Benthic macrofauna classification system for Faroese fjords

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Menning av flokkingarskipan fyri botndjór á føroysku firðunum

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Samandráttur

Endamálið við hesari verkætlan er at menna eina flokkingarskipan fyri botndjór á teimum føroysku firðunum, sum Umhvørvisstovan skal nýta í sambandi við teirra umhvørviseftirliti av alifirðum. Við íblástri frá grannalondum okkara, sum skulu liva upp til krøvini í Vatnrammu Direktivinum hjá ES, eru tvær mannagongdir royndar; tann danska mannagongdin, sum ikki tekur hædd fyri tí náttúrliga ymisleikanum í firðunum, og ein nýggjur háttur, sum tekur hædd fyri ymiskum dýpum, gløðitapi (LOI) og botnslagi, sum man veit hevur ávirkan á botndjórasamansetingina og tættleika.

Okkara kanning vísti, at teir ymisku firðirnir eru náttúrliga sera eins, og at neyðugt er ikki at menna ymiskar flokkingarskipanir fyri teir ymisku firðirnir, so leingi at man tekur hædd fyri botnslagnum. Hettar tí, at ein botnur, sum t.d. er silt, runa ella móra hevur náttúrliga eitt lægri index virði, samanborið við ein sandbotn, sum inniheldur eitt meiri fjølbroytt umhvørvi og harvið eisini meiri fjølbroytt botndjórasamfelag. Tískil verður mælt til, at tann seinna mannagongdin at menna eina flokkingarskipan verður nýtt, og at hædd verður tikin fyri botnslagnum.

Tó má flokkingarskipanin javnan endurskoðast, tí meiri vanligt verður at ala á meiri harðbalnum økjum, sum hægst sannlíkt hava eitt ørðvísi botndjórasamfelag og harvið eisini náttúrliga ørðvísi index virðir. So hvørt sum fleiri dátur verða tøkar frá slíkum harðbalnum økjum, má tað endurskoðast um m.a. flokkingarksipanin, eisini eigur at taka hædd fyri onnur viðurskiftir, so sum t.d. LOI innihaldið í sedimentinum.

Eitt annað endamál var eisini at kanna um index, sum Aquaculture Stewardship Counsel góðkennir, og sum grannalond okkara, Norra, Svøríki, Danmark og Skotland nýta, eru egnaði at nýta í Føroyum. Fyri at eitt index er egnað at nýta, má tað ávirkast av einum umhvørvistrýstið, sum t.d. Zn, sum verður tilsett fóðrinum í alivinnuni, og er tí eitt beinleiðis mát fyri dálking, sum stavar frá alivirkseminum. Okkara kanning vísti at 8 út av 10 indexum ávirkast væl av Zn, og at best egnaðu indexini vóru IQI, sum verður nýtt í Skotlandi, og NQI, sum verður nýtt í Norra. Mælt verður til, at NQI indexið verður nýtt á føroysku firðunum, tí IQI líkningin inniheldur eitt referansu virði, sum er ásett út frá lokalum viðurskiftum í Stóra Bretlandi.

Abstract

The aim of this project was to develop a classification system for benthic macrofauna analysis in the Faroese fjords, to be used by the Environmental Agency for assessing the potential impact of aquaculture. With inspiration from our neighbouring countries, who have implemented the EU´s Water Framework Directive, two different approaches were tested and compared; the Danish approach that does not incorporate natural variabilities in the fjords, and a novel approach that incorporates depth, Loss on ignition (LOI) in the sediment and sediment types, which are known to affect the natural composition and abundance of the benthic community.

Our analysis shows that when developing a classification system for the Faroese fjords, there is no need to develop individual classification systems for the different fjords, but that the classification system must differentiate between sediment type, since muddy sediments have significantly lower index values for benthic macrofauna compared to sandy sediments, which harbour a more diverse habitat environment, and therefore a more diverse and richer benthic community. Therefore, we recommend the latter approach that differentiates between sediment type to be used for developing a classification system for the Faroese fjords.

However, as new aquaculture sites generally are placed outside fjords at more exposed sites it should be kept in mind that the system will need to be re-evaluated on a regular basis, when more data from these sites become available, since some of these areas might contain a different benthic community and consequently naturally different index values, and that the classification system maybe should differentiate between other factors as well, such as LOI content in the sediment.

Since no specific Faroese multi-metric index has been developed, another aim was to test established indices that the Aquaculture Stewardship Counsel has approved, and those employed by our neighbouring countries Norway, Sweden, Denmark and Scotland. The conclusion was, that 8 out of the 10 candidate indices responded well to Zn as an environmental pressure derived from aquaculture, and that the indices IQI and NQI, which are employed in Scotland and Norway, respectively, were most sensitive to this pressure. Since IQI has a reference variable that is locally determined for Great Britain built into its formulation, it is recommended that the NQI multi-metric index is used in the Faroe Islands, as long as no Faroese index is specifically developed.

1 Background

Anthropogenic activities both on land and at sea can potentially have a huge negative impact on the aquatic environment (Halpern, Walbridge, Selkoe, Micheli, & D´Agros, 2008). Because of this many countries have well-regulated legislations regarding continuous monitoring of both marine and fresh water environments in order to ensure a sustainable usage and thereby a healthy ecological status.

Analysis of the marine benthic macrofauna community is an important tool when assessing the ecological status of the marine environment. This is because benthic macrofauna species are relatively stationary, making them sentinels of the cumulative effect of various environmental stressors. Species that are sensitive to increased environmental pressures will decrease in numbers relative to more tolerant species, and may completely disappear. Hence, increasing environmental pressure will induce a shift in the macrofauna community from sensitive towards more tolerant species. This together with the fact that most benthic macrofauna species live for many years, means that the benthic macrofauna community structure reflects the environmental conditions over longer periods (Josefson, Blomqvist, Hansen, Rosenberg, & Rygg, 2009; Leonardsson, Blomqvist, & Rosenberg, 2009; Pearson & Rosenberg, 1978; Rosenberg, Blomqvist, Nilsson, Cederwall, & Dimming, 2004; Rygg, 2002).

Most countries therefore use marine benthic macrofauna along with other supplementary analysis when assessing the ecological status of the marine environment. For example, the European Water Framework Directive (WFD, Directive 2000/60/EC) states that all member states must assess the ecological status of all types of water bodies through the assessment of biological, hydromorphological, chemical and physico-chemical quality elements. In coastal and transitional waters, benthic macrofauna is one of the most important biological quality elements.

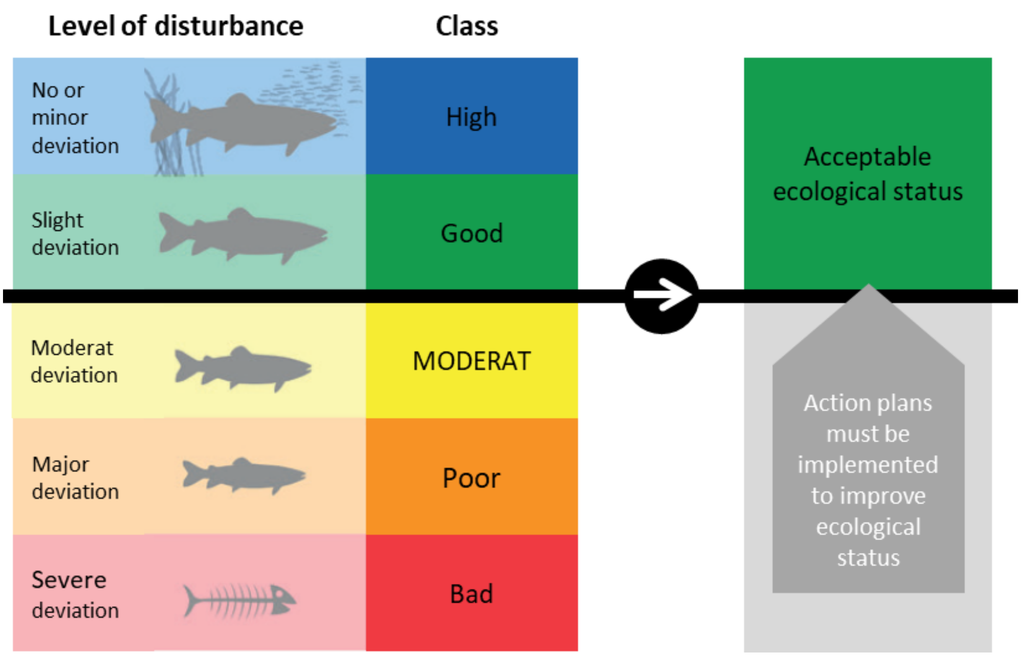


Figure 1.1. Illustration of the five status classification system of the WFD (Direktoratsgruppen Vanndirektivet, 2018).

In order to assess the ecological status of a water body by macrofauna composition, a classification system is needed. For the WFD, all EU member states must develop a classification system with five status classes (high, good, moderate, poor and bad), where the high status is characterised by no or very minor disturbance from anthropogenic activities, and the good status as a slight, but still acceptable disturbance. If the ecological status is classified as less than good, action plans should be implemented to improve the ecological status (Figure 1.1.). The WFD operates in 6-year cycles of assessment and management plans.

A fundamental pillar of the WFD is to characterise the natural ecological status, where biological communities are unaffected by human activities, also known as the reference condition, and to assess if a change from this reference condition has or is occurring in the marine environment. For benthic macrofauna, that means knowing the natural and unaffected composition and abundance of the macrofaunal community, which can then be used as a reference point to assess if changes have occurred in the benthic community due to environmental stressors.

The Faroe Islands are not part of the EU, and therefore are not required to implement the WFD. In fact, there is no legislation regarding continuous monitoring of any type of water body in the Faroes. Nevertheless, the Environmental Agency of the Faroe Islands (Umhvørvisstovan) has since 1998 required that all aquaculture companies, farming Atlantic salmon in the Faroese Fjords, must conduct benthic macrofauna community analyses in the occupied fjords along with relevant chemical and physico-chemical analysis, as part of their impact assessment program. However, the Environmental Agency cannot yet assess the ecological status of the fjords from the benthic macrofauna analyses, since no classification system has been developed for the Faroe Islands. Unfortunately, the macrofauna community was not analysed prior to the establishment of aquaculture in the fjords (i.e., no true baseline). Due to the lack of knowledge regarding the natural benthic macrofaunal composition and abundance in the different Faroese fjords, the development and implementation of a classification system has been hampered. In 2014, it was therefore decided to suspend the requirement for benthic macrofauna analyses until a classification system had been developed for the Faroese fjords.

Due to the complexity of benthic macrofauna communities, these are often characterised by means of various biological indices. Different types of biological indices have been developed and implemented in different countries. One reason for this lack of harmonisation is that the implementation of the WFD is entirely a national responsibility and the directive does not dictate any particular metric to be employed, i.e., it is a framework directive. The only requirement for the metric is that it should describe changes in abundance and composition of the macrofauna community in response to relevant pressures. In most countries, this has been achieved through multi-metric biological indices that include information on composition and abundance of invertebrate taxa and the proportion of disturbance-sensitive taxa (Á. Borja, Marín, Muxika, Pino, & Rodríguez, 2015; Gislason, Bastardie, Dinesen, Egekvist, & Eigaard, 2017; Josefson et al., 2009).

An index value, that represents the natural benthic macrofaunal community can and most often will vary among countries as well as among different regions within the same country. This is because the benthic macrofaunal community is affected by changes in e.g., salinity, depth, level of organic matter, oxygen levels, sediment type, currents etc. (Creutzberg, Wapenaar, Duineveld, & Lopez Lopez, 1984; Pearson & Rosenberg, 1978); changes that are naturally occurring and changes that are enhanced by human activities. This means that some areas will naturally exhibit high index values, whereas other areas will naturally display lower values. Hence, a classification system developed specifically for one country, can therefore not necessarily be implemented in another country without adequate modifications. Importantly, the Faroese fjords differ from the fjords of neighbouring countries by being generally smaller and shallower. This causes the wind to rapidly break down any potential stratification that may have developed. This constant mixing in the fjords enhances the nutrient supply to the surface layer, stimulating primary production. As a consequence, primary production is two to three times higher in the Faroese fjords compared to the fjords in Norway and Iceland (Gaard, Norði, & Simonsen, 2011). This high primary production causes a naturally high organic enrichment on the seafloor in the Faroese fjords (á Norði, Glud, Simonsen, & Gaard, 2018). Organic matter is an important food source for the benthic community, but too much organic matter promotes the development of hypoxia and anoxia, with severe negative effect on the benthic community (Diaz & Rosenberg, 1995; Pearson & Rosenberg, 1978).

The benthic macrofauna diversity in the Faroese fjords is poorly described in the literature, since most studies have focused on registering the different types of species but not the abundance of each species (Sørensen, Hansen, & Joensen, 2007). The availability of an extensive data set on benthic macrofauna from the aquaculture monitoring program gives a unique opportunity to characterise the Faroese benthic community and develop a benthic macrofauna classification system *sensu* the WFD.

2 Aim

The Environmental Agency of the Faroes Islands and the Faroese Aquaculture Association have commissioned Fiskaaling (Aquaculture Research Station of the Faroe Islands) to develop a classification system for soft bottom benthic macrofaunal analysis in the Faroese fjords, to be used by the Environmental Agency for assessing the potential impact of aquaculture. For this assignment Fiskaaling has collaborated with the University of Aarhus in Denmark.

By using two different approaches, described in details in section 4.1 and 4.2, two classification systems will be examined and compared. A specific Faroese multi-metric biological index will not be developed. Instead, the applicability of existing indices that neighbouring countries (Table 2.1) have developed are examined to identify the most suitable index for the Faroes.

Some farming companies are approved by the Aquacultural Stewardship Counsel (ASC) which also requires them to carry out benthic macrofauna analyses. According to the ASC standard (*ASC Salmon Standard. Version 1.3. Aquaculture Stewardship Counsel.*, 2019), the farming companies can choose one out of four predetermined indices to use. The indices that can be used according to the ASC will therefore also be examined for applicability. The indices, 10 in total, investigated in this project are described in details in Annex A.

Table 2.1 A summary of the indices that Denmark, Norway, Sweden, Scotland and the ASC use, and that are being tested in this project.

Land	**DKI**	**H´**	**ES100**	**NSI**	**ISI2012**	**NQI**	**BQI**	**IQI**	**AMBI**	**ITI**
Denmark	X									
Norway		X	X	X	X	X				
Sweden							X			
Scotland								X		
*ASC**		X					X		X	X

* According to the ASC standard, one out of the four indices must be used.

In summary, the aim of this project is to **1)** Develop classification systems, by using two different approaches, **2)** test indices for applicability, and **3)** compare the two approaches and classification systems developed in order to suggest the most suited ones to be used in the Faroe Islands.

3 Dataset

Data for this project are all the benthic macrofauna community analyses that the Environmental Agency and ASC have required from the farming companies. The sampling and taxonomic identifications were all done by an independent company.

A database was developed by the Environmental Agency of the Faroe Islands to hold all the registered taxonomic data available for each benthic macrofauna sample that they have required. A separate database was developed by Fiskaaling to hold the ASC data, that only Fiskaaling has access to.

The data were all quality checked to ensure that all the macrofauna samples were analysed exactly the same way for comparison. Only soft sediment samples taken by 0.1 m^2 Van Veen grabs, and sieved through a mesh size of 1 mm with all individuals counted in the sample were included. Macrofauna individuals were identified to the lowest possible taxon; however, according to the NS-EN ISO16665:2013 (16665:2013, n.d.) standard, which is used in the Faroe Islands for benthic macrofauna sampling, certain taxonomic groups should be registered but not included in the analysis. These taxonomic groups were therefore excluded from the dataset, so that the suggested classification system is coherent with Faroese sampling protocols. The taxonomic groups excluded are:

- Foraminifera
- Nematoda
- Cirripedia
- Colony-forming Porifera, Cnidari[i] and Bryozoa
- Planktonic organisms

All species names were harmonized with the species database WoRMS (www.marinespecies.org), to assure a common standard nomenclature. Replicate sediment samples (most commonly 2 but in cases up to 5) were taken for analysis of the macrofauna community at approximately half of the sampling occasions. A decision was made to treat each replicate as an individual sample.

Environmental data associated with the macrofauna samples were also added to the database. These were loss on ignition (LOI), which is an estimate of the organic content, pH, redox level, and concentrations of zinc (Zn) and copper (Cu) in the sediment. The Environment Agency requires Zn and Cu analysis, since Zn is added to the salmon feed and the nets are sometimes impregnated with Cu. The environmental variables were measured in the top (0-1 cm) sediment from a separate 0.025 m^2 Van Veen grab sample and associated with both replicates (also the case if more than 2 replicates were taken). Sampling position and depth were also registered. In those cases where the depth had not been recorded, the depth was interpolated to the position from bathymetric gridded maps.

Two samples had unrealistically low concentrations of Zn and Cu and were discarded. Similarly, a few extremely high concentrations of Zn and Cu were also discarded as they were most likely measurement faults.

After the data filtering a total of 741 macrofauna data samples, representing almost all the Faroese fjords, were available for this project (Figure 3.1). These samples represent the time period between

[i] Except soft sediment Anthozoa

2002 and 2020. In addition, one sample is taken in 2001, two samples in 2000 and two samples in 1998.

In the database 188 samples are assigned as reference data. According to the ASC these reference samples must be taken at least 500 m from the sea cages to assure that they are not affected by the aquaculture. The Environmental Agency, however, does not specify a specific distance that these reference sample should be taken, but requires that these samples should represent an unaffected state. 40 of the 188 reference samples are newer samples taken before aquaculture started at new farming locations, in an attempt to assess baseline conditions for these new locations. These 40 samples are taken at Sandsvág in 2002 and 2014, at Velbastað in 2013, at Hvalba in 2014, at Vestur á Víkum in 2016 and undir Neslandinum in 2019.

