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Exploration of Benthic Invertebrate Diversity Indices and Ecological Quality Ratios for defining ecological status of coastal marine waters according to the Water Framework Directive (2000/60/EC)

Pamela J. Woods, Steinunn Hilma Ólafsdóttir og Rakel Guðmundsdóttir

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Ágrip

Til þess að uppfylla kröfur laga um stjórn vatnamála (nr. 36/2011) og reglugerðar nr. 535/2011 um flokkun vatnshlota, eiginleika þeirra, álagsgreiningu og vöktun skal skilgreina líffræðilega gæðaþætti sem nota á við vistfræðilega ástandsflokkun strandsjávarvatnshlota. Í þessari úttekt voru fyrirliggjandi gögn um botnlæga hryggleysingja (botndýr) af mjúkum botni frá ýmsum fjörðum fyrir austan, norðan og vestan notuð til þess að ákvarða hentugan vísi (fjölbreytileika og/eða þolvísir) við vöktun vatnshlotagerða í strandsjó við Ísland. Ýmsir vísar voru skoðaðir og leiddi þessi vinna til þess að mælt er með því að nota samsetta gæðavísinn NQI1 (Norwegian Quality Index 1) við mat á vistfræðilegu ástandi strandsjávarvatnshlota. Fyrstu drög að viðmiðunarmörkum fyrir vistfræðilegt gæðahlutfall (EQR) eru lögð fram og byggja þau á útreikningum á NQI1 fyrirliggjandi gagna, sérfræðiáliti, þekkingu á staðháttum sýnatökustaða og mati á raski þeirra svæða sem sýnin voru tekin frá. Lagt er til að í framhaldi af þessari vinnu verði gerð frekari úttekt á vísum fyrir botnlæga hryggleysingja, endurmeta EQR viðmiðunarmörkin með frekari gagnasöfnun sem miðuð eru að markmiði vöktunar strandsjávarvatnshlota, skoðað verði hvernig vísar bregðast við mismunandi álagi og að metnar verði aðferðir sem taka mið af niðurstöðum fleiri gæðavísa.

Abstract

To facilitate monitoring of coastal waterbodies for the purpose of complying with the Icelandic Water Management Act no. 36/2011 and regulation no. 535/2011, various biological quality indices need to be defined to inform ecological status by water types. In this study, historical soft bottom benthic invertebrate data from various fjords in the eastern, northern, and western part of Iceland were analyzed to determine which quality index could be the most useful for monitoring water quality of coastal water bodies in Iceland. A series of benthic invertebrate indices were explored. The conclusion was that the NQI1 quality index (Norwegian Quality Index 1) should be used as the main invertebrate indicator of

ecological status for Icelandic coastal water bodies. Preliminary ecological quality ratio (EQR) boundaries were also proposed to define ecological statuses based on how variation in NQI1 calculated from historical samples matched local expert knowledge of the ecological condition and the degree of disturbance expected in sampled locations. It is recommended that further development of benthic invertebrate indices used for monitoring include refinement of EQR boundaries based on data specially surveyed and collected for these monitoring purposes. In addition, how quality indices respond to environmental gradients within Iceland requires further investigation and classification methods that combine results from multiple indices should be explored.

Lykilorð: *Water Framework Directive, benthic invertebrates, diversity indices, ecological quality ratio, ecological monitoring*

Undirskrift verkefnisstjóra:	**Undirskrift forstöðumanns sviðs:**
	

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1. Introduction

To comply with the Water Framework Directive (WFD, 2000/60/EC), on the ecological status of defined water bodies within the coastal waters of Iceland, the water quality indices applicable for monitoring need to be defined. Ecological status is expressed as a ratio between the values of the indices observed at a given site, and the values for these elements from a reference ecological state. A contract was made between the Environment Agency of Iceland and the Marine and Freshwater Research Institute in 2013, to suggest indicators of ecological status by biotic indices, such as those that reflect the sensitivities of different taxa to environmental gradients, and could represent the environmental status of different waterbodies. In 2020, this work was taken further to suggest methods to calculate the ecological status and the boundaries for "*Very good*", "*Good*" and "*Moderate*" quality status levels.

Macrofaunal communities of benthic invertebrates inhabiting soft sediments on the sea floor are one of the elements used as indicators of ecological quality in the WFD. The focus of this study was to test the performance of already established biotic indices to be used for further monitoring and propose boundaries between "*Very Good*", "*Good*" and "*Moderate*" quality status for coastal water bodies of Iceland. We explored patterns of benthic invertebrate diversity in soft muddy bottoms from coastal water bodies in the west fjords, east fjords, and north of Iceland. Data were taken from historical studies and samples, and are therefore not standardized in terms of sampling methods or taxonomic level of identification. Therefore, guidelines for evaluation of ecological status based on benthic invertebrate density, as presented here, should be taken as a first attempt given the constraints of data availability. It will be updated as the accumulation of standardized samples provides greater resolution of precise boundaries. For now, interpretations are largely based on expert knowledge as no data are available for analyzing index responses to gradients of environmental pressures within Iceland. Therefore, the study mostly focused on indices that have been intercalibrated, and shown to respond to environmental pressures in other locations. Within Iceland, expert knowledge of spatial variability in ecological status and likely human impact are used to indicate if the index is likely to be responsive to environmental pressures.

2. Methods

No systematic sampling of benthic invertebrates in Iceland has been conducted for the purposes of assessing nationwide diversity or quality indices. Therefore, data for this study were compiled from a variety of localized studies conducted over the past several decades. Data were collected using a variety of sampling methods mainly in the east and west fjords, as well as Eyjafjörður in the north (Table 1). Most samples represent a single temporal sample, with the exception of samples from Eyjafjörður, which were repeated through a year at a single location, and Berufjörður, which was studied during three periods in impact assessments related to the establishment of an aquaculture facility. This set of samples from Berufjörður are additionally the only samples used to reflect a measurable human impact (i.e., pollution from the aquaculture facility which could, for example, affect oxygen levels). However, these samples are only used anecdotally here: they do not represent standardized samples or a thorough analysis of human impacts.

Human population densities outside Reykjavík are quite low, and very few industrial activities beyond fishing and aluminum smelting occur along the coast. Fishing is generally not conducted within fjords (with the exception of some shrimp trawl fishing), and the samples represented here predate the establishment aluminum smelters in other studied fjords (Reyðarfjörður). Excepting Berufjörður sites, any aquaculture activities in the sampled fjords are not considered to have affected the sampling sites (e.g., in Arnarfjörður) at the time of the sampling. Therefore, using expert judgement, it is expected that most sites sampled in this study should fall into a *Very Good* or *Good* categorization (Table 1), whereas the Berufjörður samples taken closest to the aquaculture facility are expected to show some level of impact (likely a *Moderate* or *Poor* status, Table 1). Arnarfjörður is also an exception in that it is known to have naturally low oxygen concentration in the bottom layer of the water column during late autumn. Benthic diversity and abundance levels are therefore low and more likely to be reflective of a *Moderate* categorization than *Good* (Table 1).

Table 1. List of researched areas for which data were available and used to analyze diversity indices and propose status boundaries.

