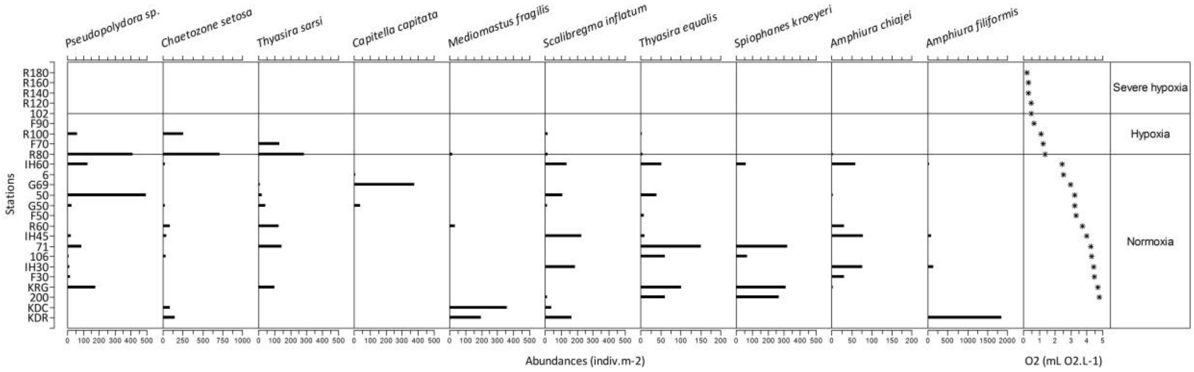


Figure7

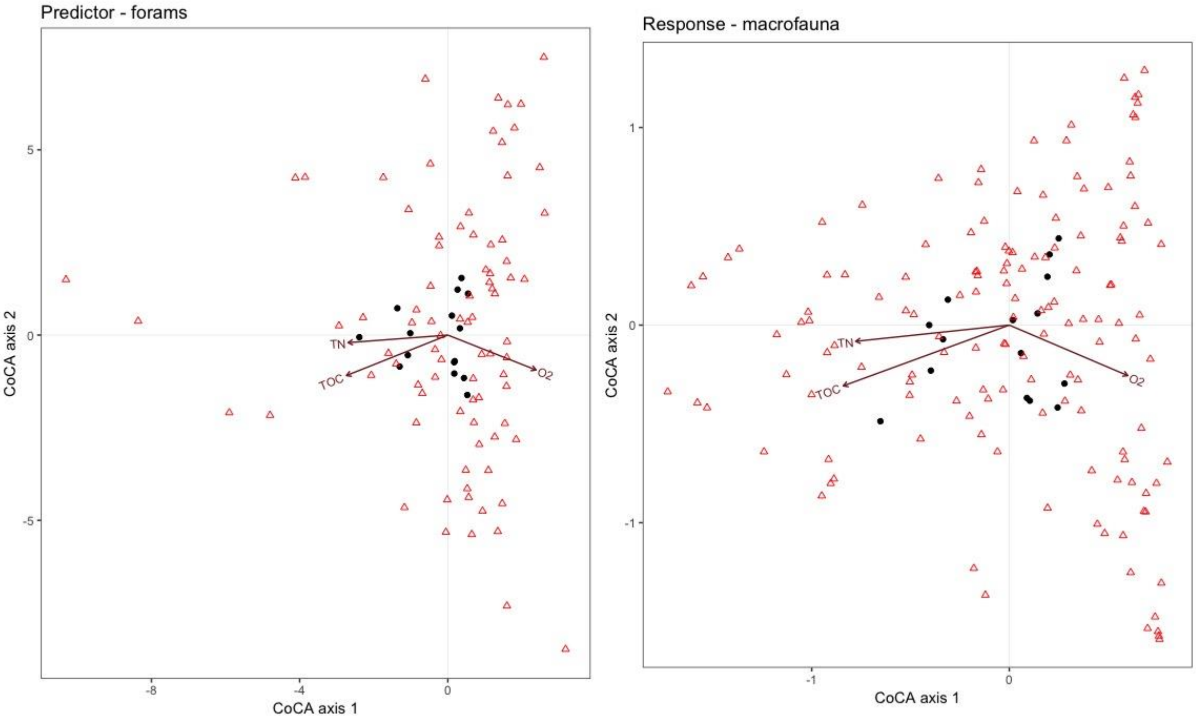

Predictor - forams
CoCA axis 2
CoCA axis 1
5
0
-5
-8
-4
0
TN
TOC
O2
Response - macrofauna
CoCA axis 2
CoCA axis 1
1
0
-1
-1
0
TN
TOC
O2

Table1

	Station	Water depth (m) at stations	Depth below threshold (m)	$[O_2]_{tos}$	Grain size	TOC	TN	C/N
Ærøydypet	200	111	59	4.76	93	3.5	0.4	9.7
Kragerøfjord	71	138	118	4.25	98	4.3	0.5	7.9
	KRG	102	79	4.67	94	3.6	0.5	7.2
Sandnesfjord	50	65	42	3.21	92	5.7	0.4	12.6
Eidangerfjord	106	103	43	4.31	96	3.7	0.2	15.5
Risørbassenget	R60	60	35	3.73	83	6.6	0.9	7.4
	R80	80	56	1.36	89	5.8	0.6	10.1
	R100	104	75	1.09	87	4.7	0.6	8.1
	R120	124	99	0.47	90	6.0	0.6	10.5
	R140	142	118	0.33	83	6.7	0.6	10.7
	R160	157	134	0.29	91	7.0	0.7	10.4
	R180	182	157	0.20	91	6.7	0.7	10.1
Håøyfjord	102	204	169	0.49	98	2.8	0.3	9.7
Topdalsfjord	6	74	42	2.52	94	6.7	0.5	12.5
Groosefjord	G40	40	17	3.85	nd	8.1	0.6	12,9
	G50	54	31	3.25	84	9.0	0.7	12.5
	G60	60	37	2.58	nd	8.8	0.7	11.7
	G69	69	47	2.95	93	8.1	0.7	10.7
Frierfjord	F30	28	8	4.48	98	2.6	0.1	32.2
	F50	52	32	3.30	92	3.2	0.2	13.3
	F70	70	50	1.25	98	3.0	0.1	21.1
	F90	91	70	0.67	98	4.2	0.2	17.8
Indre Hvaler	IH30	30	6	4.44	98	2.1	0.1	14.2
	IH45	45	20	3.98	98	2.3	0.2	14.3
	IH60	62	37	2.42	98	2.2	0.2	13.2
Kristiansandsfjord	KDR	23	nd	nd	58	0.8	0.1	14.6
	KDC	31		nd	52	2.7	0.2	14.6

Table2

FORAMINIFERA	This study EG	Foram-AMBI EG
Micrometula hyalostriata	1	na
Phainogullmia aurata	1	na
Cylindrogullmia alba	2	na
Leptohalysis scottii	4	na
Cassidulina laevigata	2	1
Liebusella goësi	3	2
Bolivinellina pseudopunctata	4	2
MACROFAUNA	This study EG	AMBI-list EG
Scalibregma inflatum	1	3
Thyasira cf. *sarsii*	4 or 5	3