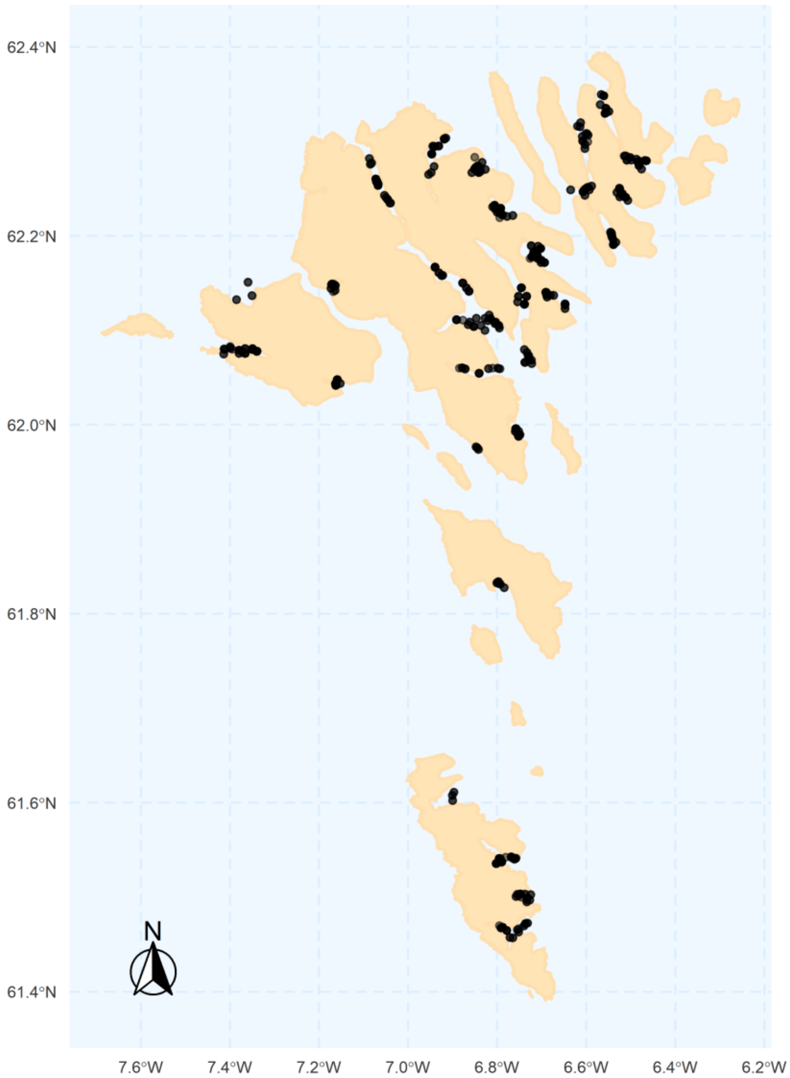


Figure 3.1. Sampling locations (black dots) for benthic macrofauna in the Faroese fjords.

4 Method

As mentioned before, according to the WFD the ecological status must be assessed relative to some deviation from a given reference condition. A classification system is therefore ideally developed by first assessing the reference condition where no or only very minor acceptable anthropogenic changes have occurred, i.e. the high ecological status (Á. Borja, Dauer, & Grémare, 2012), and from these the other status classes are typically derived by defining acceptable deviations from the reference condition.

However, pristine areas with no or even only minor changes are hard to find since most marine ecological systems have been altered in different degrees for centuries due to anthropogenic activity both on land and at sea (Birk & Hering, 2009; Á. Borja et al., 2012; Diaz & Rosenberg, 1995; Johnson, Lindegarth, & Carstensen, 2013; Josefson et al., 2009; Pearson & Rosenberg, 1978).

This challenge is something that most countries face, including the Faroe Islands where no assessments of the macrofauna community were carried out before the initiation of aquaculture in the fjords, besides the 40 references samples taken prior to the start of aquaculture at those particular locations, as mentioned above. Also, the fjords in the Faroes are small with villages or cities occupying them along the coast, inevitably affecting the ecosystem of the fjords.

Acknowledging the challenge that most countries face in finding suitable reference areas, the EU has developed a guidance that identifies four options for deriving reference conditions for transitional and coastal waters (WFD CIS Guidance document NO. 5. COAST 2003). According to the guidance, reference conditions can be estimated using:

1. Currently undisturbed sites, which is the preferred approach
2. Historical data
3. Modelling
4. Expert judgement

From a review that aimed to identify the most commonly used approaches to establish reference conditions, it is clear, that a combination of all four approaches listed above, is the most common procedure when it comes to marine environments (Johnson et al., 2013). However, because of the difficulty in finding pristine reference conditions, many countries do not find their reference data suitable to define the high ecological class. Instead they assume that their reference data contain data of both high and good status, and therefore use their reference data to carefully define the boundary between the good and moderate classes, which also marks the critical threshold where a community changes from an acceptable to an unacceptable state (Johnson et al., 2013). Consequently, the type of data used as reference data varies substantially among countries, as does the methods employed to derive the boundaries in their classification system.

A literature study was therefore carried out at the beginning of this project, to examine how our neighbouring countries Norway, Sweden, Denmark and Scotland have set their reference conditions and developed their classification systems. A decision was made to focus on these countries, due to similar climate and close geographical proximity; particularly Norway and Scotland with similar fjord systems.

While it was possible to find literature that describes the Danish, Norwegian and Swedish approach in details, this was not the case regarding the Scottish approach. Denmark and Sweden use similar data as the ones available for this project, to set their reference conditions, and use them to set the boundary between good and moderate (Blomqvist & Leonardsson, 2016; Henriksen et al., 2014; Leonardsson et al., 2009; Leonardsson, Blomqvist, & Rosenberg, 2016). Norway on the other hand sets higher criteria for their reference data, and use their reference data to set the boundary between good and high. For example, only data taken more than 1 km from industries, waste water effluents, villages (<100.000 inhabitants) and aquaculture facilities, and more than 5 km from larger cities (>100.000 inhabitants) can be used as reference data in Norway (Pedersen et al., 2016). Since the data available for this project are more similar to the ones Denmark and Sweden use, the decision was made to test the Danish and Swedish approach on the data from the Faroe Islands, to develop a classification system. However, after testing the Swedish approach on our data, it became evident that this approach was not suitable (see Annex D for further details).

Therefore, only the Danish approach will be tested, according to the Danish approach a pressure gradient is used to identify thresholds typifying marked changes/deteriorations in the benthic community due to the increased pressure. In other words, threshold levels signifying the change from a tolerable to a non-tolerable situation or alternatively, which can be used to set the boundary between the good and moderate ecological status, and from this, other classes can be derived.

The Danish approach does not include and account for the natural variability that may exist between different areas/fjords, due to, as mentioned above, different depths, levels of organic matter, sediment types etc. Factors all known to affect the natural benthic community. Instead, the Danish approach assumes that the identified data that is unaffected by a pressure gradient represent the same population.

Another novel approach will therefore also be tested for developing a classification system, that incorporates environmental factors associated with each sample (depth, LOI in the sediment and the sediment type), accounting partly for the large natural variability among samples. Hence, two different methods, described in details in section 4.1 and 4.2 will be tested and compared.

4.1 The Danish approach

The Danish approach was developed by Josefson et al., (2009) as an alternative approach to set the boundary between good and moderate, when no pristine reference conditions are available. This approach builds on the assumption that a pressure gradient can identify thresholds reflecting marked changes/deteriorations in the benthic community due to the increased pressure; typically, the change from a tolerable to a non-tolerable situation, and therefore can be used to identify good and high ecological statuses.

In this approach, the different index values are regressed against a pressure variable, using the LOESS smoothing method. The point on the smoothed regression line where a clear change occurs is identified visually, and beyond this point the samples are assumed to reflect an ecological state below good. Data on the regression line before this change commences are assumed to be on the less effected site and therefore represent an at least good ecological status. Using a bootstrap approach, the data on the less impacted site, are randomly resampled with replacement with the same number as the sample size. The lower 5-percentile of each resample is calculated and stored. This procedure is repeated 9.999 times, and finally the median of all the calculated 5-percentiles is calculated, which is used to set the boundary between good and moderate. The statistical software program R (R Core Team, 2021) was used for this purpose.

Denmark has used this approach to set the boundary between good and moderate. The pressure gradient that they used was the distance from a sewage plant in Marselisborg at Aarhus Bight (Figure 4.1) (Henriksen et al., 2014).

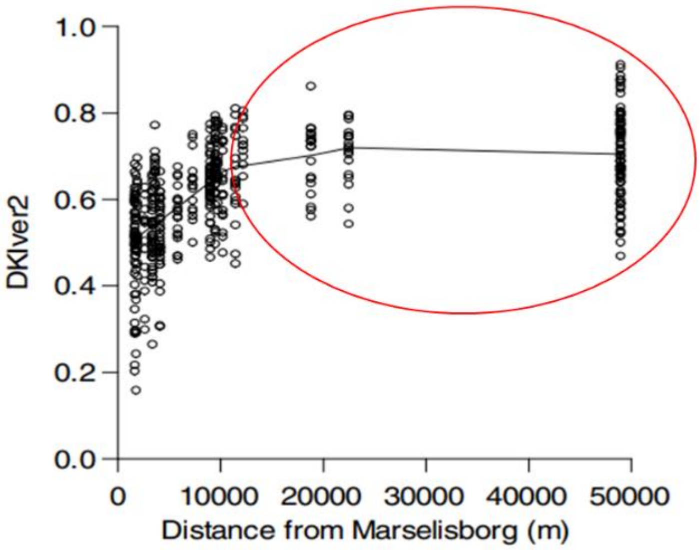


Figure 4.1 In Denmark the distance from a sewage plant in Aarhus bight is used as pressure gradient. In this example it is shown that with increasing distance from the sewage plant the ecological status improves, with a threshold distance around 11 km. The less impacted data (red circle) are resampled 9.999 times, and the 5-percentile is calculated for each resample. Then the median of all the 5-percentiles is calculated and used for setting the G-M boundary (Henriksen et al., 2014).

4.2 Modelling natural and aquacultural influences

As mentioned above the benthic macrofaunal community is affected by changes in e.g., salinity, depth, level of organic matter, oxygen levels, sediment type, currents etc. This means that some areas will naturally exhibit high index values, whereas other areas will naturally display lower values. The Danish approach does not take this in to consideration, but assumes that data on the less impacted site represent the same population. In this approach the natural influencing factors of depth, LOI content in the sediment and the sediment type are accounted for and incorporated when assessing reference conditions used to develop a classification system.

In the database 188 samples are, as mentioned above, grouped as reference data. However, when examining these reference data, it becomes apparent that many of them are taken too close to the aquaculture facilities, with some of them clearly affected, with for example high Zn and Cu concentrations in the sediment. The first step in this approach is therefore to first identify reference data not affected by aquaculture. The method used to identify reference data not affected by aquacultural builds on the development of a pressure gradient with the basic assumption that sediment concentrations of LOI, Zn and Cu are enhanced in the vicinity of aquaculture sites, and that elevated concentrations of LOI, Zn and Cu affect the macrofauna community. This cause-effect chain corresponds to the pressure-state-impact chain of the DPSIR framework (EEA, 1995; OECD, 1993) with aquaculture being the pressure leading to a change in the environmental state causing an impact on the benthic macrofauna.

Section 4.2.1 explains the method used to examine the pressure-state relationship between aquaculture and LOI and Zn, in order to assess data with normal background levels of LOI and Zn. Thus, observations sampled sufficiently far away from the sea cages are considered relatively unaffected by aquaculture. Although aquaculture enhances both LOI, Zn and Cu concentrations in the sediment, only LOI and Zn are considered, since LOI and Zn are both a more general state response in aquaculture, whereas Cu is currently being used less frequently as an anti-fouling agent. However, sediment concentrations of Zn and Cu are strongly correlated such that Zn concentrations will, to a large extent, represent the combined effect of both metal ions. The 40 reference data samples taken before the aquaculture started are assumed not to be affected by aquaculture and are therefore included in the dataset representing reference data.

Section 4.2.2 explains how the model examines how the natural influencing factors of depth, LOI content in the sediment and sediment type effect the reference conditions, and incorporates this into the development of a classification system. This section also explains the method used to examine the 10 indices for applicability.

4.2.1 Pressure-state relationship between aquaculture and LOI/Zn concentrations

Zn ions typically adsorb to organic particles and therefore, the Zn concentrations depend on the amount of organic material in the sediment, proxied by LOI. However, aquaculture also enriches the sediment with organic material, implying that there are causal links between aquaculture and Zn concentrations as well as between aquaculture and LOI. Therefore, relationships between aquaculture and Zn concentrations have to consider both the direct effect of Zn losses from aquaculture on sediment concentrations and the indirect effect through organic enrichment of the sediments from aquaculture that promotes absorption of Zn to sediment particles.

In general, LOI increases with depth as organic particles, because of relatively lower density, are more easily resuspended and deposited at deeper depth than mineral particles (á Norði et al., 2018). In addition to this natural depth gradient, it is hypothesized that there is a gradient with distance from aquaculture, suggesting higher LOI near the aquaculture location and decreasing with distance to approach natural levels. The relationship of LOI versus depth and distance to aquaculture was formulated as:

$$LOI = \left(\frac{a}{1+b \cdot dist^2} + c\right) \cdot depth^d \cdot exp\,(\varepsilon) \qquad \text{(Eq. 4.1)}$$

where the first factor describes the effect of aquaculture on LOI as a sigmoid function of the squared distance ($dist^2$), the second factor describes the depth relationship with a power function, and the third factor describes the residual error with a lognormal distribution. In the first factor, the parameter c describes the background LOI at a given depth, the parameter a describes the LOI enrichment under the cages, and the parameter b describes how fast the change from elevated to natural LOI levels occurs with distance. Thus, $a + c$ describes the LOI under the cages as the combination of a background level and an enrichment from the aquaculture. The reason for squaring the distance in the sigmoid function is that the effect of a point source generally decreases with the squared distance (inverse-square law). The parameter d describes how LOI scales with depth, e.g., a linear relationship for $d = 1$.

Since LOI typically follows a lognormal distribution, the relationship can be formulated for normal distributed variables by applying the log-transformation to Eq. (4.1). Consequently, the model becomes additive

$$ln(LOI) = ln\left(\frac{a}{1+b \cdot dist^2} + c\right) + d \cdot ln(depth) + \varepsilon \qquad \text{(Eq. 4.2)}$$

Similarly, Zn concentrations were also assumed to be higher in the proximity of aquaculture, in addition to naturally higher Zn concentrations in samples taken at deeper depths and with higher LOI. The relationship for Zn concentration versus depth, LOI and distance from aquaculture was formulated as:

$$Zn = \left(\frac{a}{1+b \cdot dist^2} + c\right) \cdot depth^d \cdot LOI^e \cdot exp\,(\varepsilon\,) \qquad \text{(Eq. 4.3)}$$

where the first factor describes the effect of aquaculture on Zn as a sigmoid function of the squared distance ($dist^2$), the second and third factor describe the relationship to depth and LOI, respectively, by means of power functions, and the last factor describes the residual error with a lognormal distribution. The interpretation of the parameters follows the model for LOI (Eq. 4.1). Since Zn concentrations typically follow a lognormal distribution, the relationship can be formulated for normal distributed variables by applying the log-transformation to Eq. (4.3).

$$ln(Zn) = ln\left(\frac{a}{1+b \cdot dist^2} + c\right) + d \cdot ln(depth) + e \cdot ln(LOI) + \varepsilon \qquad \text{(Eq. 4.4)}$$

Marginal relationships for each of the distance, depth and LOI dependencies (Eq. 4.2 and 4.4) were shown by subtracting variations caused by all other effects from the dependent variable (LOI or Zn concentration) and plotting the estimated relationship. For example, the marginal relationship for distance in Eq. (4.2) can be illustrated by normalizing observations to an average depth ($ln(LOI) - \hat{d} \cdot (ln(depth) - \ln(\overline{depth}))$) and calculating the distance relationship $ln\left(\frac{\hat{a}}{1+\hat{b} \cdot dist^2} + \hat{c}\right) + \hat{d} \cdot ln\left(\overline{depth}\right)$ for the average depth.

Both models for LOI and Zn concentration describe a decreasing effect with distance from aquaculture, which at some distance reaches a small proportion (p) of the background concentration. By reformulating the factor for the distance relationship, the range of influence, defined as concentrations exceeding $1 + p$ of the background concentration, can be calculated

$$dist = \sqrt{\left(\frac{a}{c \cdot p} - 1\right) \Big/ b} \qquad \text{(Eq. 4.5)}$$

Here $p = 1\%$ was used to define the range of influence, i.e., beyond the range of influence the effect of aquaculture is less than 1%. Observations beyond the range of influence were considered reference data, representing good and high status (cf. discussion above on lack of pristine conditions).

Reference conditions and boundaries for high-good and good-moderate for LOI and Zn were calculated by estimating simplified versions of models (Eq. 4.2 and 4.4) without the distance relationship using only observations that are sufficiently far away from the cages ($dist_{RI}$=range of influence) such that they represent reference data (or background levels). These observations represent the high and good status classes and therefore, the depth relationship was chosen to represent the boundary between high and good, whereas the upper 95-percentile of the distribution around the regression line was chosen to represent the boundary between good and moderate and the lower 5-percentile was chosen to represent reference conditions. The percentiles were chosen for consistency with methods employed in other countries (e.g., Denmark and Sweden).

The significance of the distance relationships in Eq. 4.2 and 4.4 can be tested by setting $a = 0$ and $b = 0$ using the likelihood ratio test, whereas t-test were employed for the other parameters. These approximate tests are valid given the relatively high number of observations. The models for LOI and Zn (Eq. 4.2 and 4.4) were estimated with non-linear regression using PROC MODEL in SAS software, Version 9.3.

4.2.2 State-impact relationships for benthic fauna

Zn concentrations above the background level represent an affected state, which may influence the benthic faunal community. As described above, 10 candidate indices are proposed for assessing ecological status. These indices have been developed to represent pressure-response relationships between different types of pressures in other countries and consequently, these indices will respond differently to changes in sediment Zn concentrations when applied to Faroese data. In addition to the potential effect of Zn concentrations on benthic fauna, it is also hypothesized that depth, LOI and sediment type (mud versus sand) may influence the benthic community, but the exact nature of these relationships is not known. Therefore, a GAM (Generalized Additive Models) approach was pursued using non-parametric spline functions ($S()$, consisting of a linear and a non-linear component) for Zn, depth and LOI (all log-transformed) and two parameters to describe differences between the sediment types.

$$BFindex = S(\ln(Zn)) + S(ln(depth)) + S(ln(LOI)) + sedtype + \varepsilon \qquad \text{(Eq. 4.6)}$$

This generic model was estimated for all 10 indices and the deviances (i.e., measure of variation) explained by the model and specifically, the component related to Zn, were calculated. The best indices are characterized by high deviance explained by the entire model and importantly, by $S(\ln(Zn))$.