	Year	Sampling equipment	Expected ecological status	References
Northern and eastern areas (CN1152 & CN1352)				
North - Eyjafjörður	**1992-1993**	**van Veen grab 0,1 m^2**	Very good-Good	Kristinn Guðmundsson et al., 2002.
East fjords				
Berufjörður	**2002,2006, 2016**	**van Veen grab 250 cm^2, van Veen 195 cm^2**	Good - Poor	Þorleifur Eiríksson and Böðvar Þórisson, 2004; Þorleifur Eiríksson et al., 2007; Þorleifur Eiríksson et al., 2017
Borgarfjörður Eystri	**2006**	**Sea boss 0,09 m^2**	Very good-Good	Steinunn Hilma Ólafsdóttir and Sigmar Arnar Steingrímsson, 2007
Héraðsflói	**2006**	**Sea boss 0,09 m^2**	Very good-Good	Steinunn Hilma Ólafsdóttir and Sigmar Arnar Steingrímsson, 2007
Reyðarfjörður	**2000**	**Shipek grab 0.041 m^2**	Very good-Good	Hafsteinn Guðfinnsson et al., 2001
Seyðisfjörður	**2002**	**Shipek grab 0.041 m^2**	Very good-Good	Sigmar Arnar Steingrímsson, 2006
Vopnafjörður	2006	Sea boss 0,09 m^2	Very good-Good	Steinunn Hilma Ólafsdóttir and Sigmar Arnar Steingrímsson, 2007
Southern and western areas (CS2152 & CS2352)				
West fjords				
Arnarfjörður	2018	Shipek grab 0.041 m^2	Moderate	Stefán Áki Ragnarsson et. al., in prep.
Ísafjarðardjúp	2002-2012	van Veen grab	Very good-Good	Þorleifur Eiríksson et al., 2012 (compilation of studies)
Ísafjarðardjúp (2018)	2018	Shipek grab 0.041 m^2	Very good-Good	Stefán Áki Ragnarsson et. al., in prep.
Tálknafjörður	2009	Shipek grab 0.041 m^2	Very good-Good	Steinunn Hilma Ólafsdóttir, 2015
Patreksfjörður	2009	Shipek grab 0.041 m^2	Very good-Good	Steinunn Hilma Ólafsdóttir, 2015

In the studies included, taxonomic counts of benthic invertebrates were sometimes reported as averages or totals over 2 - 3 repetitions when raw data by site and repetition were not given. Over time, names and taxonomic status of species change so the names were updated according to the World Register of Marine Species (WoRMS, 2020). However, the taxonomic level to which data were classified were very similar across studies. Data were standardized among studies as well as possible, by aligning taxonomic naming conventions and converting reported counts to a common measure of average counts per repetition per square meter (by multiplying or dividing by repetition numbers and dividing by gear dimensions). All sites were assigned to one of four water body types, which were defined by Sólveig R. Ólafsdóttir et al. (2019): open water bodies off the west and south coast of Iceland ("CS2152"), sheltered water bodies off the west and south coast of Iceland ("CS2352"), open water bodies off the northeast and east coast of Iceland ("CN1152"), and sheltered water bodies off the northeast and east coast of Iceland ("CN1352").

Standardized count data were then used to calculate candidate biotic indices that could be used for monitoring sample ecological quality of locations and within each water body type. The indices explored included (Table 2): 1) NSI (mean species NSI values, weighted by abundances, Rygg & Norling, 2013), 2) the IQI (UKTAG, 2008), 3) NQI1 (Vann 2018, Van Hoey et al., 2019), 4) NQI2 (Rygg, 2006) and 5) DKI (Van Hoey et al., 2019). All except NSI range 0 – 1 and use a quality reference value of 1, meaning that they can be directly interpreted as ecological quality ratios (EQRs). These indices have been used in the WFD process in Europe and it was our intention to test how well they perform with Icelandic data.

To analyze potential boundaries among "*Very Good*," "*Good*" and "*Moderate*" statuses for each index, expert knowledge regarding whether the samples reflected human disturbance were compared with distributions of index values calculated by site within a water body (usually a fjord or fjord complex), according to the normative definitions for these ecological statuses as shown in Box 1 (quote from COAST 2003, p. 60).

For each index, distributions were analyzed visually, using the median, 25%, and 5% quantiles, to determine potential boundaries between "*Very Good*," "*Good*" and "*Moderate*" statuses. Not enough samples of impacted areas within Iceland were available to analyze boundaries reflecting "*Poor*" or "*Bad*" ecological conditions. Most samples were thought to reflect pristine or undisturbed areas, although some samples within areas could reflect very light disturbance. As discussed previously, exceptions include Berufjörður and Arnarfjörður, which could reflect (respectively) greater disturbance or unusually poor-quality natural conditions. As discussed earlier, Berufjörður and Arnarfjörður are more likely to reflect a range of samples indicating *Good* - *Poor* statuses. Therefore, quantiles were calculated excluding samples from Berufjörður and Arnarfjörður to more closely reflect *Very Good* and *Good* status distributions and the two boundaries formed from them (*Very Good*/*Good* and *Good*/*Moderate*). Candidate index distributions

Normative definitions of ecological status

Very good (High)
The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions.

All the disturbance-sensitive taxa associated with undisturbed conditions are present.

Good
The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions.

Most of the sensitive taxa of the type-specific communities are present.

Moderate
The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with the type-specific conditions.

Taxa indicative of pollution are present. Many of the sensitive taxa of the type specific communities are absent.

were compared for their ability to satisfy three criteria in relation to expert judgement: to 1) show on average the expected categorizations based on expert judgement, 2) yield a broad range of values within each category (i.e., so resulting ranges of status values between boundaries are not unnecessarily narrow), and 3) show no large unexpected or unexplained differences among fjords in value distributions. Of the analyzed indices, IQI, NQI1, and DKI have undergone intercalibration (Van Hoey et al., 2019), so intercalibrated boundaries were also compared in figures with calculated boundaries presented here. A final index that best fit the expected categorizations based on expert judgement (Table 1) was then chosen, and the relationship between this index and several raw diversity indices was analyzed (see section 3.4). Analyses were performed in R statistical software version 4.0.2. (R Core Team, 2020) and using the Benthos package for most index calculations (Walvoort, 2019).

Table 2. Biotic index definitions. Component diversity measures include the Shannon-Wiener index calculated with a base 2 log (*H'*), Simpsons index (λ'), and species diversity (S).

Biotic index	Equation	Source
NSI	$NSI = \sum_{i}^{S} \left(\frac{N_i * NSI_i}{N_{NSI}} \right)$ where N_i is the number of individuals of species *i*, NSI_i is the sensitivity value of species *i*, N_{NSI} is the number of individuals with assigned values, and sensitivity values were taken from NIVA 2013	Rygg et al. 2013
IQI	$IQI = \left(\left(0.38 * \frac{1 - \left(\frac{AMBI}{7}\right)}{1 - \left(\frac{AMBI_{Ref}}{7}\right)} \right) + \left(0.8 * \frac{1 - \lambda'}{1 - \lambda'_{Ref}} \right) + \left(0.54 * \left(\frac{S}{S_{Ref}} \right)^{0.1} \right) - 0.4 \right) / 0.6$ Where $1 - \left(\frac{AMBI_{Ref}}{7}\right) = 0.96$, $1 - \lambda'_{Ref} = 0.97$, and $S_{Ref} = 68$	UKTAG 2008; Van Hoey et al. 2019
NQI1	$NQI1 = \left(0.5 * \left(1 - \frac{AMBI}{7} \right) + 0.5 * \left(\frac{SN}{2.7} \right) * \left(\frac{N}{N+5} \right) \right)$ $SN = lnS / \ln(\ln N))$; N = total number of individuals	Vannportalen 2018; Van Hoey et al. 2019
NQI2	$NQI2 = \left(0.5 * \left(1 - \frac{AMBI}{7} \right) + 0.5 * \left(\frac{H'}{6} \right) \right)$	Rygg 2006
DKI	$DKI = \left(\frac{\left(1 - \frac{AMBI}{7}\right) + \left(\frac{H'}{H'max}\right)}{2} \right) * \left(\frac{\left(1 - \frac{1}{N}\right) + \left(1 - \frac{1}{S}\right)}{2} \right)$ Where $H' \max = 5$; N = total number of individuals	Van Hoey et al. 2019

3. Results and Discussion

3.1. Distributions of candidate indices

Most of the samples used for this work were collected in areas that fall into the *open water body* type category, yielding little data with which to set boundaries specific to *sheltered water body* types. Therefore, although analyses were conducted and results shown for sheltered water body types, recommendations on the choice of water quality indexes for the Icelandic coastal regions are based on the open water body type results within geographic regions.