The models for BFindex (Eq. 4.6) were estimated with PROC GAM in SAS software, Version 9.3.

For assessing the pressure-response of the different indices, a sensitivity index was calculated from the slope ($b_{BFindex}$) of the linear component of $S(\ln(Zn))$ normalized by the range in the benthic fauna index.

$$sensitivity(BFindex) = \frac{b_{BFindex}}{range(BFindex)} \quad \text{(Eq. 4.7)}$$

The sensitivity index describes the change in the index relative to its range of variation when the Zn concentration is changing by one unit on the log-scale (i.e., a factor of 2.71).

Reference conditions for the selected indices were calculated as function of depth using two approaches: 1) a simplified version of Eq. (4.6), including only $S(ln(depth))$ and $sedtype$, estimated from observations beyond the range of influence for aquaculture ($dist_{RI}$) and 2) the full model of Eq. 4.6 with reference conditions of LOI and Zn concentrations predicted as function of depth (see above). Since the two approaches produced similar results for the reference conditions (Annex C), the simpler approach (1) was chosen and used for determining boundaries between ecological status classes. As above for LOI and Zn, the relationship with depth was used to define the reference condition (95-percentile), the boundary between high and good (the median) and the boundary between good and moderate (5-percentile).

Importantly, observations of BFindex did not belong to a single population as they were sampled in different fjords, at different sites and with replicates in most cases. Therefore, three random variations were included to describe variation among fjords, variation among sites within fjords and variation among replicate samples at the same site. For characterizing the distribution of reference data, only the variation among sites within fjords was considered, as variations among fjords apply to spatial units equal to or larger than water bodies to be assessed, and variation among replicates was interpreted as a 'measurement error'. Consequently, the natural variability within an assessment unit (e.g., fjord) was described by the variation among sampling sites within fjords. The moderate-poor and poor-bad boundaries were found by employing equidistant status classes for moderate, poor and bad status, i.e., diving the range between the good-moderate boundary and the lowest possible value into three classes of equal size.

5 Results and discussion

5.1 The Danish approach

The pressure variables tested were LOI, Cu and Zn. Out of the total of 741 macrofauna data samples available 520 samples had measured LOI values. The Cu and Zn concentration in the sediment was measured in 512 and 516 samples respectively.

5.1.1 Copper

The Cu pressure gradient ranged between 29.3 and 206 mg kg^{-1} DW, and when this gradient was regressed against the index values, there was a tendency of decreased index values with increasing Cu concentrations in the sediment. No clear break point however, was observed on the regression curve, that could indicate a clear shift from a tolerable to a non-tolerable ecological state.

One reason for the lack of a clear break point on the regression curve can possibly be explained by that fact that the 94% of the 512 samples have a Cu concentration measured below 100 mg kg^{-1} DW, which is normal background level for Cu (Johansen, Hansen, Olsen, & Hoydal, 2006). I.e., the pressure gradient does not contain observations with a high enough Cu concentration.

5.1.2 Loss on ignition

Regarding LOI, there also seems to be a tendency for decreased index value with increased LOI. However, no clear breaking point is observed on the regression line. Consequently, LOI is not considered suitable to be used as a pressure gradient.

5.1.3 Zinc

The Zn pressure gradient ranged between 29 and 485 mg kg^{-1} DW, and when this gradient was regressed against the index values, there was a tendency of decreased index values with increasing Zn concentrations in the sediment. At approximately 60 mg kg^{-1} DW on the regression curve a breakpoint is observed (visually) for all ten indices (Figure 5.1).

In 2006 a study by Johansen and co-workers, sediment samples were taken and analysed at five different Faroese fjords that were considered minimally disturbed by anthropological activity. The aim was to identify normal background levels of different chemicals. That study showed, that the mean Zn concentration in these five fjords was 55 mg kg^{-1} DW, ranging between 41 and 73 mg kg^{-1} DW (Johansen et al., 2006). This fits well with the observed breakpoint at 60 mg kg^{-1} DW, and that samples below this break point are not affected by Zn, and can be used to represent samples of an at least good status.

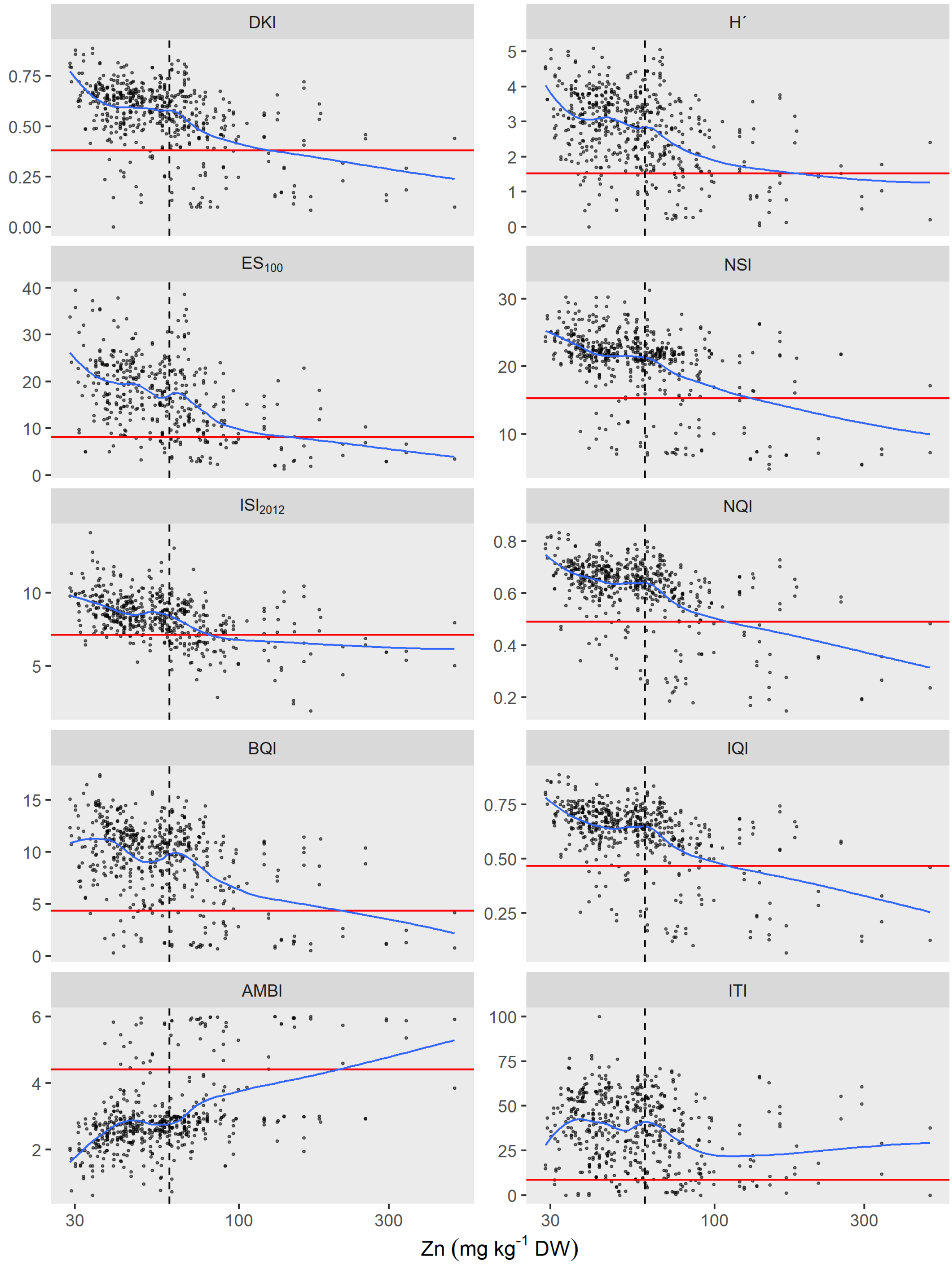


Figure 5.1. Plots of index values against sediment Zn concentrations (log-scale) with a LOESS smoothing nonlinear regression (solid blue curve, span=0.5). Dashed black vertical line at 60 mg kg-1 DW Zn indicates a break point (treshhold) on the regression curve. Horizontal red line denotes the median of the 5-percentile of bootstrapped data on the less impacted side of the threshold, and consequently the boundary between good and moderate classes.

5.1.4 Suggested classification system

Cu and LOI could not be used as a pressure gradient using the Danish approach, since no clear break point was observed on the regression curve. Therefore, the bootstrap approach was only done on data that had Zn concentrations below 60 mg kg^{-1} DW (n=328) which was the visually observed threshold point. The median of the lower 5-percentiles after bootstrapping was calculated and used to set the boundary between good and moderate. AMBI uses a reverse scale. Therefore 95-percentile is used (Figure 5.1).

Table 5.1 summarises the suggested boundaries for the ten indices. The boundary between high and good is set as 2/3 of the interval between the good and moderate boundary and the maximum value. The boundary between moderate/poor and poor/bad were set as 2/3 and 1/3 respectively of the interval below the good/moderate boundary. This is the same approach as used in Denmark and Sweden (Henriksen et al., 2014; Johnson et al., 2013).

Table 5.1. Suggested classification boundaries determined using the Danish approach.

Index	H-G	G-M	M-P	P-B
DKI	0.690	0.379	0.253	0.126
H´	3.760	1.520	1.013	0.507
ES100	26.546	8.092	5.395	2.697
NSI	23.651	15.302	10.201	5.101
ISI2012	13.563	7.126	4.751	2.375
NQI	0.746	0.491	0.327	0.164
BQI	12.178	4.356	2.904	1.452
IQI	0.733	0.466	0.311	0.155
Ambi	1.453	2.907	4.360	5.180
ITI	54.309	8.617	5.745	2.872

5.2 Modelling natural and aquacultural influences

5.2.1 Pressure-state relationship between aquaculture and LOI

LOI was significantly related to both distance from aquaculture ($\chi^2(2)$ = 15.13; P = 0.0005) and depth ($t(1)$ = 9.00; P<0.0001), displaying higher levels near aquaculture sites and at deeper depths (Figure 5.2). The two explanatory variables accounted for 26.1% of the total variation in $ln(LOI)$ (Eq. 4.2), and accounting for variations in one variable yields a clearer relationship for the other (by comparing left and right panel in Figure 5.2).

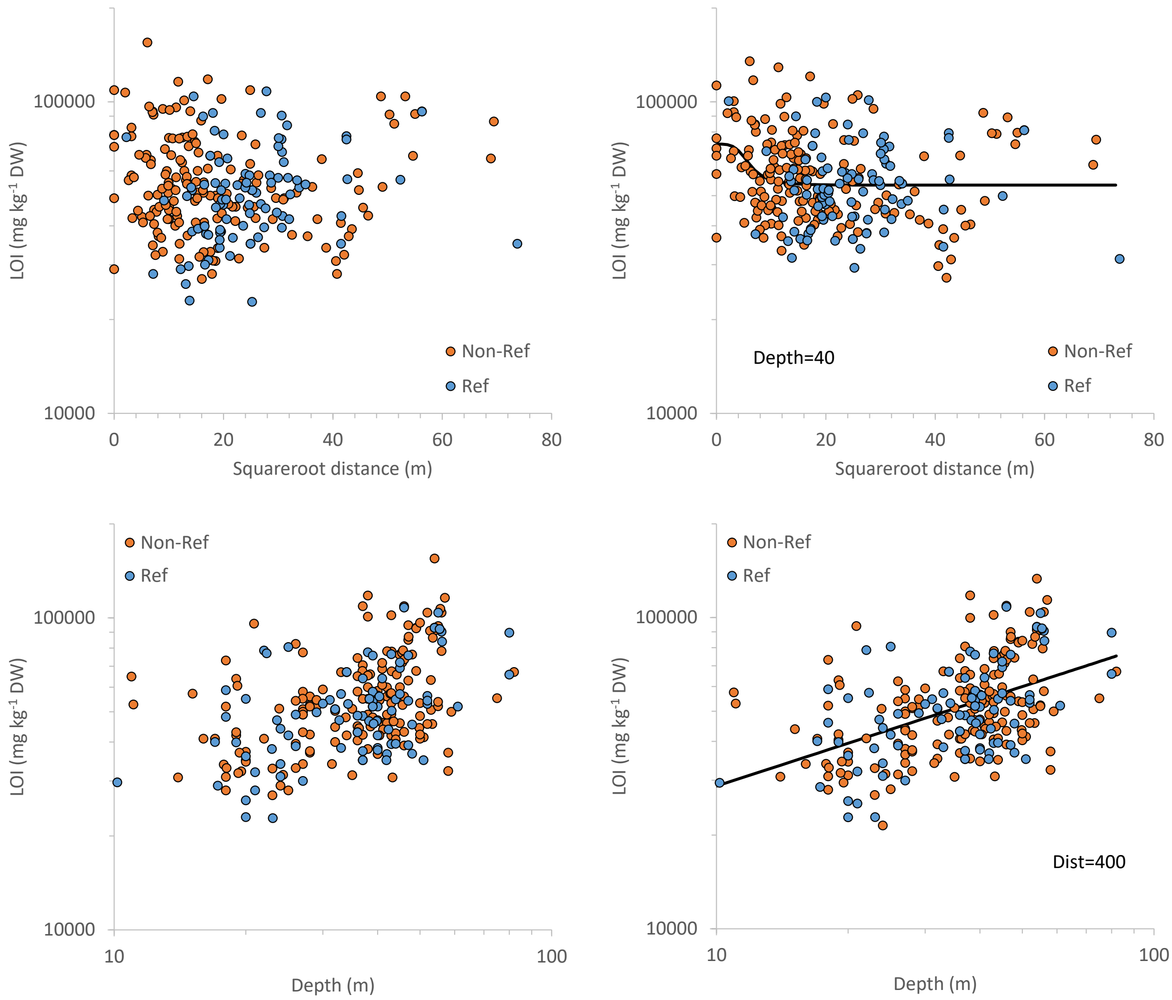


Figure 5.2. Loss-on-Ignition (LOI) in relation to distance from aquaculture and depth. Left: Raw scatter plots of LOI versus distance from aquaculture and depth for reference (blue) and non-reference locations (red). Right: Marginal relationships for LOI versus distance from aquaculture and depth with observations adjusted for variations in the other explanatory variable. Marginal relationships are shown for typical values of the other explanatory variable. In all plots, only observations with both depth and distance recorded are shown. Note that LOI and depth are on a log-scale and that the square root transformation has been applied to distance from aquaculture.

The estimated relationship with distance from aquaculture suggested that the influence of aquaculture was reduced to less than 1% at distances exceeding 204 m. Hence, aquaculture enhances organic matter in the sediments within a range of approximately 200 m. Furthermore, the ratio between the parameter estimates $\hat{a}$ and $\hat{c}$ suggests that LOI is 35% higher under the sea cages relative to the background levels, when depth differences are accounted for. This is consistent with Gillibrand et al. (1996), suggesting that aquaculture may contribute up to 50% of the sediment organic pool.

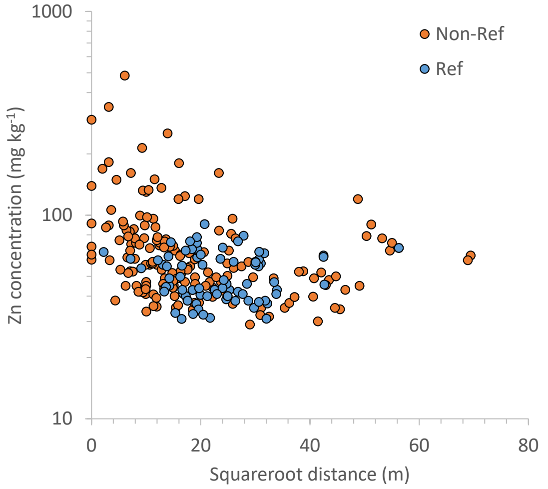
The effect of organic enrichment from aquaculture depends on farming practices (Holmer, Wildish, & Hargrave, 2005) and local environmental conditions (Mayor, Zuur, Solan, Paton, & Killham, 2010). Thus, the spatial range of aquaculture influence varies among the Faroese fjords and the estimated maximum range of 200 m represents an average across all studied fjords. This is supported by the study and modelling of the Faroese fjord Kaldbaksfjørð, that showed that in this particular fjord, organic particles stemming from aquaculture travelled a maximum distance of 116 m from the rings before settling (á Norði, Glud, Gaard, & Simonsen, 2011).

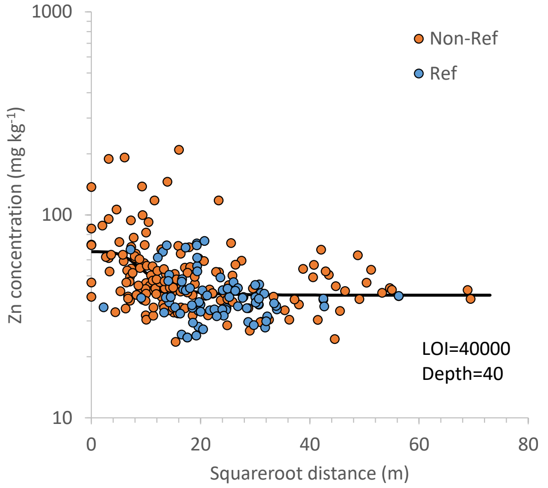
A similar range of influence (~250 m) was found in Kutti et al. (2007) for a Norwegian fjord. However, estimates of such spatial ranges also depend on how the influence is defined. For example, Carroll et al. (2003) investigated organic enrichment of sediments in relation to salmon aquaculture in Norwegian fjords and found significantly higher total organic carbon (TOC) levels in the immediate vicinity of the cages, but this enrichment became less clear (non-significant) at distances of 50-100 m. Using significance testing as a measure to define the range of influence is problematic, as the significance depends on sampling effort within the study. Overall, significant environmental impacts are typically found within 100-200 m from the cages (Mayor et al., 2010; Mente, Pierce, Santos, & Neofitou, 2006). Hence, the estimated range of 200 m in this study is consistent with the current knowledge.

It could be argued that the distance relationship is affected by inaccurate definition of the distance measure, which is assessed at the time of sediment sampling. Cages are typically moved around within an area of the fjord allowed for aquaculture, in order to let the local seabed below the cages fallow frequently, as it was a general evaluation that this was the better strategy when the monitoring started, thus sediment samples could potentially be affected if cages had been placed over the sampling site in the past, i.e., a legacy that is not included in the distance measure. However, Macleod et al. (2004) found that sediments recover relatively fast from organic enrichment (about 2 months). Another study at Kalbaksfjørð also showed substantial improvement in surface sediment condition after only a 39 day break from farming activity, due to combined effect of mineralization and resuspension (á Norði et al., 2011). Therefore, the distance relationship is unlikely affected by such fallowing practices.

5.2.2 Pressure-state relationship between aquaculture and Zn concentration

Zn concentration was significantly related to distance from aquaculture ($\chi^2(2) = 15.13$; P<0.0001), LOI ($t(1) = 11.03$; P<0.0001) and depth ($t(1) = 4.02$; P<0.0001), displaying higher levels near aquaculture sites, organically enriched sediments and at shallower depths (Figure 5.3). The three explanatory variables accounted for 46.8% of the total variation in $ln(Zn)$ (Eq. 4.4), and accounting for variations in the other explanatory variable produces a clearer relationship for the explanatory variable in question (by comparing left and right panel in Figure 5.3).





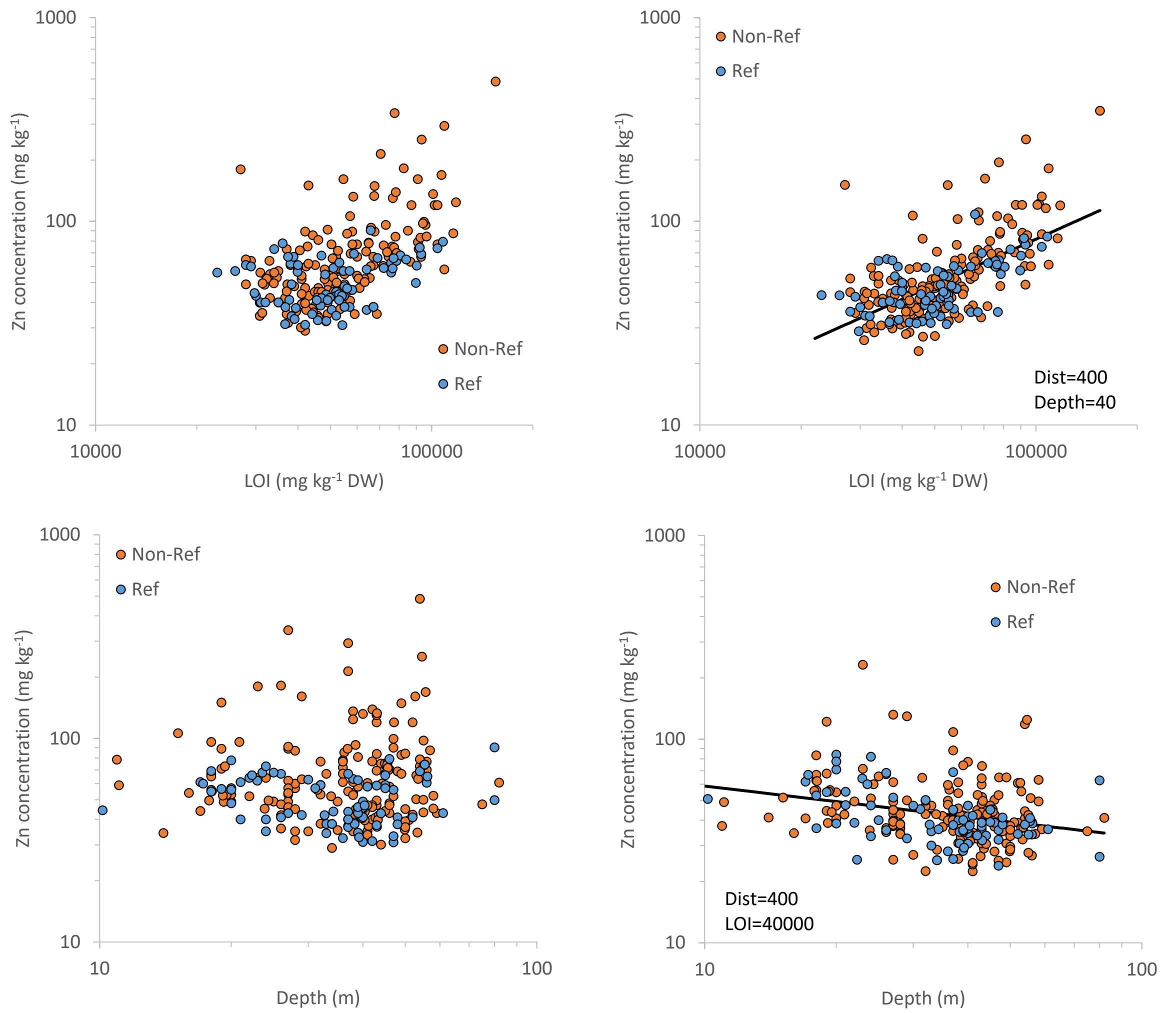


Figure 5.3. Zn concentrations in relation to distance from aquaculture (top), LOI (middle) and depth (bottom). Left: Raw scatter plots of Zn concentration versus distance from aquaculture, LOI and depth for reference and non-reference locations. Right: Marginal relationships for Zn concentration versus distance from aquaculture, LOI and depth with observations adjusted for variations in the other two explanatory variables. Marginal relationships are shown for typical values of the other explanatory variable. In all plots, only observations with depth, LOI and distance recorded are shown. Note that LOI and depth are on a log-scale and that the square root transformation has been applied to distance from aquaculture.

The estimated relationship with distance from aquaculture suggested that the influence of aquaculture was reduced to less than 1% at distances exceeding 723 m. Hence, higher Zn concentrations are observed within approximately 700 m of the cages. This range of influence is larger than for LOI, because organic matter is degraded as opposed to Zn having a longer life in the environment. Furthermore, the ratio between the parameter estimates $\hat{a}$ and $\hat{c}$ suggests that Zn concentrations are 64% higher under the sea cages relative to the background levels, when differences in depth and LOI are accounted for.

Hamoutene et al., (2021) found enriched trace-metal concentrations within 150 m of aquaculture cages in Canada, reaching >600 mg kg^{-1} under the cages for Zn. Dean et al. (2007) estimated that Zn concentrations in Scottish lochs were enriched by 87% under the cages and returned to background levels at 300 m distance. Although these estimates are comparable to our results, it should be stressed that the dispersion of particles and Zn is determined by the prevailing hydrodynamics and consequently, Zn is not uniformly distributed around the cages. The spatial range of influence will naturally be larger for narrow, long-stretched fjords where dispersion is restricted along the major axis, as is the case for several of the Faroese fjords. Hence, it is likely that Zn concentrations are enriched up to 700 m from cages in the Faroese fjords.

The lifetime of Zn in the surface sediments is substantially longer than for organic matter and moving cages within fjords will contribute to further dispersion of Zn. Unfortunately, it has not been possible to retrieve historical records for the exact placement of cages over time. Nevertheless, whereas fallowing practices in which the cages are moved around are good for allowing sediments to recover from organic enrichment, this may also promote spreading of more persistent pollutants such as Zn.

The decreasing relationship with depth, when accounting for variations due to distance and LOI, is most likely caused by increasing dispersion with deeper depths. Export particles from the cages sink with a relatively constant velocity, which for cages located at deeper depths will allow for larger dispersion of Zn because the particles are subject to longer time in the water column. Another possible explanation is that the dispersion is more restricted in the inner fjords that are typically shallower than the outer part.

5.2.3 Reference conditions for LOI and Zn

The simplified model for estimating reference conditions for LOI included only one effect (depth). LOI was significantly related to depth ($t(1) = 6.41$; $P<0.0001$; $R^2 = 19.9\%$) for observations beyond the range of influence of the cages ($dist_{RI}$>200 m). The linear relationship with depth (Figure 5.4) was similar to the marginal relationship with depth estimated using all observations (Figure 5.2) with only minor differences in the slope estimates ($\hat{d}$ = 0.41 versus $\hat{d}$ = 0.46). This suggests that the LOI model (Eq. 4.2) is robust, as the depth relationship is not affected by including/excluding observations influenced by sedimentation of organic matter from the cages. Most observations, including those within 200 m from the cages, were within the range from the good-moderate boundary to the reference condition, indicating that LOI is not severely affected by aquaculture.

The simplified model for estimating reference conditions for Zn concentrations included both LOI and depth. However, the depth relationship was not significant ($t(1) = 0.07$; $P = 0.9464$) when the model was estimated using only observations beyond the range of influence for Zn ($dist_{RI}$>700 m). This suggests that the depth dependency in Eq. (4.4) is associated with aquaculture, i.e., the depth dependency is related to the dispersion from the sea cages rather than a natural depth gradient suggesting that the dispersion is larger at deeper depths. Consequently, LOI appeared to be the only natural source of variation.

Zn was significantly related to LOI ($t(1) = 8.21$; $P<0.0001$; $R^2 = 52.1\%$). The linear relationship with LOI (Figure 5.4) was similar to the marginal relationship with LOI estimated using all observations (Figure 5.3) with only minor differences in the slope estimates ($\hat{e}$ = 0.63 versus $\hat{e}$ = 0.74). Similar to the LOI model, this suggests that the estimated relationship between Zn and LOI is robust and represents a natural gradient unaffected by emissions from aquaculture. A substantial portion of the observations were within the boundaries of good and high status, but there were also a decent number of Zn concentrations that exceeded the boundary line between good and moderate. Such concentrations could potentially render a chemical status less than good for some fjords.

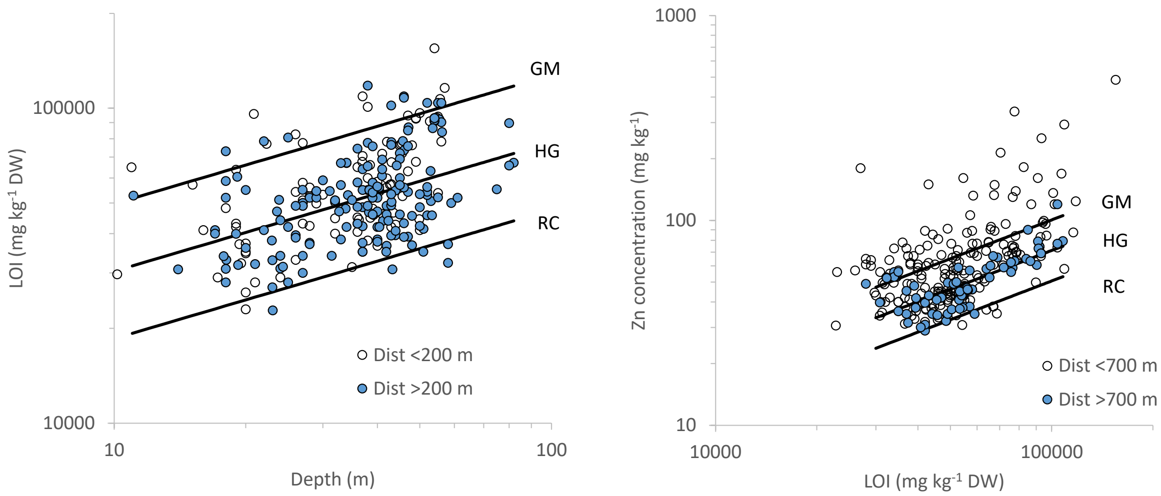


Figure 5.4. Relationship between LOI and depth (left) and between Zn concentration and LOI (right) using only distances sufficiently far away from sea cages to represent background levels. The relationship and the 5- and 95-percentiles of the distribution around the regression line define boundaries and reference conditions. RC= reference conditions, HG = boundary between high and good classes, and GM = boundary between good and moderate classes.

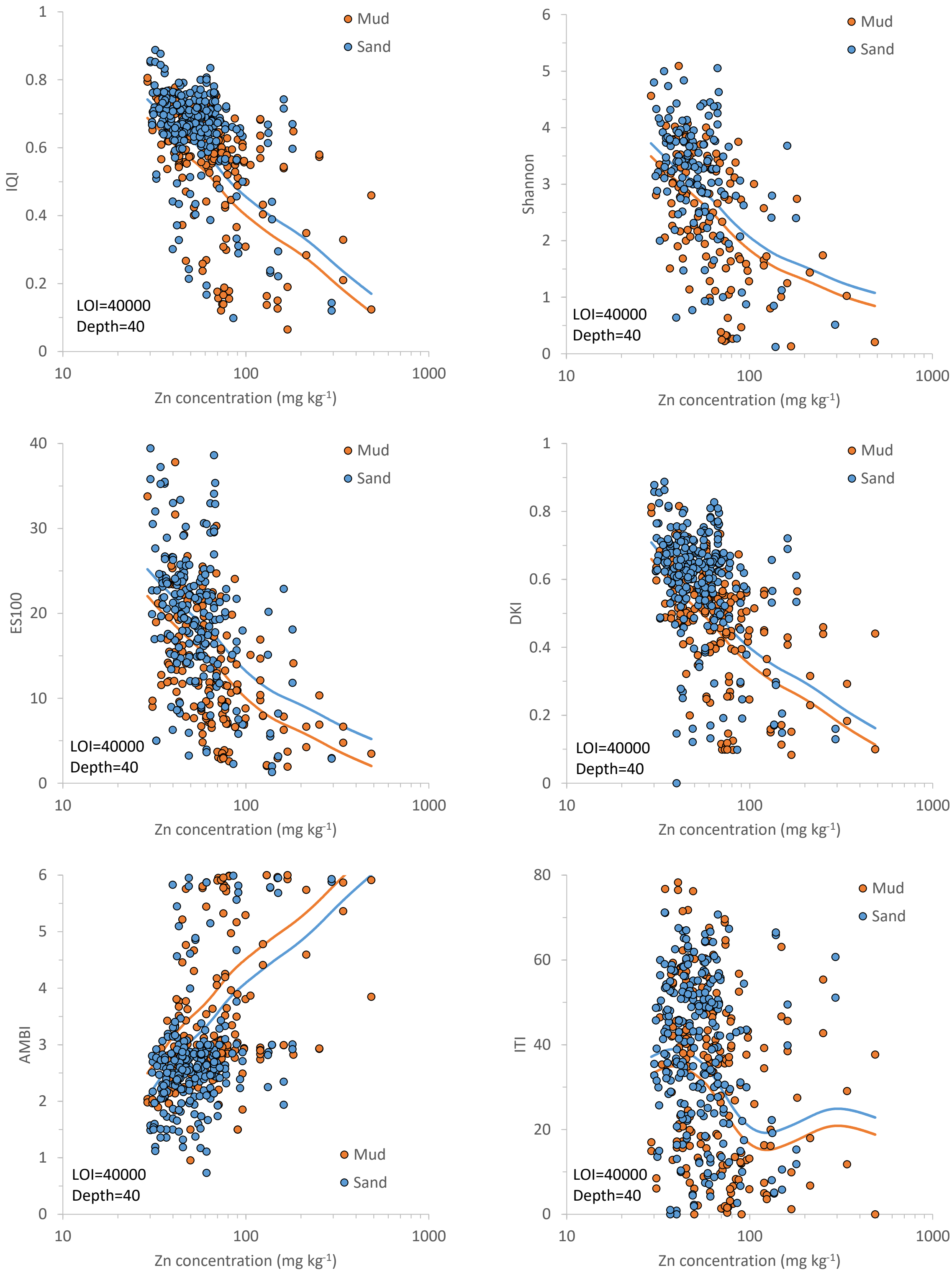
5.2.4 State-impact relationships for benthic fauna

All benthic indices responded significantly to changing Zn concentrations and these responses were primarily linear (Table 5.2). One index (ITI) had a more complex (i.e., non-linear) relationship with Zn, but this was primarily driven by few observations and for a relatively poor model (Figure 5.5). All indices, except AMBI, displayed negative correlations with Zn concentrations, i.e., decreasing index values with increasing Zn concentrations. This is because AMBI uses a reverse scale compared to the other indices. Depth relationships were more variable, but mostly displayed decreasing tendencies with increasing depth (Annex B). Relationships with LOI were also variable and mostly showed increasing tendencies with LOI (Annex B). In addition to these quantitative explanatory variables there was also a significant effect of sediment type for all indices, showing that benthic fauna in sandy sediments generally had better a status when differences in Zn, depth and LOI were accounted for.

Table 5.2. P-values for the different explanatory variables in the benthic index model (Eq. 4.6) and the deviance explained by the model and the spline function for Zn alone. Significant effects (P<0.05) are highlighted in bold.

BF index	$S(\ln(Zn))$		$S(\ln(depth))$		$S(\ln(LOI))$		$sedtype$	Deviance explained	
	Linear	Non-lin.	Linear	Non-lin.	Linear	Non-lin.		Model	$S(\ln(Zn))$
IQI	**<0.0001**	0.2045	**<0.0001**	**0.0172**	**0.0002**	0.0567	**0.0001**	37.0%	21.3%
Shannon	**<0.0001**	0.2307	**<0.0001**	**0.0032**	0.1001	0.0828	**0.0214**	35.3%	12.8%
ES100	**<0.0001**	0.2613	0.0038	**0.0163**	0.3411	**0.0057**	**<0.0001**	37.1%	12.9%
DKI	**<0.0001**	0.1261	**<0.0001**	**0.0207**	**0.0018**	0.1034	**0.0015**	36.2%	18.8%
AMBI	**<0.0001**	0.1458	0.2143	**<0.0001**	**0.0014**	**0.0208**	**<0.0001**	31.7%	18.8%
ITI	**<0.0001**	**0.0003**	**0.0115**	**0.0279**	**0.0102**	0.1579	**0.0485**	14.3%	7.3%
NQI	**<0.0001**	0.2308	**0.0006**	**0.0094**	**0.0014**	**0.0282**	**<0.0001**	38.1%	21.0%
ISI2012	**<0.0001**	0.0684	**<0.0001**	0.6033	0.1439	0.2027	**<0.0001**	43.9%	9.6%
NSI	**<0.0001**	0.4825	0.0854	**<0.0001**	**0.0057**	0.0810	**0.0018**	32.3%	19.0%
BQI2009	**<0.0001**	0.0948	**0.0067**	**0.0170**	**<0.0001**	0.0591	**0.0017**	28.0%	16.5%

The model (Eq. 4.6) could explain >35% of the variation for 6 out of 10 indices, and for two of these (IQI and NQI), variations in Zn concentrations alone explained more than 20% of the variation (Table 5.2). This suggests that these two indices have the strongest responses to the aquaculture pressure, described with the Zn concentration in the sediment. However, other indices (DKI, AMBI and NSI) also performed well, reaching almost similar goodness-of-fit statistics.