The distributions of sample NSI (Figures 1 & 2) were able to differentiate several samples in Arnarfjörður and Berufjörður as having rather low ecological quality and mostly responded similarly across fjords (criteria 1 & 3), but it also had a very small dynamic range across samples expected to be categorized as *Very Good*, *Good*, or *Moderate* (criteria 2). The IQI and NQI1 followed similar patterns for all water body types (Figures 3 – 6). Both were able to differentiate several samples in Arnarfjörður and Berufjörður as having rather low ecological quality, had a larger dynamic range across samples, and mostly responded similarly across fjords (i.e., no fjord stood out with unexpected means or variability among sites in index values), indicating that all expert knowledge criteria were fulfilled. The NQI2 and DKI indices followed similar patterns for all water body types (Figures 7 – 10). Both were able to differentiate several samples in Arnarfjörður and Berufjörður as having rather low ecological quality and had a very small dynamic range across samples (criteria 1 & 2). However, both indices indicated Ísafjarðardjúp (2018) samples as having a rather lower quality, which was not expected according to expert knowledge, and therefore did not fulfill criteria 3.

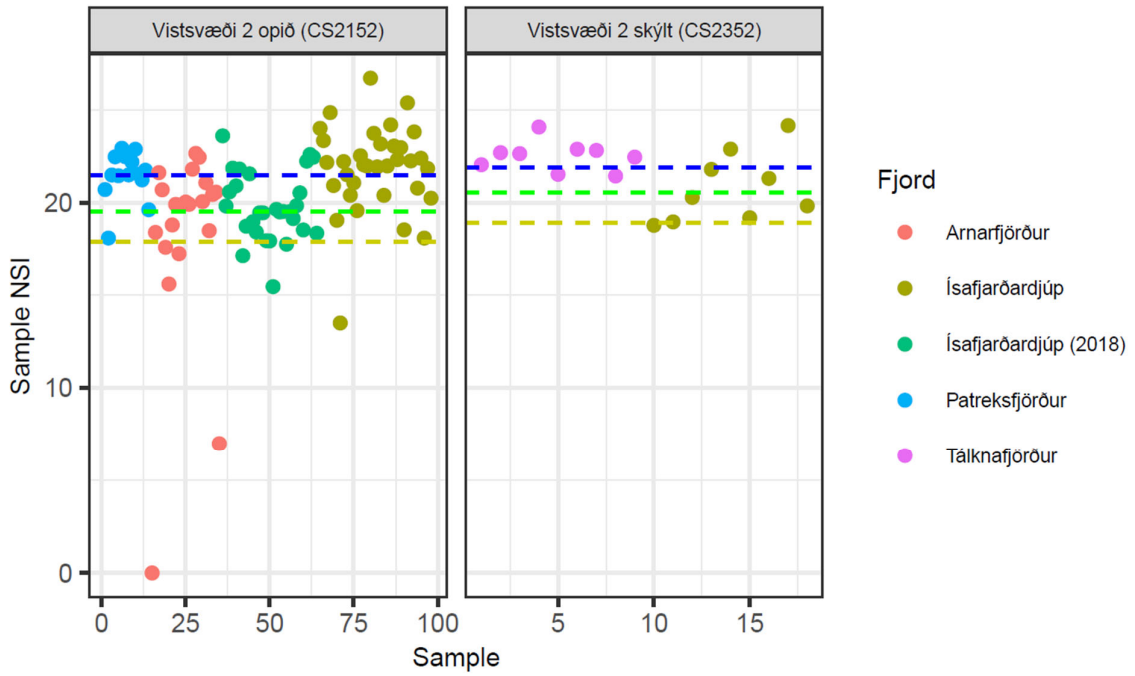

Figure 1. Sample NSI values plotted by site in the western waters off Iceland (Vistsvæði 2 opið / skýlt: water bodies of ecological type 2 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður.

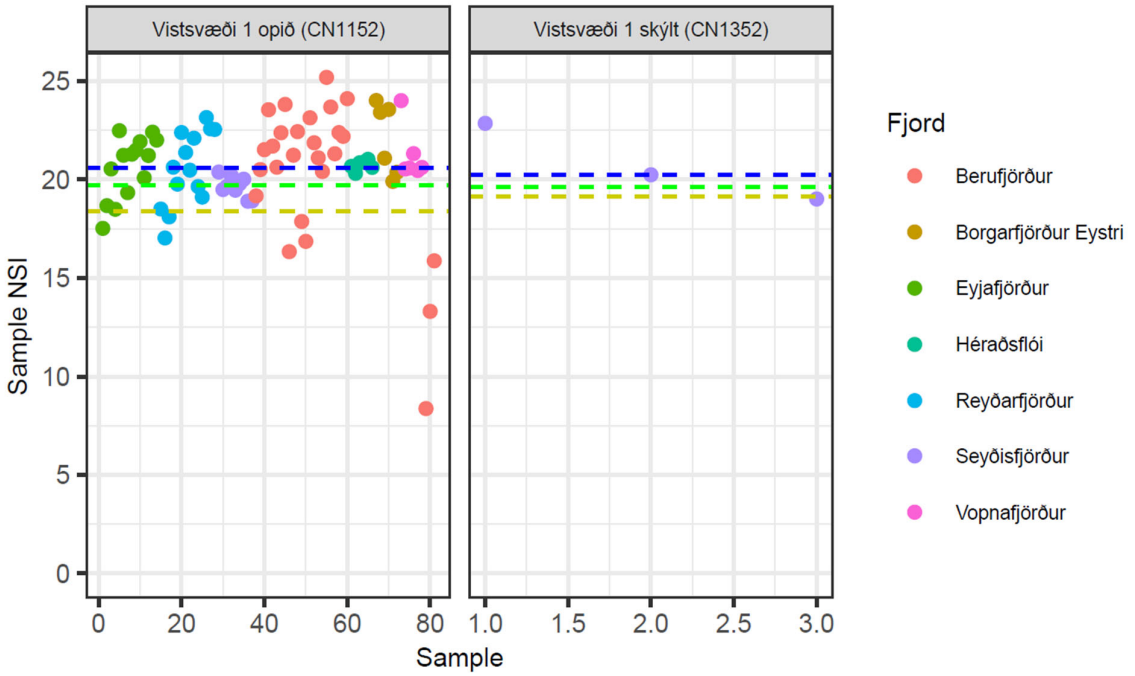

Figure 2. Sample NSI values plotted by site in the eastern and northern waters off Iceland(Vistsvæði 1 opið / skýlt: water bodies of ecological type 1 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður.

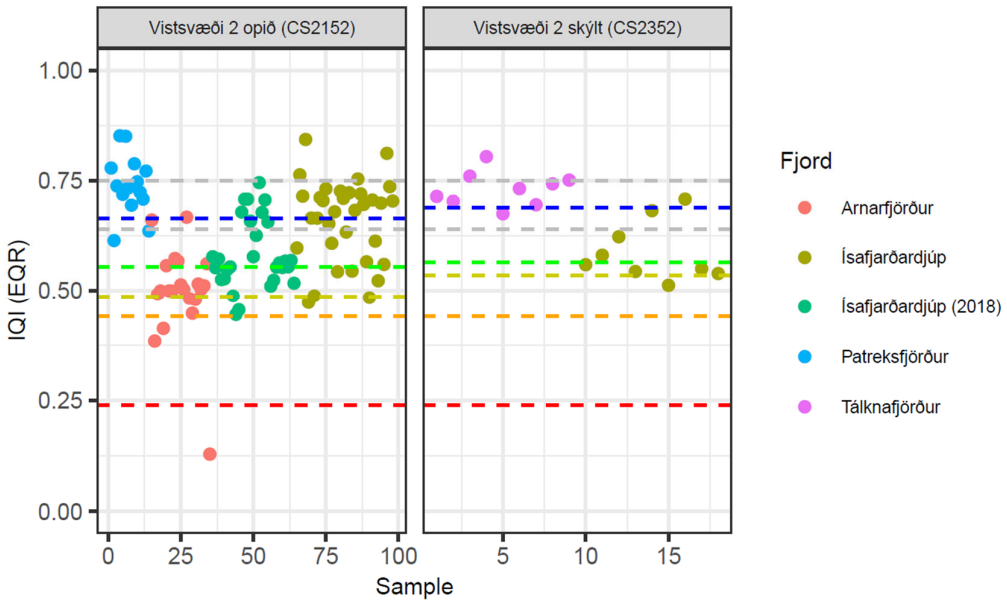

Figure 3. IQI values plotted by site in the western waters off Iceland(Vistsvæði 2 opið / skýlt: water bodies of ecological type 2 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al., 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al., 2019).

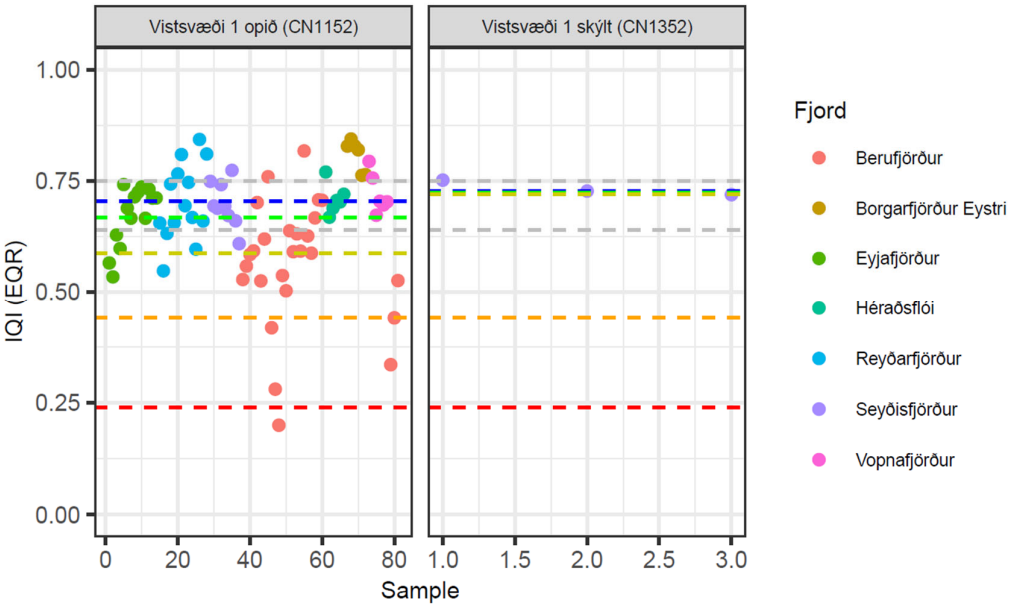

Figure 4. IQI values plotted by site in the eastern and northern waters off Iceland(Vistsvæði 1 opið / skýlt: water bodies of ecological type 1 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al., 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al., 2019).

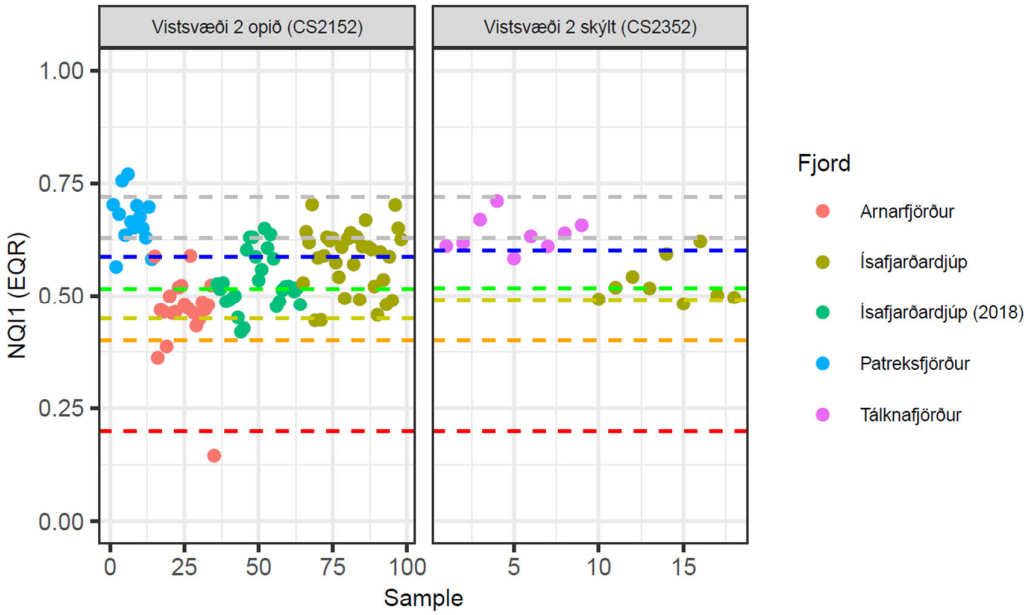

Figure 5. NQI1 values plotted by site in the western waters off Iceland. Sample numbers are a simple ordered identification number within water body type (Vistsvæði 2 opið / skýlt: water bodies of ecological type 2 open / sheltered). The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al. 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al. 2019).

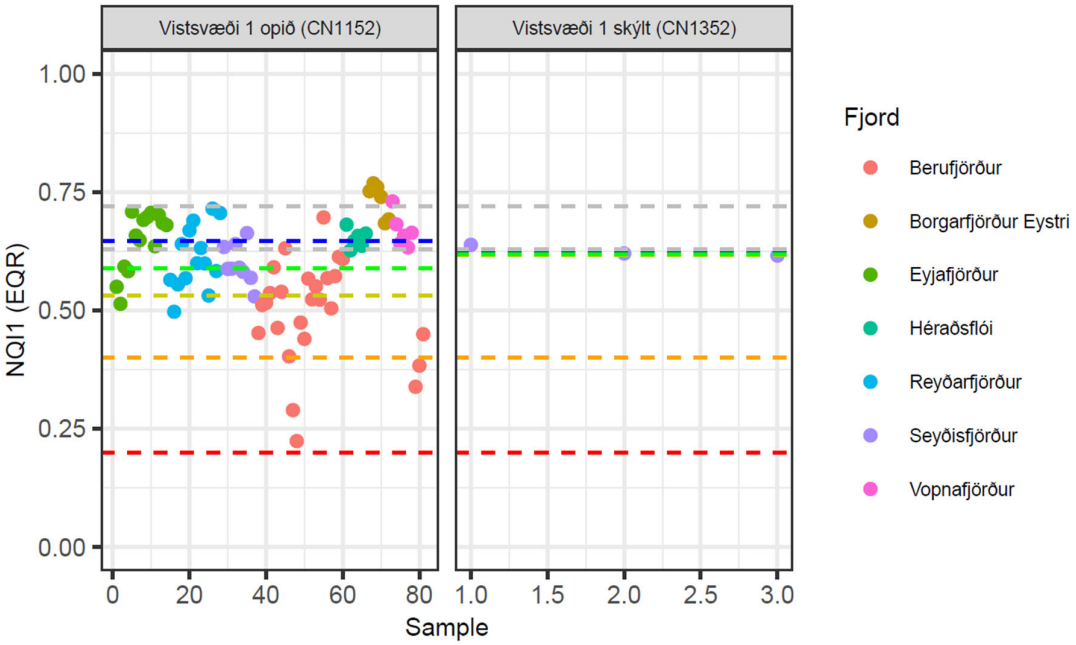

Figure 6. NQI1 values plotted by site in the eastern and northern waters off Iceland(Vistsvæði 1 opið / skýlt: water bodies of ecological type 1 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al., 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al., 2019).