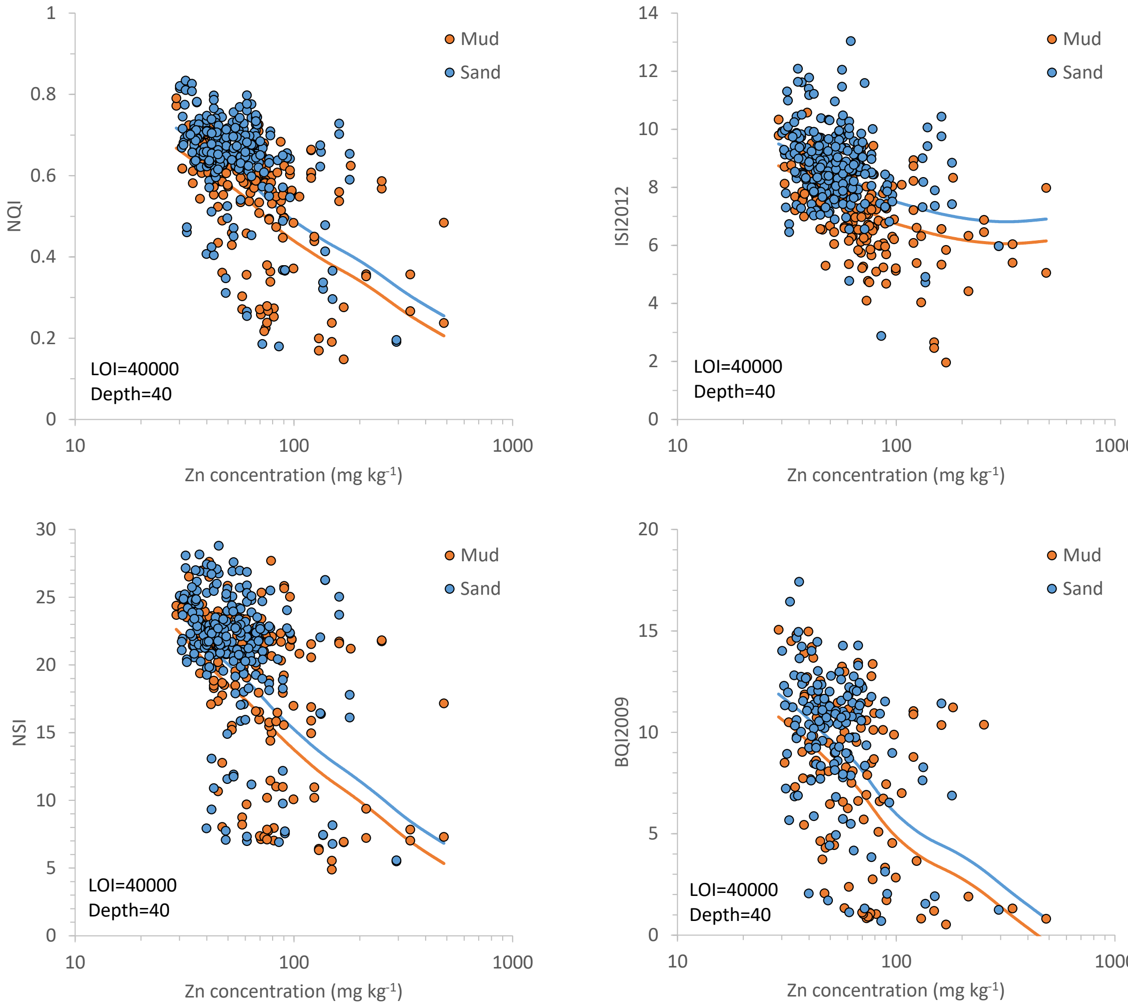


Figure 5.5. Marginal relationships for the 10 benthic fauna indices (predicted for LOI = 40000 mg kg^{-1} DW and depth = 40 m) versus Zn concentration (Eq. 4.6) plotted with the raw observations. Observations and relationships for mud and sand use same colour code, red and blue respectively. Note that Zn concentrations are shown on a log-scale. Marginal relationships for LOI and depth are found in Annex C.

The sensitivity of the indices to changes in Zn concentrations was similar for eight of the 10 indices candidates; only ITI and ISI2012 performed considerably poorer (Figure 5.6). Thus, the majority of the indices respond with the same sensitivity across the Zn scale. However, despite a well-defined gradient of Zn concentrations in the data set, not all indices exhibited an expected continuous decline for all Zn ranges (Figure 5.5). DKI, AMBI, NSI and BQI2009 showed stronger tendency to separate observations into distinct clusters with overlapping ranges of Zn concentrations, whereas IQI and NQI appeared to produce more gradual responses to changing Zn concentrations (Figure 5.5). Hence, the marginal relationships for IQI and NQI correspond better to the expected pressure-response behaviour of the benthic fauna.

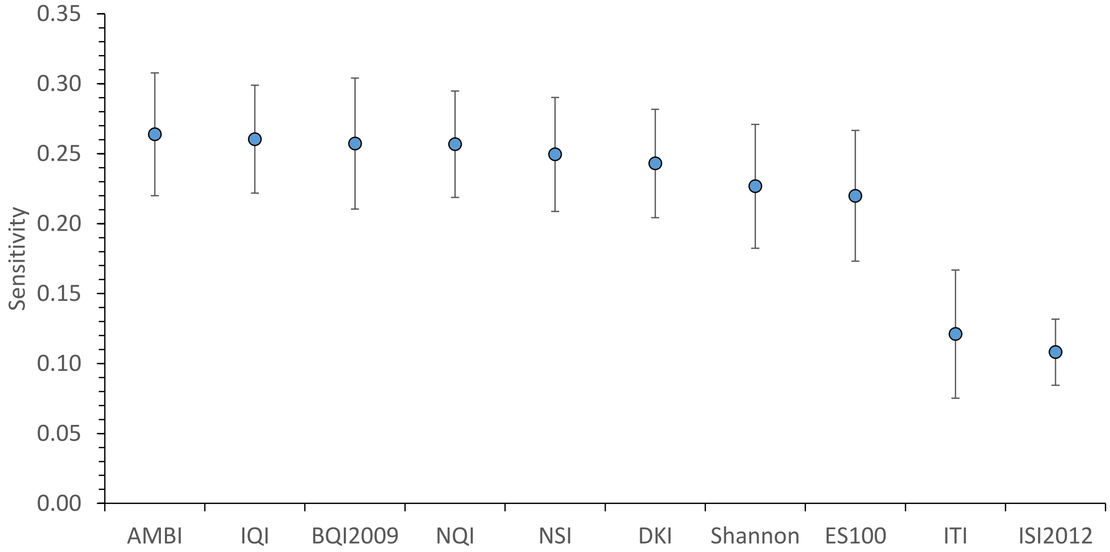


Figure 5.6. The sensitivity index for the 10 candidate indices in ranked order. Error bars mark the 95% confidence interval of the sensitivity index based on the standard error of the slope estimate ($b_{BFindex}$).

5.2.5 Suggested classification system for the most applicable indices

Our analysis showed that all indices responded well to Zn as an environmental pressure, with the exception of ITI and ISI2012 (Table 5.2). However, NQI and IQI had the strongest response to Zn. How the boundaries in the suggested classification system are set for all the indices is described below, by using two of most promising benthic fauna indices, IQI and NQI, as examples.

First the distribution of the IQI and NQI index values were examined for all observations more than 700 m from the sea cages representing a background or reference distribution, and for the 34 out of 40 samples taken prior to the aquaculture started (6 of the 40 samples did not have a depth registration and were therefore not included, these 6 samples excluded are taken Vestur á Víkum); all assumed to represent reference data (Figure 5.7). One sampling occasion in one fjord was excluded from this analysis as the benthic fauna exhibited clear signs of impoverishment. The two approaches for calculating reference conditions produced similar results for the two best candidates (Annex C) and therefore, the simple model approach was chosen, including only depth and sediment type (Figure 5.8).

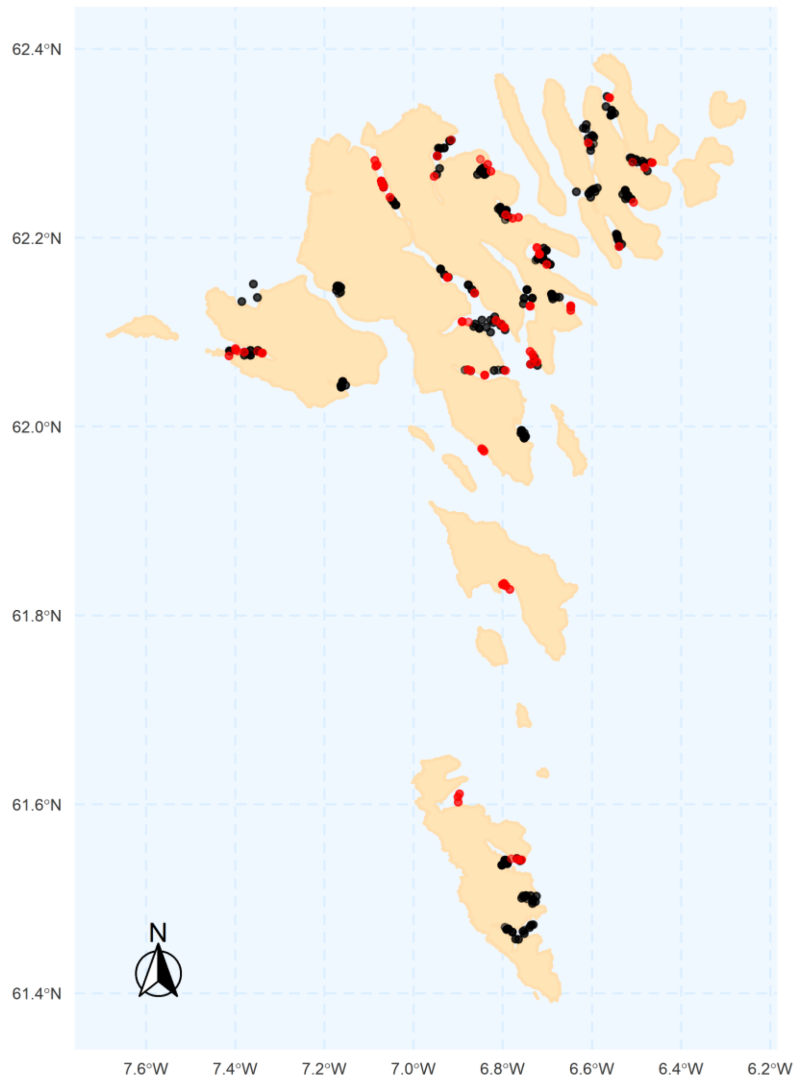


Figure 5.7 Sampling locations (black dots) for benthic macrofauna in the Faroese fjords. Red dots represents refecerence samples, assumed not affected by aquaculture.

For the simplified version of Eq. (4.6), including only $S(ln(depth))$ and $sedtype$, the non-linear components of $S(ln(depth))$ were significant for both IQI and NQI (P<0.0001 for both), showing a tendency for higher values at intermediate depths at around 30 m (Figure 5.8). The linear components of $S(ln(depth))$ were not significant (P = 0.9511 and P = 0.7167), but there was a highly significant difference between sediment type for the two indices (P<0.0001 for both the linear and non-linear), with sandy sediments having higher index values compared to muddy sediments. Same tendency regarding depth and sediment type was also observed for all other indices except ES100, ISI2012 and ITI, which were the three indices with the poorest performance in responding to Zn concentrations (Figure 5.6).

The reason for why the index values seem higher at intermediate depths at around 30 m is not clear. One reason can be that the two muddy samples taken at ~11 m values (Figure 5.8) have inaccurate depth registrations, since muddy samples are more characteristic for samples taken at deeper depths, and typically have lower index. Other samples taken at that particular location in Sørvág have been taken at 30-40 m depths, supporting the theory that these two depths registrations are most likely inaccurate.

Another reason for why index values seem to be higher at intermediate depths, and lower at shallower depths (<30 m) can also be explained by the fact that some of these shallower samples (<30 m) have very low LOI values (<20.000), which is significantly lower compared to all the other samples in our dataset. When these samples with low LOI values are examined closer it becomes clear that these samples are relatively new and have been taken prior to onset of fish farming (triangles in Figure 5.8) at Sandoy and Velbastað. New fish farming sites are primarily located in exposed areas with strong tidal currents and high exposure to waves, which is characteristic for these two particular locations. The explanation might therefore just be, that even though these samples are taken at shallower depths and are registered as sandy sediment types, and therefore are expected to have higher index values, they have lower index values, simply because they derive from a different and more exposed environment, compared to the other samples taken at more protected areas, and therefore contain a different benthic community. When all the samples, taken prior to onset of farming, are excluded from our dataset, the depth variable becomes non-significant.

Since the current number of samples from such exposed sites is relatively low and that the tendency is mostly a result from two of the new sites, it was chosen to only use sediment type as governing factor for now (Figure 5.8). This was also chosen, since excluding data from new sites taken prior to onset of aquaculture, did not significantly change the boundary values (see Annex E that contains boundary levels for IQI and NQI when pre-aquaculture samples are excluded). In the future when more samples from such sites have been gathered, it is possible that the tendency for higher values at intermediate depths will become even more clear and that the classification system will need to be grouped even further.

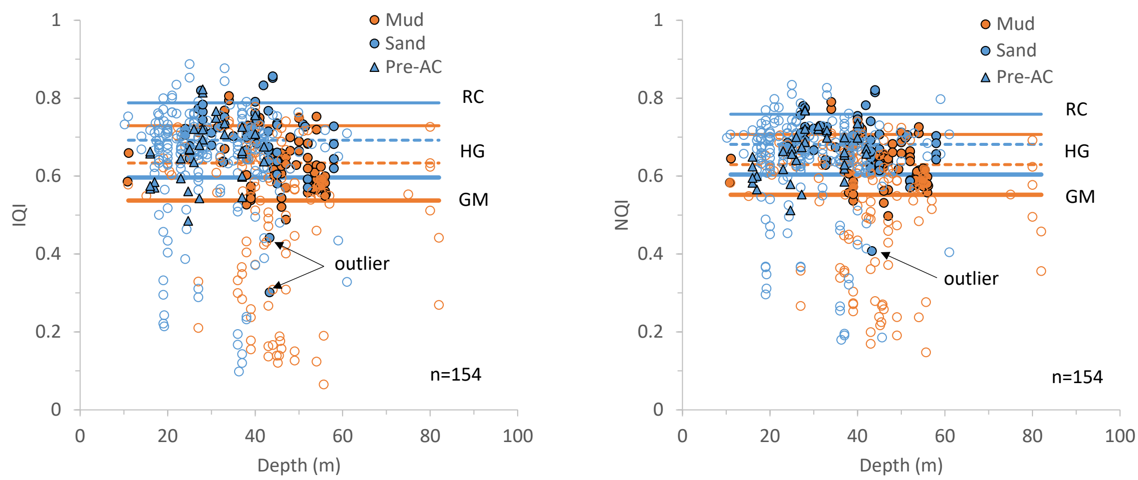


Figure 5.8. Estimated relationships for reference conditions (RC, solid lines) and boundaries between high and good (HG, dashed lines) and between good and moderate (GM, solid lines) for the two best candidate indices (IQI and NQI) versus depth. Observations and relationships for mud and sand use same colour code, red and blue respectively. Open symbols are observations within 700 m from sea cages and were not used for estimating reference conditions and boundaries. Triangle symbols show observations taken before aquaculture was established in the fjord. The numbers of observations for estimating reference conditions, HG and GM boundaries are inserted.

Consequently, reference conditions and boundaries depended only on sediment type (Table 5.3). Whereas reference conditions, high-good (HG) and good-moderate (GM) boundaries were found from the distribution of the indices using observations more than 700 m from the sea cages and observation done prior to aquaculture, moderate-poor (MP) and poor-bad (PB) boundaries were found as 2/3 and 1/3 of the good-moderate (GM) boundary, respectively.

Table 5.3 Proposed values for reference conditions and class boundaries for the indices in different sediment types.

	IQI		NQI	
	Mud	Sand	Mud	Sand
Reference conditions	0.729	0.788	0.707	0.759
H-G boundary	0.633	0.692	0.630	0.681
G-M boundary	0.537	0.596	0.552	0.604
M-P boundary	0.358	0.397	0.368	0.403
P-B boundary	0.179	0.199	0.184	0.201
	ISI2012		NSI	
	Mud	Sand	Mud	Sand
Reference conditions	9.288	10.678	25.853	26.811
H-G boundary	7.901	9.291	22.685	23.643
G-M boundary	6.514	7.903	19.516	20.474
M-P boundary	4.343	5.269	13.011	13.649
P-B boundary	2.171	2.634	6.505	6.825
	Shannon		ES100	
	Mud	Sand	Mud	Sand
Reference conditions	3.636	4.229	24.221	30.896
H-G boundary	2.661	3.255	15.630	22.305
G-M boundary	1.687	2.281	7.039	13.714
M-P boundary	1.125	1.521	4.693	9.143
P-B boundary	0.562	0.760	2.346	4.571
	DKI		AMBI	
	Mud	Sand	Mud	Sand
Reference conditions	0.687	0.760	2.001	1.696
H-G boundary	0.567	0.640	2.740	2.435
G-M boundary	0.567	0.519	3.479	3.173
M-P boundary	0.378	0.346	4.319	4.115
P-B boundary	0.189	0.173	5.160	5.058
	ITI		BQI	
	Mud	Sand	Mud	Sand
Reference conditions	59.122	59.873	12.446	14.480
H-G boundary	37.228	37.979	9.334	11.368
G-M boundary	15.334	16.086	6.223	8.256
M-P boundary	10.223	10.724	4.148	5.504
P-B boundary	5.111	5.362	2.074	2.752

The natural variability of the indices within a fjord was estimated to ±0.058 and ±0.047 for IQI and NQI, respectively (Table 5.4). In comparison, the random variability among fjords was ±0.029 and ±0.022 for IQI and NQI, whereas the variation among replicates was ±0.033 and ±0.027 for IQI and NQI. These estimates show that the variation among sites within a fjord is the largest source of variation and approximately twice as high as the variation among samples taken at the same site. Similar results were obtained for the other benthic fauna indices. The variation among fjords was generally low and not significant ($P>0.05$) for most indices and only weakly significant for the four remaining indices (0.0<<P<0.05), highlighting that undisturbed benthic fauna communities are similar across Faroese fjords. This means that when it comes to a classification system, there is no need to develop one system specific for each fjord in the Faroe Islands, but that the classification system must, as mentioned above, differentiate between sediment type.