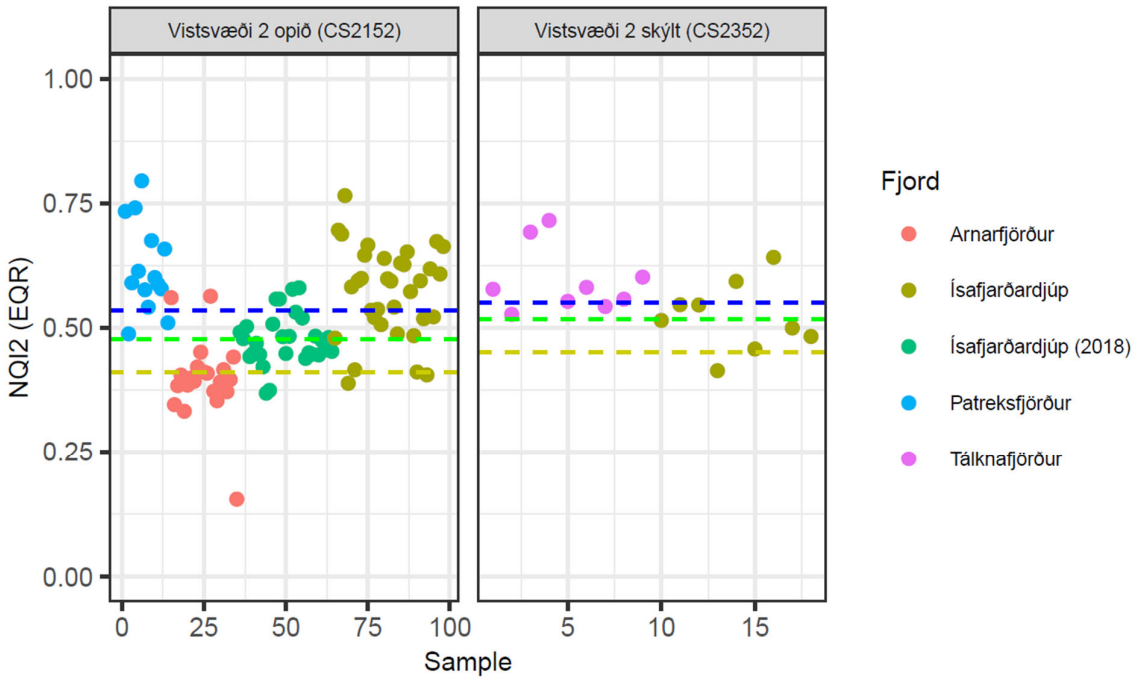

Figure 7. NQI2 values plotted by site in the western waters off Iceland(Vistsvæði 2 opið / skýlt: water bodies of ecological type 2 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður.

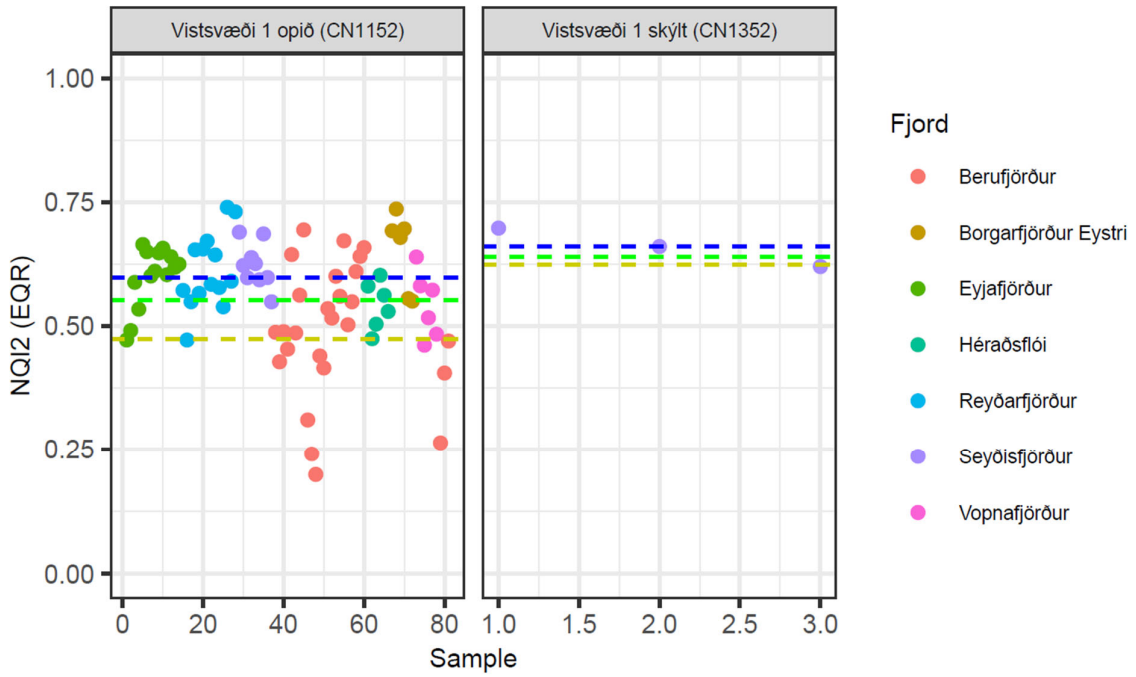

Figure 8. NQI2 values plotted by site in the eastern and northern waters off Iceland(Vistsvæði 1 opið / skýlt: water bodies of ecological type 1 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður.

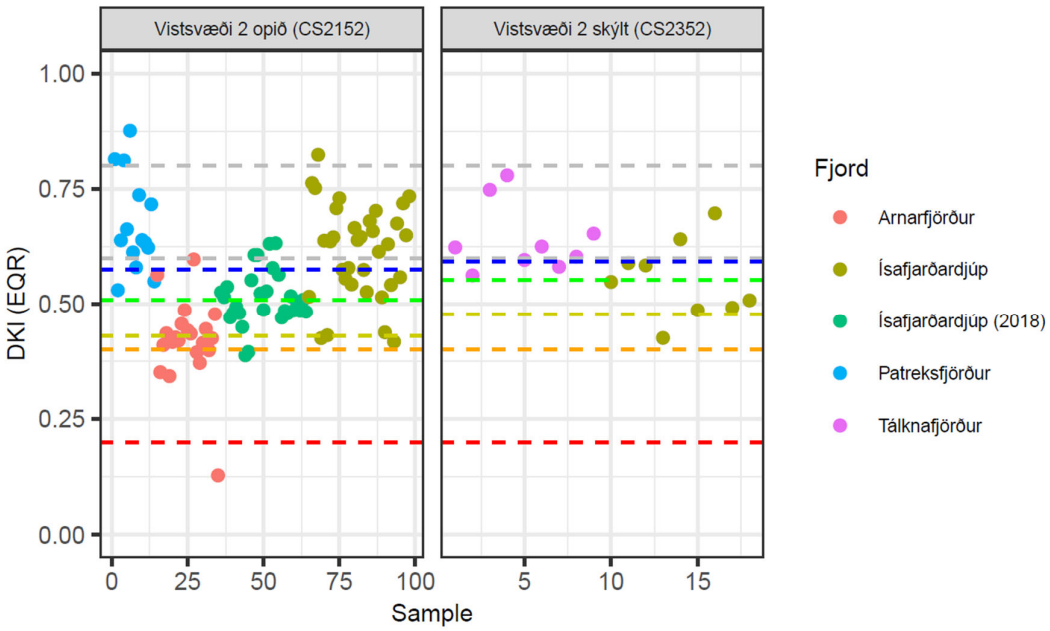

Figure 9. DKI values plotted by site in the western waters off Iceland(Vistsvæði 2 opið / skýlt: water bodies of ecological type 2 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al., 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al., 2019).

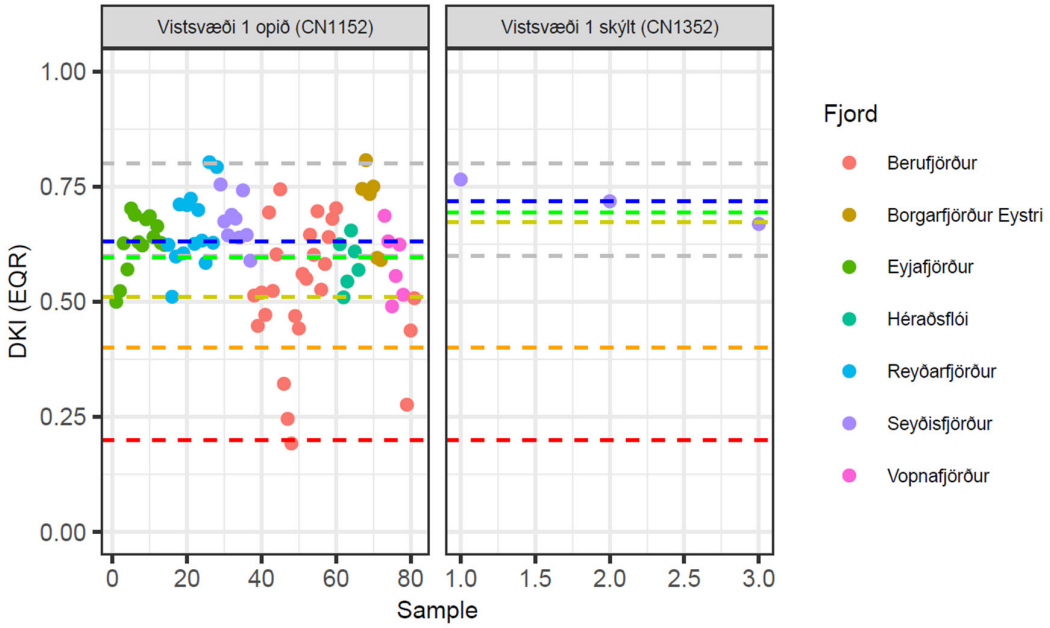

Figure 10. DKI values plotted by site in the eastern and northern waters off Iceland(Vistsvæði 1 opið / skýlt: water bodies of ecological type 1 open / sheltered). Sample numbers are a simple ordered identification number within water body type. The median (dark blue), 25% quantile (green), and 5% quantile (yellow) are plotted as horizontal dashed lines and were calculated excluding Berufjörður and Arnarfjörður. Orange and red dashed lines indicated intercalibrated values between the 'Moderate', 'Poor', and 'Bad' categories (Van Hoey et al., 2019). Grey dashed lines indicate the intercalibrated boundaries between the 'Very Good', 'Good', and 'Moderate' categories (Van Hoey et al., 2019).

3.2. **Proposed ecological status boundaries**

Both IQI and NQI1 were concluded as the best candidate indices, but only one was chosen for further analysis as they showed very similar patterns. It is possible to choose multiple indices, set boundaries for each index, and calculate a combined status using all indices when evaluating ecological status by first normalizing EQR ranges (following methods exemplified by Vannportalen, 2018, p. NQI1128). However, there is high uncertainty in setting boundaries in this study due to the unstandardized nature of data collection used in this study. Therefore, it was decided to continue only with a single best index until more standardized data became available with which status boundaries could be re-evaluated. NQI1 was chosen as the index to explore further and base ecological quality ratio boundaries on, based on similar usage in the neighboring country Norway.

Based on Figures 5 and 6, NQI1 values in Icelandic waters appear lower than values in other locations, as using the intercalibrated boundary values would yield very few sampled falling into the *Very Good* category. Rather, most would fall into the *Good* or *Moderate* categories. Possible reasons for this discrepancy between values found in Iceland versus other locations include 1) naturally lower diversity in Iceland, 2) lower taxonomic resolution of taxa included in this study due to high rate of endemic unclassified species, 3) lower taxonomic resolution of taxa due to the study designs within originating studies, or 4) smaller sample sizes and differences in sampling equipment. Therefore, it is suggested that the boundaries between *Very Good*, *Good*, and *Moderate* categories be shifted downward to fit the natural variation exhibited within Iceland to date, but the boundaries may need to be modified later if more standardized and detailed sampling methods yield higher diversity levels. To do this, it is proposed to use the median as the boundary between *Very Good* and *Good*, the 5% quantile between *Good* and *Moderate*. The 25% quantile was excluded because it often yields only a narrow range in index values between it and the median. At this time, the intercalibrated boundaries defining the boundaries between *Moderate*, *Poor*, and *Bad* (0,4 and 0,2, respectively) could be tentatively retained, with better evaluation upon collection of appropriate data, including a gradient of human impact and index response to it, with which to analyze these boundaries. In addition, because data are generally lacking for sheltered area water bodies in both the western and eastern regions, it is suggested that the values for open water bodies within the same ecological water body type (1 or 2) are used until more data are collected. A summary of proposed EQR boundary definitions based on the NQI1 index are presented in Table 3.

Table 3. Ecological quality ratios (EQR) for benthic invertebrates on soft bottom in coastal water bodies around Iceland based on the distributions of NQI1 index values by site within open water body types. Medians define the Very good / Good boundary, and 5% quantiles define the Good / Moderate boundary.

		EQR boundaries			
Water body type	**Description**	**Reference value**	**Very good**	**Good**	**Moderate**
Open North/East (CN1152)	Coastline in northern and eastern regions where the coast is exposed to wave action	1	1-0,65	0,65-0,53	<0,53
Sheltered North/East (CN1352)	Coastline in northern and eastern regions where the coast is sheltered	1	1-0,65	0,65-0,53	<0,53
Open South/West (CS2152)	Coastline in southern and western regions where the coast is exposed to wave action	1	1-0,58	0,58-0,45	<0,45
Sheltered South/West (CS2352)	Coastline in southern and western regions where the coast is sheltered	1	1-0,58	0,58-0,45	<0,45

3.3. **Further analysis of NQI1 and definition of boundaries of ecological quality ratios**

The NQI1 index showed that spatial patterns in the west fjords are not unexpected, such as localized clusters of high or low NQI1 values. Ísafjarðardjúp was found to be highly variable across its range, Arnarfjörður had rather low values (as expected), and Patreksfjörður and Tálknafjörður exhibited rather high-quality values, but similar to some samples from Ísafjarðardjúp (Figure 11). In the east fjords, variability could also be seen within fjords, with generally higher quality in outer waters (Figure 12).