Table 5.4 Variability in the 10 benthic fauna indices estimated as variance components in a mixed model.

	Estimated standard deviations for different sources of variability		
Index	Fjords	Sites within fjords	Replicate samples
IQI	±0.029 (NS)	±0.058	±0.033
NQI	±0.022 (NS)	±0.047	±0.027
ISI2012	±0.436 (NS)	±0.843	±0.759
NSI	±1.801	±1.926	±1.153
Shannon-Wiener	±0.588	±0.592	±0.396
ES100	±2.968 (NS)	±5.223	±2.791
DKI	±0.055	±0.073	±0.047
Ambi	±0.000 (NS)	±0.449	±0.258
ITI	±5.134 (NS)	±13.310	±8.656
BQI	±1.677	±1.892	±1.113

5.3 Classification examples

For illustrating the application of the class boundaries for IQI and NQI (Table 5.3) in practice, data from two fjords (labelled A and B) were selected to exemplify status classification (Figure 5.9). About half of the observations were below the good-moderate (GM) boundary for both IQI and NQI in Fjord A, whereas only 2 out of 12 observations were below the good-moderate boundary for both IQI and NQI in Fjord B. In Fjord A, observations below the good-moderate boundary were mostly from mud sediments. There was also a stronger gradient with distance from sea cages in Fjord A compared to Fjord B (Figure 5.10), which resulted in a generally poorer status. Noteworthy, the two indices displayed very similar values for the two fjords, indicating that they express the same features of the benthic fauna community.

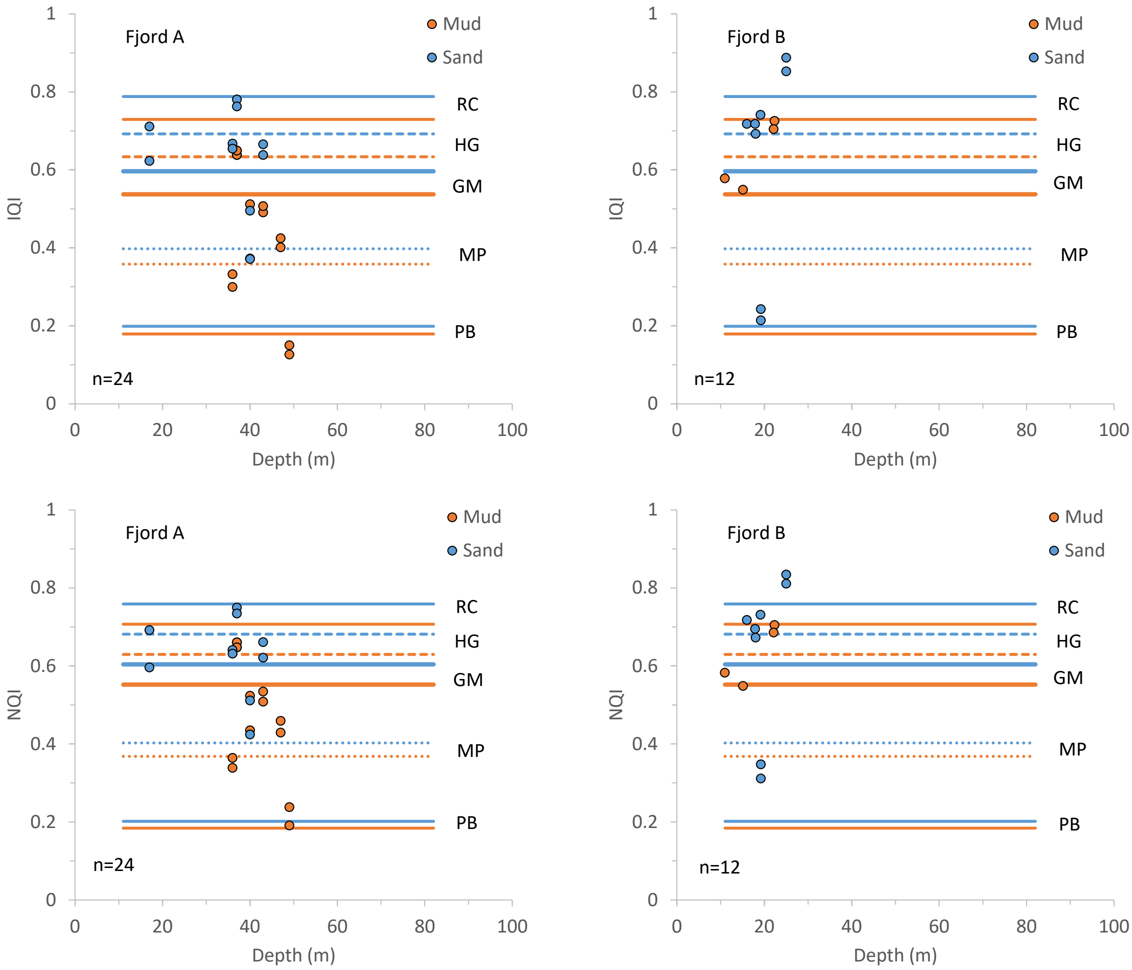


Figure 5.9. IQI (top) and NQI (bottom) observations from two fjords versus depth with proposed reference conditions and class boundaries (Table 5.3). Observations and boundaries for mud and sand use same colour code, red and blue respectivly.

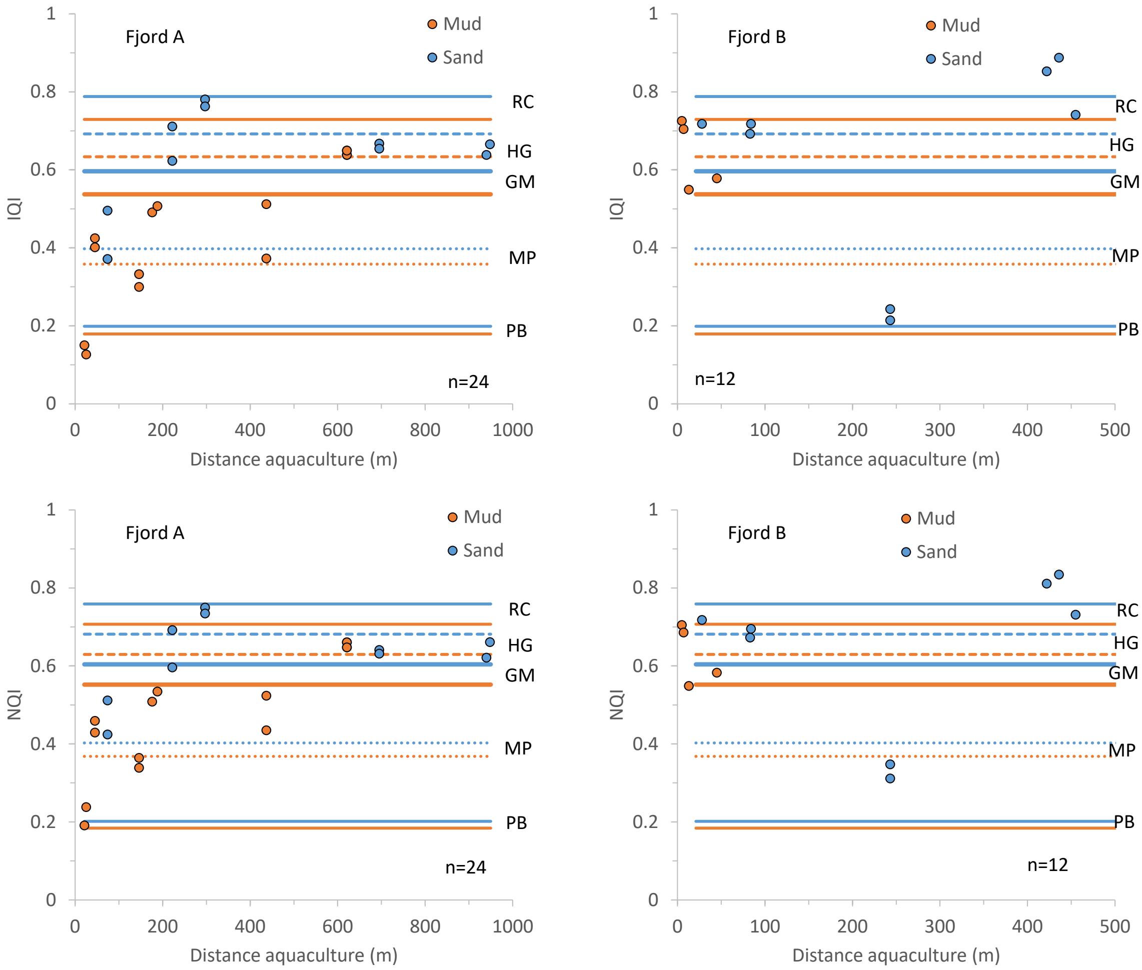


Figure 5.10. IQI (top) and NQI (bottom) observations from two fjords versus distance from aquaculture with proposed reference conditions and class boundaries (Table 5.3). Observations and boundaries for mud and sand use same colour code, red and blue respectivly.

Fjord-specific distributions of the IQI and NQI means were calculated for mud and sand sediment separately, using the estimated variances for variation among sites within fjords and variation among replicate samples (see above), and compared with the suggested boundaries (Figure 5.11 and Figure 5.12). In Fjord A, the benthic fauna community had moderate status in mud sediments, when assessed with both IQI and NQI. The distributions of the means were entirely located within the boundaries of moderate status. For sand sediments, the distributions of the means spanned over three status classes (moderate, good and high) with the highest probability of good status. For IQI, the status was good with a probability of 86.4%, whereas it was 77.0% for NQI.

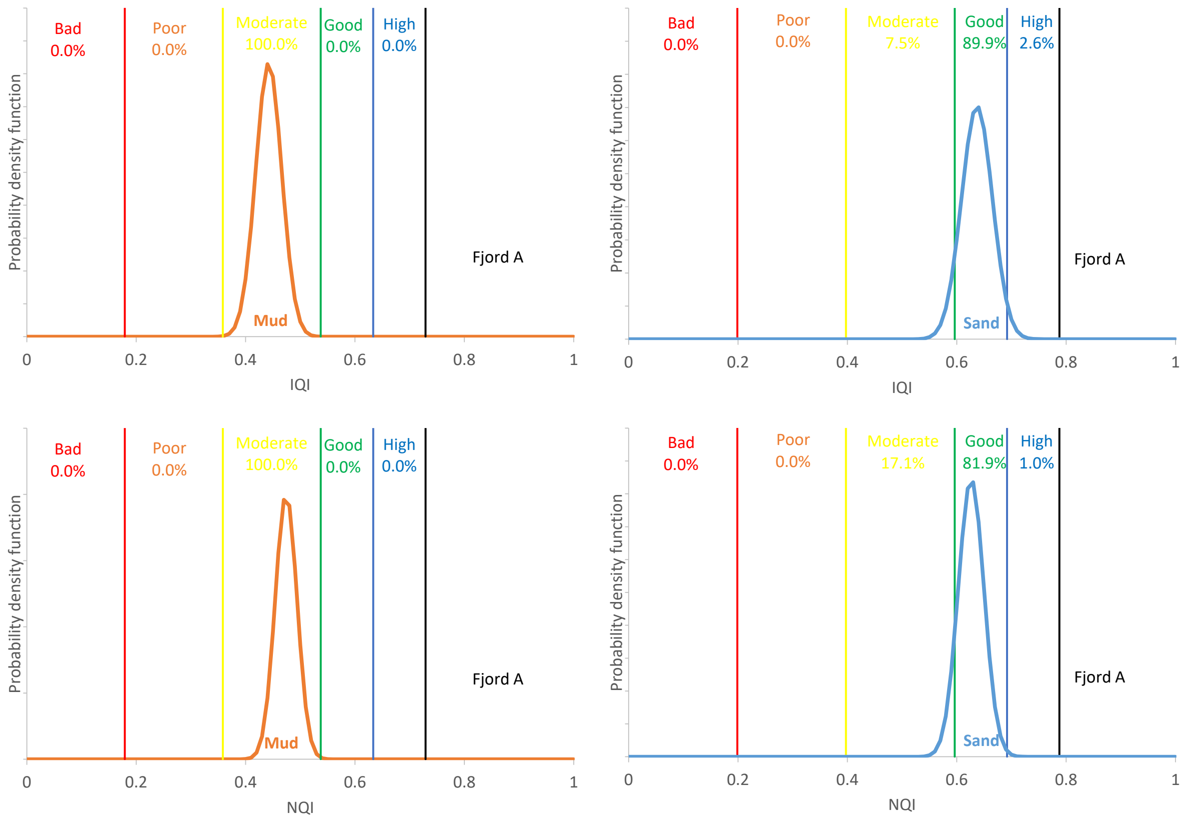


Figure 5.11. Distribution of means for IQI (top) and NQI (bottom) observations from Fjord A with proposed reference conditions and class boundaries (Table 5.3). The probability mass within each status class is listed above the curve.

For Fjord B, the distribution of the means for IQI and NQI also spanned over three classes with good status having the highest probability for both mud and sand (Figure 5.12). However, there was also a considerable probability of high status for mud sediments, whereas the probability of moderate status was low for both indices and sediment types.

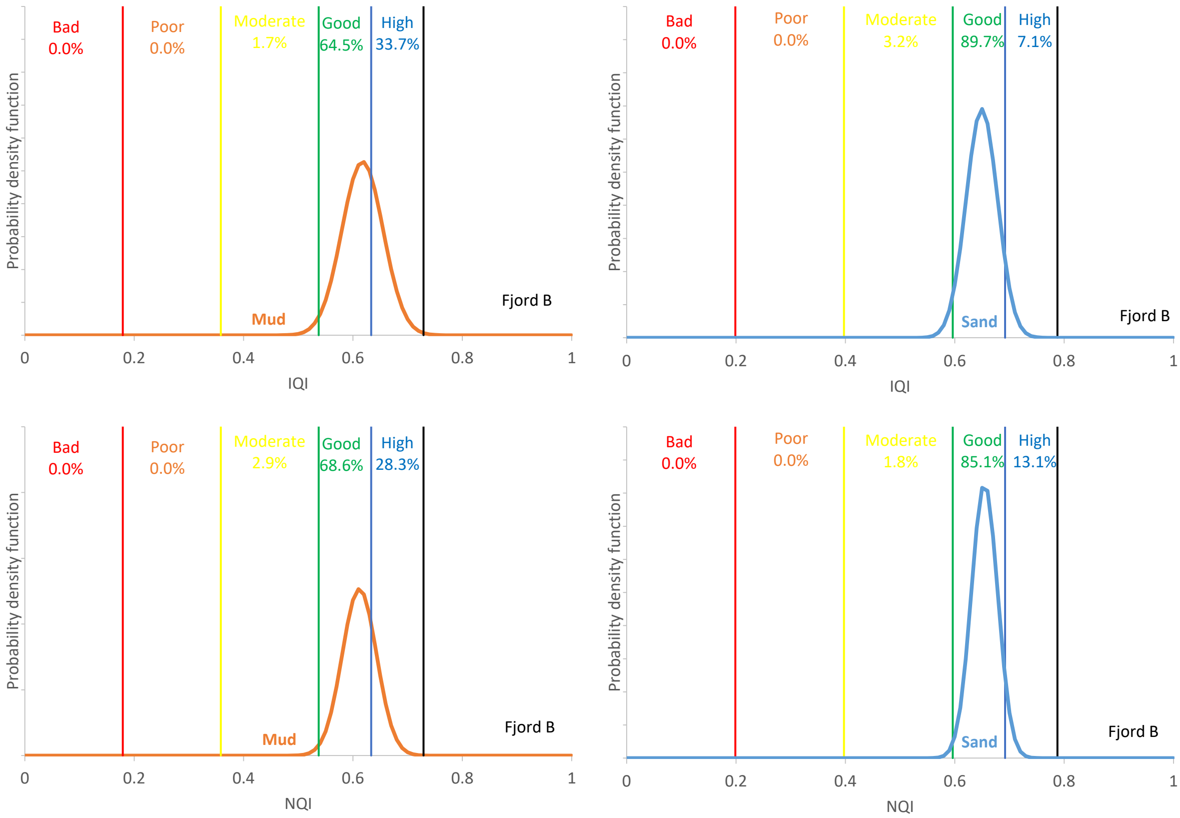


Figure 5.12. Distribution of means for IQI (top) and NQI (bottom) observations from Fjord B with proposed reference conditions and class boundaries (Table 5.3). The probability mass within each status class is listed above the curve.

The distributions of IQI and NQI means for mud and sand sediments can be combined by averaging the probabilities of individual status classes (Figure 5.13). For Fjord A, both indices show that there is about 50-60% probability of moderate status and 40-45% probability of good status. For Fjord B, there is about 2.5% probability of moderate status, 77% probability of good status, and 20.5% probability of high status.