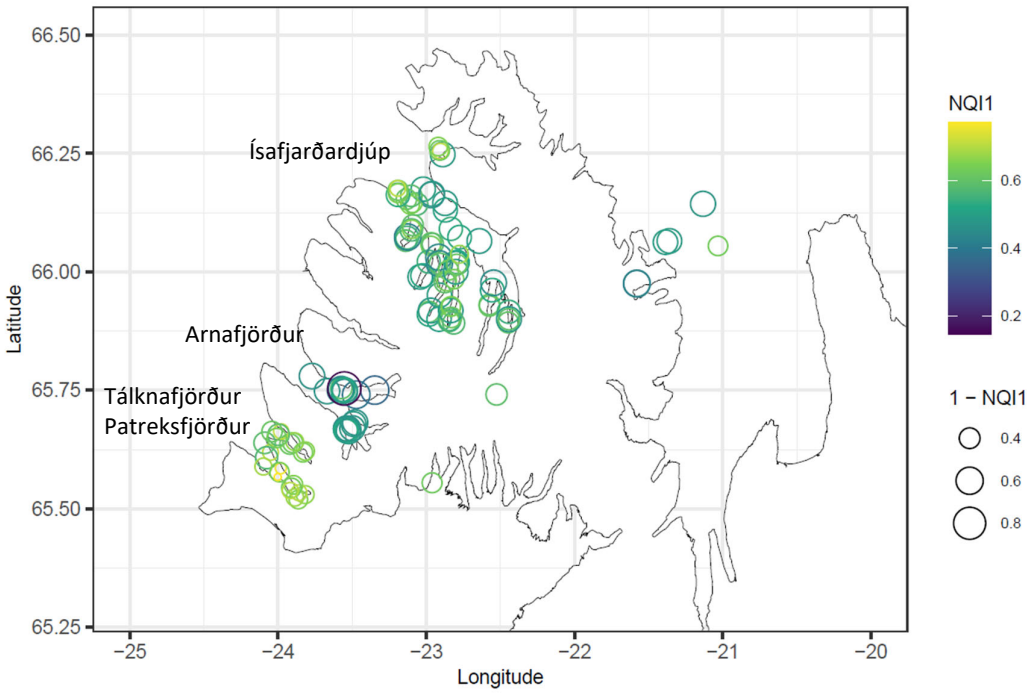

Figure 11. Spatial patterns in NQI1 in the west fjords. Large dark circles indicate low ecological quality; small light circles indicate high ecological quality. Points lying on land or to the northeast of west fjords have falsely recorded coordinate values from Ísafjarðardjúp; the point south of the west fjords has a falsely recorded coordinate value from Arnarfjörður.

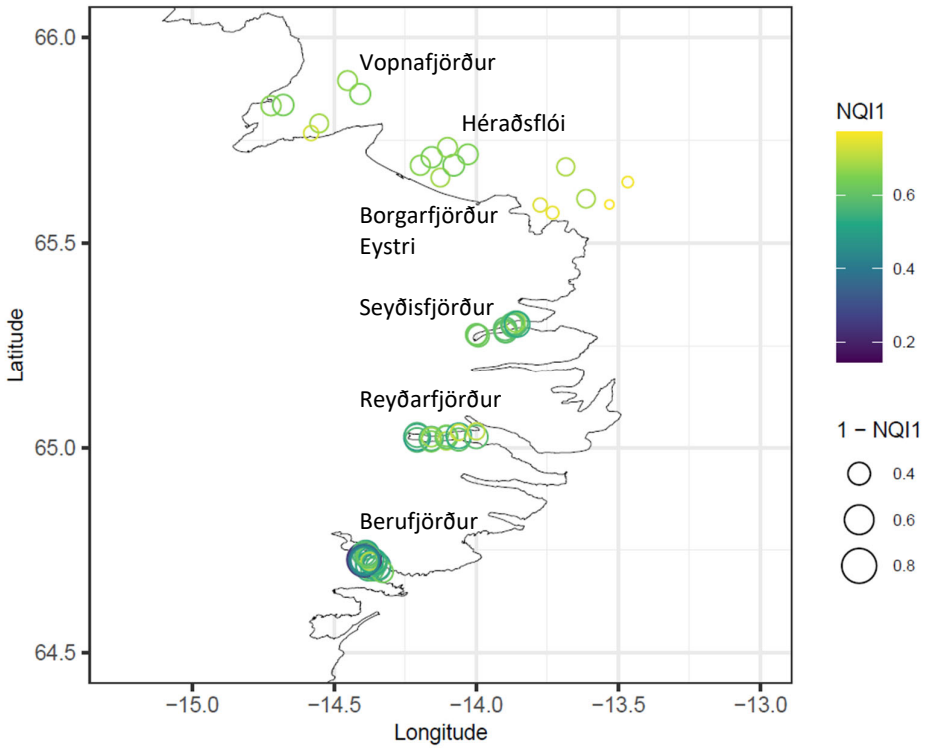

Figure 12. Spatial patterns in NQI1 in the east fjords. Large dark circles indicate low ecological quality; small light circles indicate high ecological quality.

When NQI1 values were translated into ecological status based on the proposed boundaries (Figures 13 – 14), these general trends were confirmed. Fjords overall showed a *Good* or *Very Good* status, confirming except for Arnarfjörður, which appeared highly variable but overall *Good - Moderate*, and Berufjörður, which contained isolated areas of '*Poor*' or '*Bad*' samples. The only low-quality indications were close to the source of pollution in Berufjörður, a pattern that was stable through multiple studies in which samples were taken during different time periods (Figure 15). These patterns agreed with expert judgement. Figure 16 shows high variability of samples taken over time at the same location within Eyjafjörður. Although there appears to be an increase in status over time, not enough is known about potential causes for an increased status to determine whether this trend has an underlying cause or is due to natural variation.

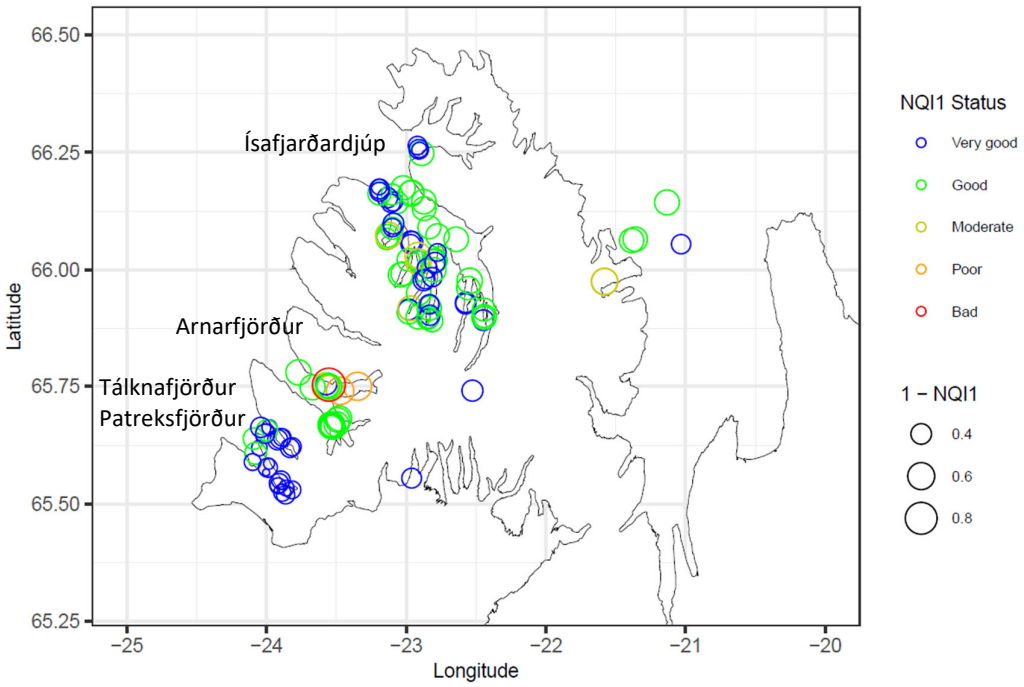

Figure 13. Spatial patterns in NQI1 categorizations in the west fjords based on proposed boundaries. Points lying on land or to the northeast of west fjords have falsely recorded coordinate values from Ísafjarðardjúp; the point south of the west fjords has a falsely recorded coordinate value from Arnarfjörður.