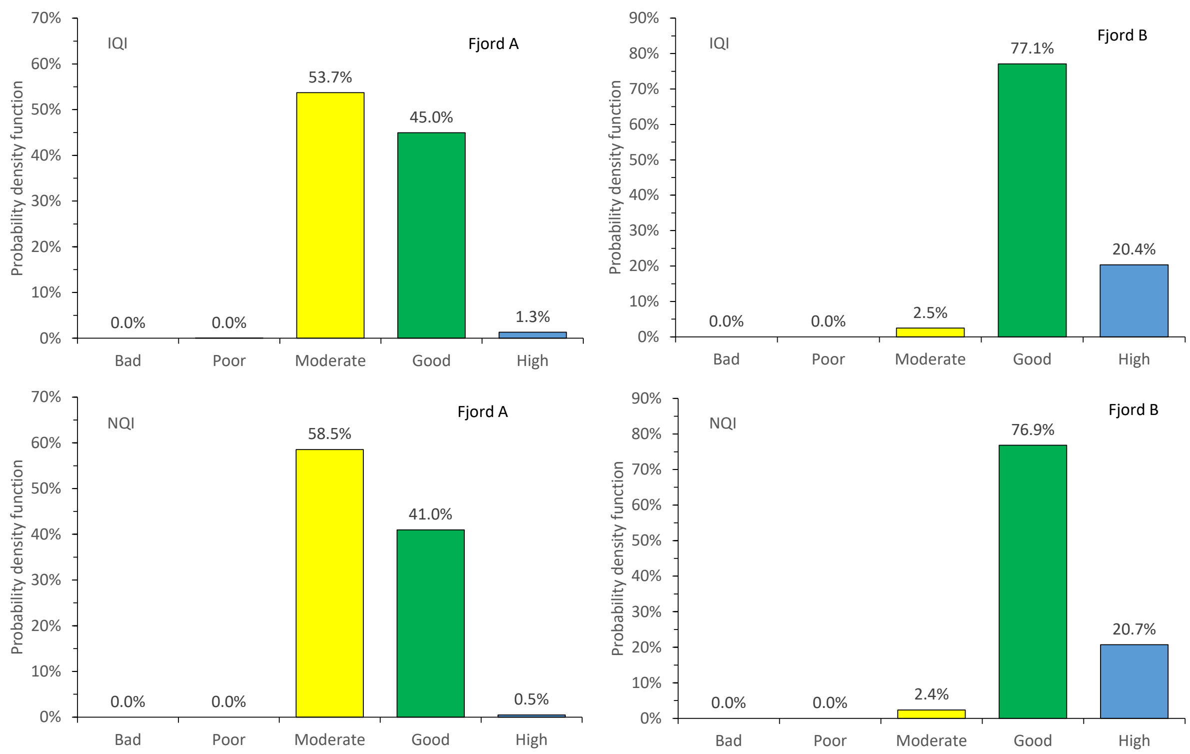


Figure 5.13. Discrete probability distributions for IQI (top) and NQI (bottom) in Fjord A (left) and Fjord B (right) combined from the continuous distributions for mud and sand sediment (Figure 5.11 and Figure 5.12). The probability mass within each status class is listed above the curve.

The CIS guidance #7 (CIS, 2003) outlines three different approaches for status classification based on the probability distribution over status classes: 1) benefit-of-doubt, 2) face-value, and 3) fail-safe. The difference between these approaches lies in the interpretation of confidence (Carstensen, 2007). The face-value approach uses the median of the distribution for classification, whereas the two other approaches use a percentile from the distribution that depends on the level of confidence desired in the classification (e.g., 90% or 95%). The face-value approach reaches moderate status for Fjord A and good status for Fjord B, assessed by the location of the median in the distributions. The benefit-of-doubt is the polluter's option, where the status is considered high or good unless it can be demonstrated with sufficient confidence that the status is worse. Using a 95% confidence level, this corresponds to considering the 95-percentile of the distributions, which is good for Fjord A and high for Fjord B. The fail-safe approach, on the other hand, is the environment's option, where the status is considered bad, poor or moderate unless it can be shown with sufficient confidence that status is better. Using a 95% confidence level, this corresponds to considering the 5-percentile of the distributions, which is moderate for Fjord A and good for Fjord B. Consequently, the choice of classification option can lead to different status classes, and the choice of option is not scientific, but political. The 'precautionary principle' in the EU legislation calls for employing the fail-safe approach.

6 Conclusion and recommendations

The aim of this project was to develop a classification system for benthic macrofaunal analysis in the Faroese fjords, to be used by the Environmental Agency for assessing the potential impact of aquaculture. Two approaches were tested; the Danish approach, that does not take into consideration the natural variability that may exists, and a novel approach that takes into account the natural variability of depth, LOI content in the sediment and sediment type.

The latter approach showed, that there was a low and mostly non-significant variability in the natural benthic community (index values) between fjords when considering data sufficiently far away from aquaculture only, meaning that undisturbed benthic fauna communities are similar across Faroese fjords. However, we found that there was a high significant variability between sites within the same fjord, and that this was primarily due to different sediment types, with sandy sediments having significant higher index values compared to muddy sediments. This underlines that there is no need to develop a classification system for each fjord individually, but that one classification system is sufficient as long as it includes the habitat type, i.e., mud versus sandy sediments.

We therefore recommend that the classification system suggested in Table 5.3, which differentiates between sediment types, is used in the Faroese fjords and not the one developed using the Danish approach that does not account for sediment type, but assumes that all samples derive from the same population. However, as new aquaculture sites generally are placed outside fjords at more exposed sites it should be kept in mind that the system will need to be re-evaluated on a regular basis, since some of these areas might contain a different benthic community and consequently naturally different index values, compared to more protected sites in the fjords, and that the classification system maybe should incorporate other factors as well, e.g., LOI. Re-evaluation of such a classification system, when more data is available, is a very common practice.

Up until now the classification of sediment type has been subjectively estimated. Some sediment samples have been classified as only muddy or sandy, while some have been classified as a combination of both, without clarifying whether one type is dominating. This may result in a misclassification, since a combined sediment type, dominated by mud will have a lower index value compared to a combined sediment sample dominated by sand. We therefore recommend that in the future, an objective sediment classification system is developed, that also specifies the dominating type, when both types of sediment are present, so a more precise classification and assessment can be obtained.

Since no Faroese multi-metric index is developed for the Faroe Islands, another aim of the project was to test the applicability of different existing indices that neighbouring countries have developed and used. Our state-impact analysis showed that all indices responded well to Zn as an environmental pressure, with the exception of ITI and ISI2012. However, NQI and IQI had the strongest responses. The NQI and IQI index equations are very similar, but the IQI equation contains a reference variable that is locally determined for Great Britain. It is unsure if this reference value can be used in the Faroese fjords without adjustment. It is therefore recommended that the NQI index is used in the Faroese fjords, which does not contain a locally determined variable in the equation.

Norway uses a combination of five indices. However, aggregating indices does not lend to more precision to the combined index, since all indices are based on the same raw data. Also, it is more difficult to communicate an index that is an aggregation of five complex indices. If decision is made to use a combination of many indices, one has to take into consideration that if one or more of the indices included have a less strong response the pressure, e.g., ITI and ISI2012, the combined index will be less efficient than selecting the best index. In other words, combining indices may produce a non-optimal index for determining responses to changing states.

The pressure-state analysis showed, that samples taken more than 700 m from the sea cages can be used to represent high and good ecological status. At this distance, the effect of aquaculture on elevated Zn concentration was on average less than 1%. Both the Environmental Agency of the Faroes Islands and the ASC requires that the Aquaculture companies sample from reference areas. ASC states that reference samples must be located at least 500 m from the sea cages, while the Environmental agency does not specify a specific distance. This can, as we have shown in this project, result in reference samples being taken too close to the Aquaculture facilities for them to be used as reference data. We therefore recommend that precautions are taken to prevent reference samples being taken too close to the sea cages.

The pressure-state analysis also showed that normal background levels for Zn ranged between approximately 30 and 100 mg kg^{-1} DW, depending on the LOI concentration. This is important knowledge if the boundary levels for the chemical parameters in reference samples one day are to be re-evaluated.

And finally, if the suggested classification system is to be used as part of an environmental impact assessment protocol by the Environmental Agency, careful considerations have to be made regarding interpretations of the benthic macrofauna analysis as to when the environmental goal is achieved, as discussed in section 5.3.

Also, worth considering is if a general national monitoring program of the Faroese fjords ought to be recommended for the Faroe Islands. Currently all impact assessments analysis in the Faroese fjords are being done in connection with aquaculture, and the assessment is only done in close proximity to the aquaculture facilities. This is unfortunate, since there may be other factors that affect the fjords' ecosystem, besides aquaculture, that could explain a change in the macrofaunal community. I.e., not knowing the ecological status of the entire fjord can be unfortunate for the farming companies. The interpretation of the results of an impact assessment would only be stronger if a general monitoring of the Faroese fjords was also done, since this would give a better understanding of the entire ecosystem and its dynamic.

7 Acknowledgment

For this project a steering committee was established with representatives from the Environmental Agency, the Faroese Aquaculture Association, the Faroe Marine Research Institute and Biofar, a private company that has been in charge of the sampling and taxonomic analysis of most of the data used in this study.

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Annex A - Description of the indices used

Denmark

Denmark uses the multimetric **DKI index** (dansk kvalitets indeks) for benthic macrouna analysis. The DKI equation contains the Shannon-Wiener diversity index, *H´*, the number of species (*S*) and the number of individuals per species (N) (incorporated into the Shannon-Wiener diversity index). The DKI index also incorporates the porportion of disturbance-senstitive taxa by using AMBI (Carstensen, Krause-Jensen, & Josefson, 2014; Henriksen et al., 2014). The Shannon-Wiener index and AMBI is described section 6.2 and 6.5 respectively.

In 2014 the DKI index was updated to work for areas with low salinity, typical of the Baltic sea (Carstensen et al., 2014) .

$$\mathrm{DKI} = \frac{\left(1 - \left(\frac{\mathrm{AMBI} - \mathrm{AMBI}_{\mathrm{min}}}{7}\right) + \frac{H}{H_{\mathrm{max}}}\right.}{2} \cdot \left(1 - \frac{1}{N}\right) \qquad \text{Eq. 6.1}$$

Where H_{max} = 2,117 + 0,086 x salinity (*psu*), og $AMBI_{min}$ = 3,083 – 0,111 x salinity (*psu*).

The faroese fjords do not have areas with very low salinity like in the Baltic Sea, instead the salinity is constantly around 35 *psu*. Therefore, the salinity is set at a constant 35 *psu*, when using Faroese data.

One prerequisite when using DKI is that H/H_{max} must never exeed 1, and if so, the value of 1 must be used. Also, $AMBI_{min}$ must never be negative, but if so 0 must be used (Carstensen et al., 2014). This means that H_{max} og $AMBI_{min}$ have the constant values of 5.127 and 0, when the salinity is 35 *psu*.

Norway

Norway uses a combination of 5 indices. They use two diversity indices; the Shannon-Wiener (**H´)** index and the Hulberts diveristy index (**ES100)**, two sensitivity indices; the Norwegian sensitivity index (**NSI**) and the Indicator species index (**ISI2012**), and a index that is a multimetric diversity- and sensitivity index called the Norwegian Quality index (**NQI)** (Direktoratsgruppen Vanndirektivet, 2018).

Shannon-Wiener (H´):

$$H = \sum_{i=1}^{S} -\frac{N_i}{N_{\mathrm{total}}} \log_2\left(\frac{N_i}{N_{\mathrm{total}}}\right) \qquad \text{Eq. 6.2}$$

S is the number of species, N_i is the number of individuals per species *i* and N_{total} is the total number of individuals (total abundance).

Hulbert's diversity index (ES100):

$$\mathrm{ES}_{100} = \sum_{i=1}^{S} \left[1 - \frac{\binom{N - N_i}{100}}{\binom{N}{100}} \right] \qquad \text{Eq. 6.1}$$

S is the number of species, N_i is the number of individuals per species. ES100 calculates the expected number of species among 100 randomly picked individuals in a sample. This means that ES100 only works for samples with at least 100 individuals (Pedersen et al., 2016; Rosenberg et al., 2004).

Norwegian Sensitivty index (NSI)

$$\mathrm{NSI} = \sum_{i=1}^{S} \frac{N_i \cdot \mathrm{NSI}_i}{N_{\mathrm{NSI}}} \qquad \text{Eq. 6.3}$$

Where *S* is the number of species, N_i is the number of individuals and NSI_i is the number of individuals assigned a sensitivity value (Rygg & Norling, 2013).

Indication Species Index (ISI2012)

$$\mathrm{ISI}_{2012} = \sum_{i=1}^{S} \frac{\mathrm{ISI}_i}{S_{\mathrm{ISI}}} \qquad \text{Eq. 6.4}$$

Where *S* is the number of species, ISI_i i s the sensitivity value of specie *i*, and S_{ISI} is the number of species that have a sensitivity value assigned (Rygg, 2002), (Pedersen et al., 2016). The sensitivity values used are found in Rygg og Norling, 2013 (Rygg & Norling, 2013).

Norwegian Quality Index (NQI1)

$$NQI1 = \left[0{,}5 * \left(1 - \frac{AMBI}{7}\right) + 0{,}5 * \left(\frac{\left[\frac{ln(S)}{\ln(\ln(N))} \right]}{2{,}7} \right) * \left(\frac{N}{N+5} \right) \right] \qquad \text{Eq. 6.5}$$

S is the number of species, *N* is the number of individuals per species, and AMBI is used to assing the sensitivity value of the differetn specie (Direktoratsgruppen Vanndirektivet, 2018).

Sweden

Sweden uses the multimetric **BQI index** (Bentic Quality index) (Josefson et al., 2009; Leonardsson et al., 2009; Naturvårdsverket, 2011; Rosenberg et al., 2004).

$$\mathrm{BQI} = \left(\sum_{i=1}^{S_{\mathrm{Classified}}} \frac{N_i}{N_{\mathrm{Classified}}} \cdot \mathrm{BQI}_i \right) \cdot \log_{10}(S+1) \cdot \left(\frac{N_{\mathrm{total}}}{N_{\mathrm{total}} + 5} \right) \qquad \text{Eq. 6.6}$$

S is the number of species, $S_{\mathrm{Classified}}$ is the number of species that have assigned a sensitivity value (BQI_i), $N_{\mathrm{Classified}}$ is the total number of individuals that have assigned a sensitivity value, N_i is the number of individuals per species *i*, and N_{total} is the total number of individuals in the sample.

There are two types of sensitivity values for BQI. For this assignment table 2.1 in appendix 2 in the handbook 2007:4 is used (Naturvårdsverket, 2011) .

Scotland

Great Britain (England, Scotland, Wales and Northern Ireland) uses the multimetric **IQI index** (Infaunal quality index) (Phillips et al., 2014; Water Framework Directive - United Kingdom Advisory Group (WFD-UKTAG), 2008). The IQI index incorporates AMBI, Simpson´s Evenness index (1-Ʌ´), number of species (S) and the number of individuals (incorporated into Simpson´s Evenness index).

$$\mathrm{IQI} = \frac{0.38 \frac{1-(\mathrm{AMBI}/7)}{1-(\mathrm{AMBI_{Ref}}/7)} + 0.08 \frac{1-\lambda'}{1-\lambda'_{\mathrm{Ref}}} - 0.54 \left(\frac{S}{S_{\mathrm{Ref}}} \right)^{0.1} - 0.4}{0.6} \qquad \text{Eq. 6.7}$$

In IQI the observed value for AMBI, Simpson´s Evenness and number of species (S) is compared to the expected value *Ref*. The expected value *Ref* depends on the type of sediment. For the sediment types fine sand, sand and mud with a sample size of 0.1 m^2 is (Phillips et al., 2014):

S_{Ref}	$(1-\mathrm{AMBI_{Ref}}/7)$	$1-\Lambda'_{Ref}$
68	0,96	0,97

Simpson´s Evenness indeksið (1-Ʌ´):

$$1 - \lambda' = \frac{\sum_{p=1}^{S} n_p \cdot (n_p - 1)}{N \cdot (N-1)} \qquad \text{Eq. 6.8}$$

S is the number of species, *N* is the number of individuals in total and, n_P is the number of individuals per species *p*.

Aquaculture Stewardship Council (ASC)

According to the ASC standard (*ASC Salmon Standard. Version 1.3. Aquaculture Stewardship Counsil.*, 2019), the ASC approved farming companies must choose one out of the four indices listed below:

1. Shannon- Wiener (H´)
2. BQI,
3. AMBI
4. ITI

Shannon-Wiener and BQI have already been described.

AZTI marine biotic index (AMBI)

AMBI is a sensitivity index, that is designed as a tool to be used by itself http://ambi.azti.es. However most EU countries have incorporated AMBI into their multimetric index that they have developed to express the proportion of disturbance-senstitive taxa, as required in the WFD (Gislason et al., 2017).

In AMBI the different species are grouped into five ecological groups (EGVI – EGVV) according to their sensitivity to eutrophication (A. Borja, Franco, & Pérez, 2000).

$$\mathrm{AMBI} = \frac{(0 \cdot \%\mathrm{EGI}) + (1.5 \cdot \%\mathrm{EGII}) + (3 \cdot \%\mathrm{EGIII}) + (4.5 \cdot \%\mathrm{EGIV}) + (6 \cdot \%\mathrm{EGV})}{100}$$ Eq. 6.9

Infauna Tropic Index (ITI)

The ITI index groups the different species to four different trophic levels according to their feeding strategy. The theory behind ITI is, that as the environmental pressure increases there is a shift in the benthic community from filter feeders to deposit feeders (Word, 1978, 1980).

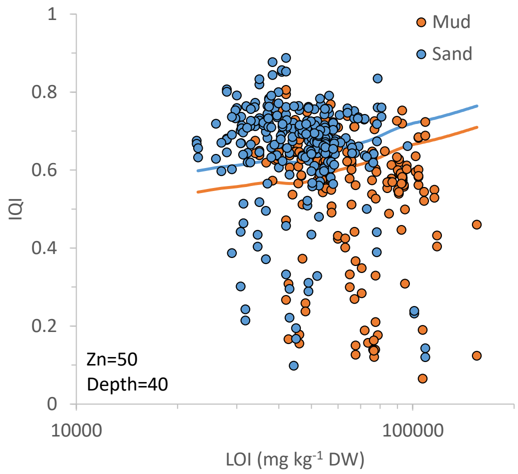
Trophic level	Feeding strategy
Group 1	Suspension feeder
Group 2	Surface detritus feeders
Group 3	Surface deposit feeders
Group 4	Sub surface deposit feeders

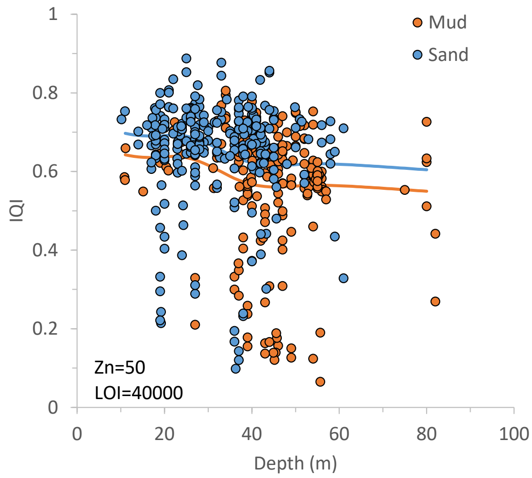
In the "Benthos" package in the statistical program R, there is a list of different Taxa and their trophic level. This list was used for this assignment (Gittenberger & Loon, 2013).

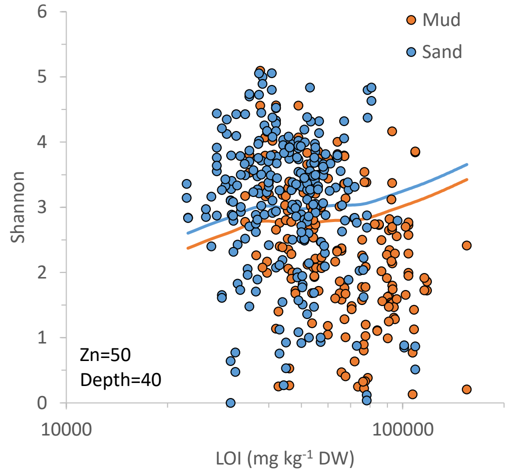
$$\mathrm{ITI} = 100 - \left(33.3 \frac{N_2 + 2N_3 + 3N_4}{N_1 + N_2 + N_3 + N_4}\right)$$ Eq. 6.10

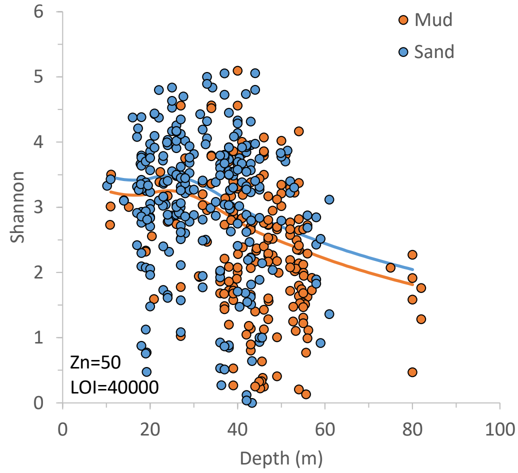
Where N_1, N_2, N_3 …., is the number of individuals in each trophic group.

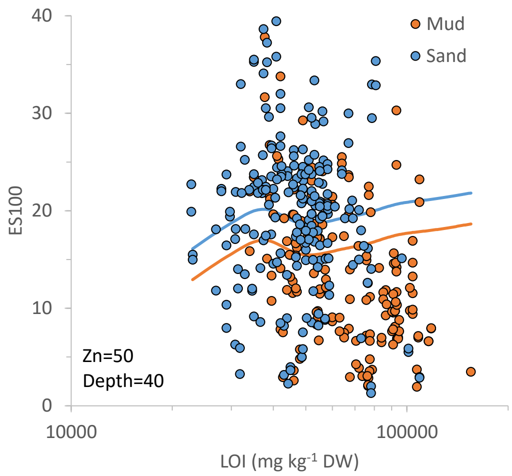
Annex B - Relationships benthic fauna indices versus LOI and depth

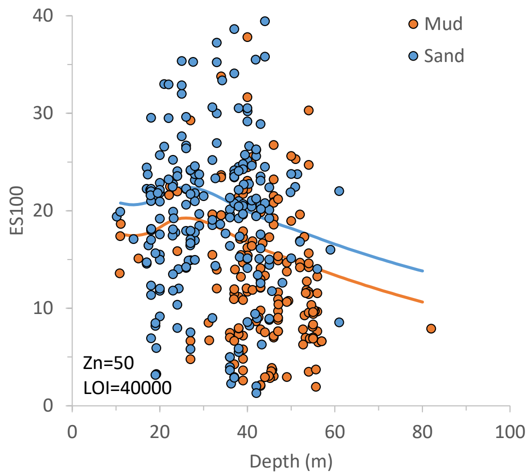


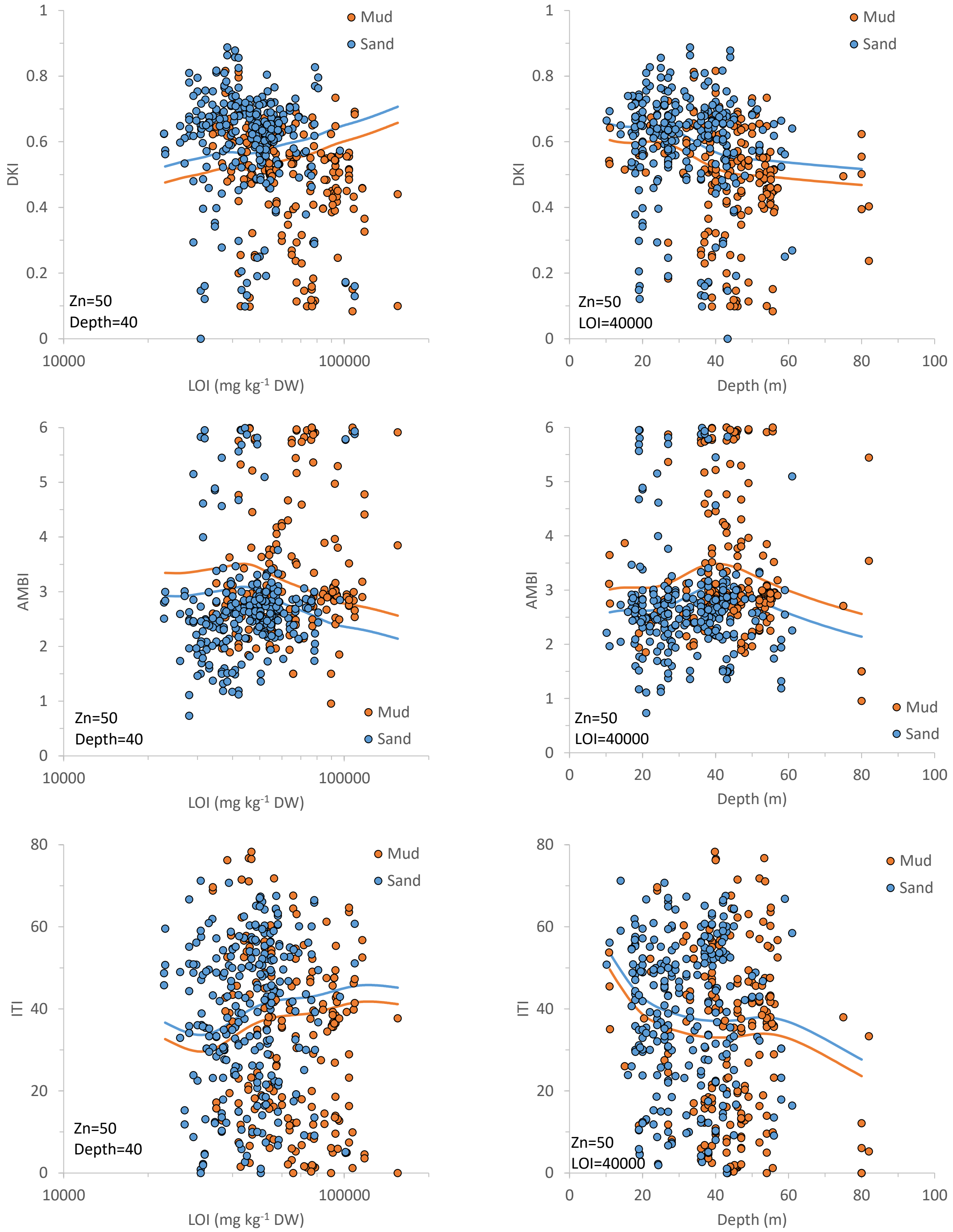


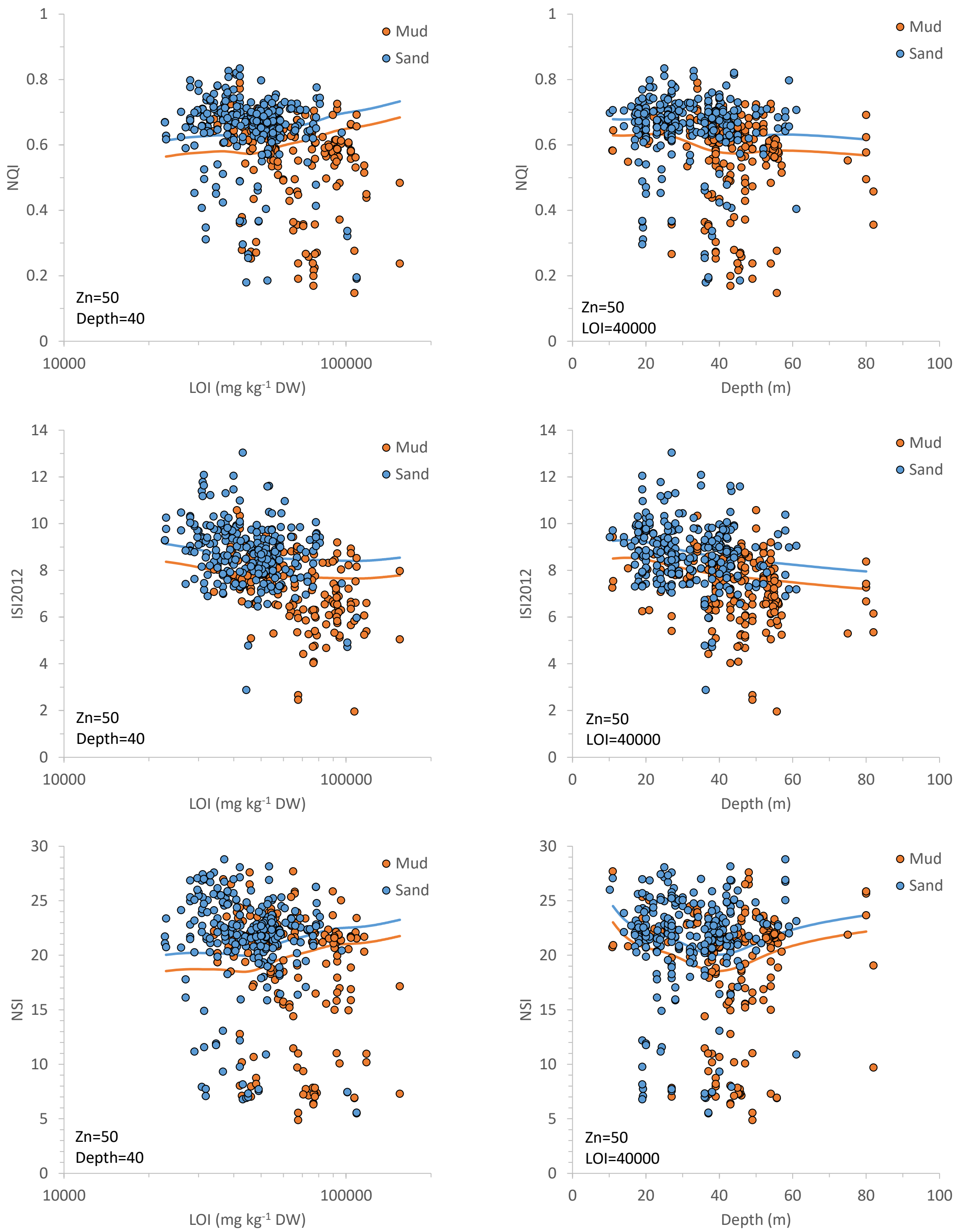









DKI
LOI (mg kg-1 DW)
Zn=50
Depth=40
Mud
Sand
DKI
Depth (m)
Zn=50
LOI=40000
Mud
Sand
AMBI
LOI (mg kg-1 DW)
Zn=50
Depth=40
Mud
Sand
AMBI
Depth (m)
Zn=50
LOI=40000
Mud
Sand
ITI
LOI (mg kg-1 DW)
Zn=50
Depth=40
Mud
Sand
ITI
Depth (m)
Zn=50
LOI=40000
Mud
Sand


NQI
LOI (mg kg⁻¹ DW)
Depth (m)
Mud
Sand
Zn=50
Depth=40
Zn=50
LOI=40000
ISI2012
NSI
10000
100000

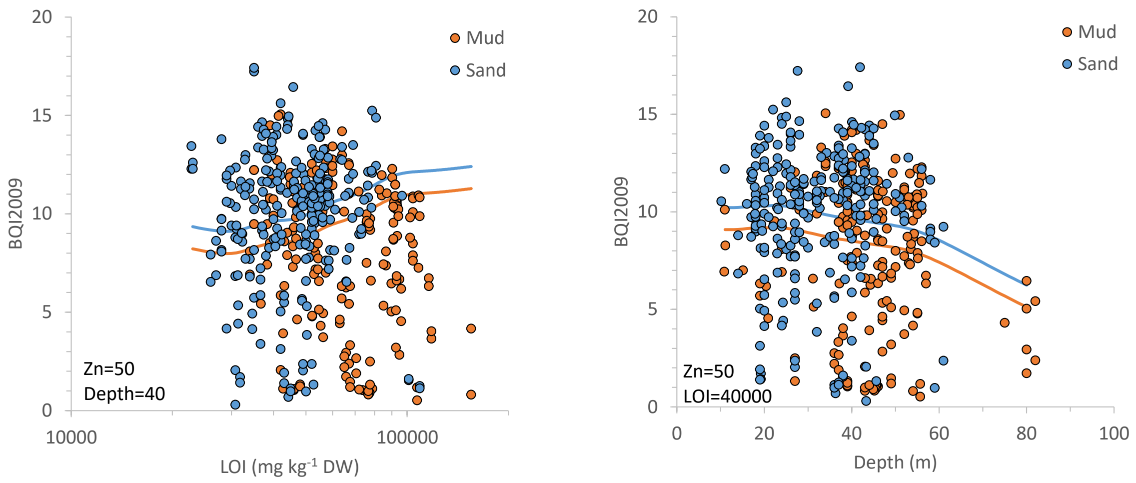


Fig. B1: Marginal relationships for the 10 benthic fauna indices versus LOI (left) and depth (right) (Eq. 4.6) plotted with the raw observations. Prediction levels for other explanatory variables are shown as inserts. Observations and relationships for mud and sand use same colour code, red and blue respectively. Note that LOI is shown on log-scale.

Annex C - Comparison of two approaches for calculating reference conditions

The relationships for reference conditions versus depth estimated with the simple and full model of Eq. (4.6) showed same tendency with depth, although the relationships based on the full model displayed more curvature. This curvature originates from the non-parametric components of $S(ln(LOI))$ and $S(ln(depth))$.

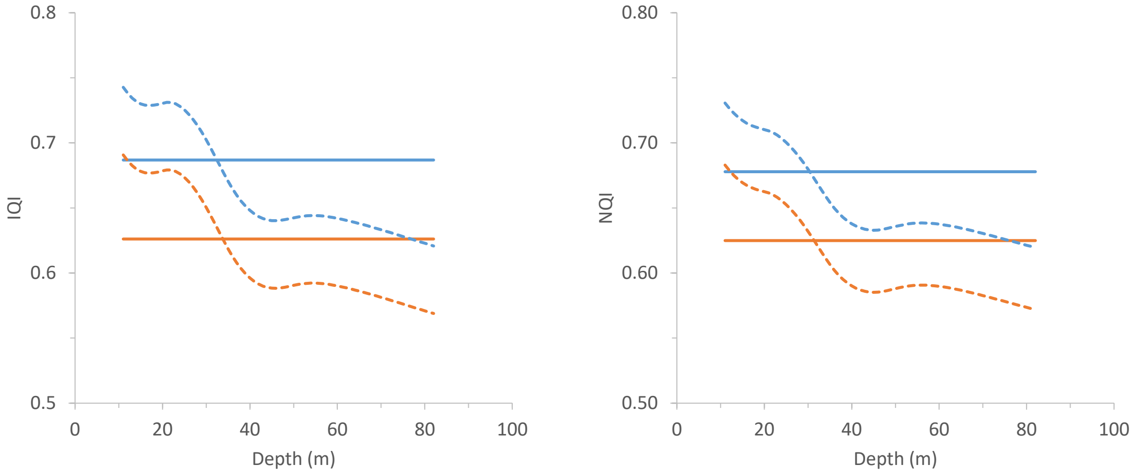


Fig. C1: Estimated relationships for reference conditions of IQI (left) and NQI (right) versus depth. Solid lines show the simple model (only depth) and dashed lines show the full model (Eq. 4.6). Sediment type is shown in blue (sand) and orange (mud).

Annex D - The Swedish approach

The Swedish approach builds on the assumption that available reference data (defined as specific time periods with low disturbance) represent an at least good ecological status, i.e., both high and good status, and can therefore be used to set the boundary between good and moderate (GM). The overall metric used is the 20-percentile of the mean BQI for a given assessment area, and the 20-percentile of the mean is calculated using a nested bootstrap approach that takes into consideration both temporal and spatial variation by clustering the different areas together according to e.g., depth, sediment types, salinity and year (Blomqvist & Leonardsson, 2016; Leonardsson et al., 2016). This is difficult to do on Faroese data, since we have too few data from each fjord and year, to conduct a nested bootstrap analysis. Since it is not possible to cluster our reference data, a simple bootstrap approach would be used instead, and this approach would assume that the reference data represented the same population. i.e., it would not take into consideration natural spatial variations.

This, however, is not the only concern regarding using the same approach as Sweden. The Swedish bootstrapping approach in setting the boundary between good and moderate is in itself questionable. Their approach goes as following: reference samples are randomly resampled with replacement with the same number as the sample size. The mean of the resample is calculated and stored. This procedure is repeated 9.999 times, and finally the lower 20-percentile of all the resampled mean values is calculated. Since this approach uses mean values of reference data to set the boundary between good and moderate, this means that approximately 50% of the reference data, that is assumed to represent an at least good ecological status would be classified as worse than good, and consequently this can result in a boundary that is set to high.

Furthermore, the use of the 20-percentile of the mean statistic is sensitive to the number of observations used in the resampling. For small sample sizes the 20-percentile of the mean will be somewhat lower than the average, but for larger sample sizes the 20-percentile of the mean will approach the average. In practise, this means that different indicator values will be obtained for the same water body by changing the number of samples. Finally, the statistical foundation, as laid out in Leonardsson et al. (2009) is not entirely transparent and appears to have a more heuristic nature.

Because of these concerns, the Swedish approach was deemed unsuitable to be used.

Annex E – Boundaries when pre-aquaculture samples are excluded

Proposed values for reference conditions and class boundaries for the two indices (IQI and NQI) in different sediment types, when samples taken at new locations pre-aquaculture are excluded

	IQI		NQI	
	Mud	Sand	Mud	Sand
Reference conditions	0.729	0.801	0.708	0.773
HG boundary	0.631	0.703	0.626	0.691
GM boundary	0.532	0.604	0.544	0.609
MP boundary	0.355	0.403	0.363	0.406
PB boundary	0.177	0.201	0.181	0.203