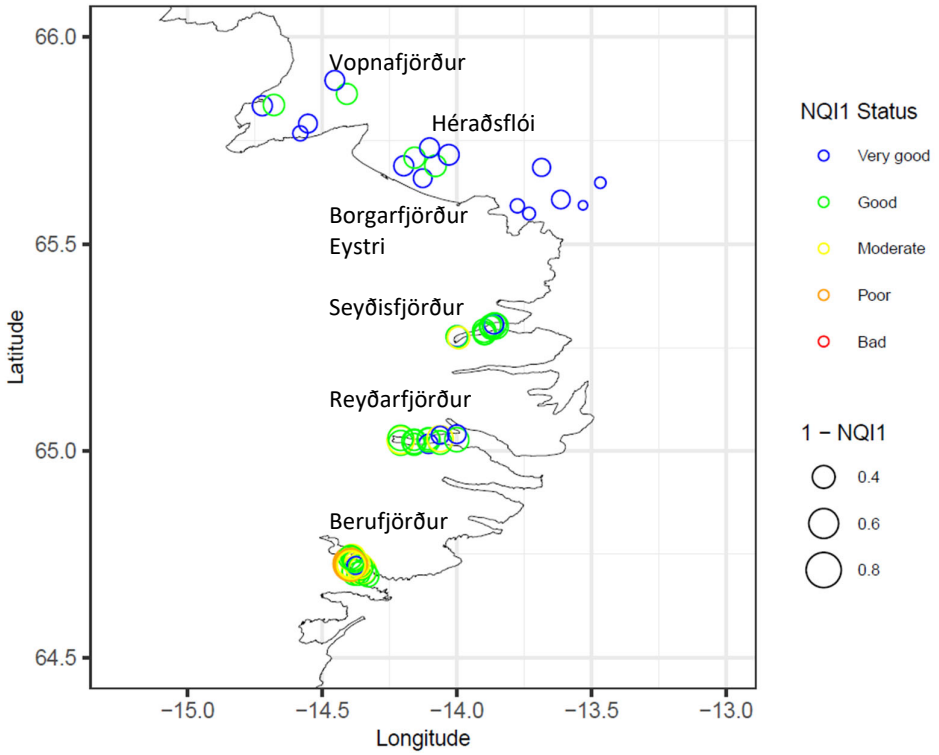

Figure 14. Spatial patterns in NQI1 categorizations in the east fjords based on proposed boundaries.

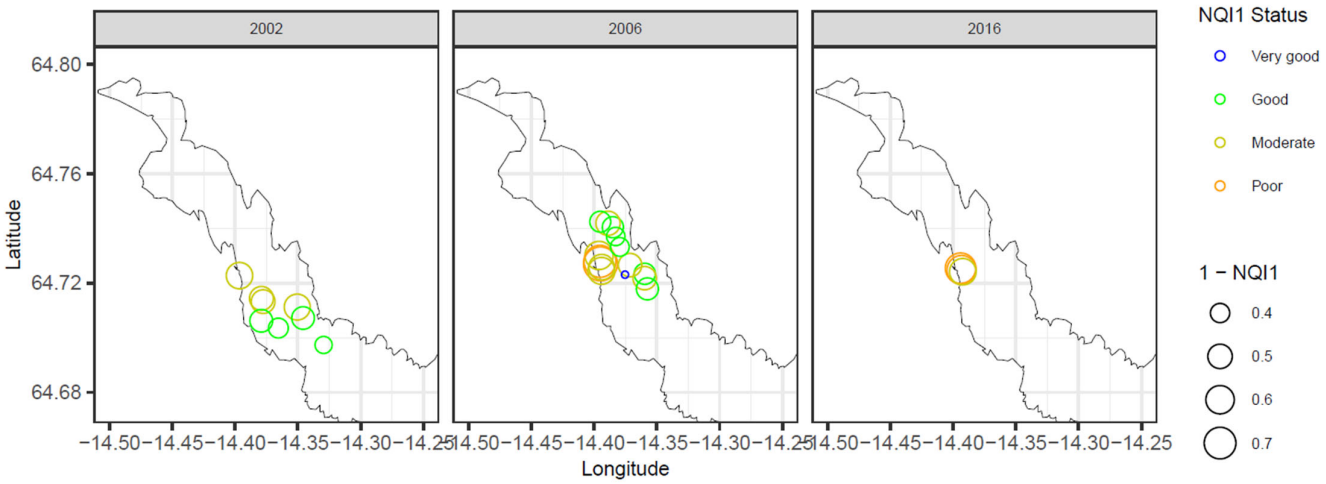

Figure 15. Spatial patterns in NQI1 categorizations in Berufjörður based on proposed boundaries, split by year (reflecting different studies).

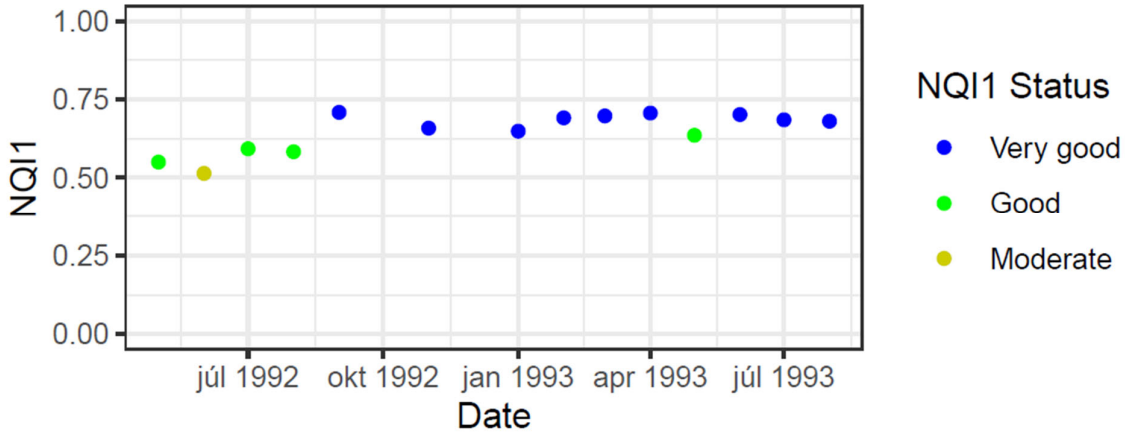

Figure 16. Temporal changes in ecological status according to NQI1 index at a single sampling location in Eyjafjörður, the only location under study with temporal replication.

3.4. **NQI1 boundaries in relation to components and other diversity indices**

Here a short comparison is given of how NQI1 boundaries translate to various common indices, some of which are included in the definition of the NQI1 boundary. These contrasts are useful when comparing historical benthic invertebrate data from Iceland that may report diversity indices, but not original count data.

The AZTI Marine Biotic Index (AMBI) is widely used to assess the quality status of benthic invertebrate assemblages and was developed in order to establish the ecological quality of European coasts and estuaries (Borja et al., 2007). The AMBI calculation is based on the sensitivity value assigned to species or groups when available, indicating the species tolerance or sensitivity to stress like pollution or other unfavorable conditions:

$$c_b = \frac{3}{2}\sum_{i=2}^{5}(i-1)p_i$$

where p_i is the proportion of species in sensitivity group i, and sensitivity groups were downloaded from the species list available at https://ambi.azti.es/descarga-de-ambi/ (Borja et al., 2000, Walvoort, 2019). AMBI is also a component of the NQI1 index, and highly reflective of resulting NQI1 Status (Figures 16 & 17). The highest AMBI values correspond with Poor or Bad statuses, which progressively improve as samples have lower values.

The Shannon-Wiener Index or Shannon's Entropy (*H'*) is a measure of diversity that combines species richness (the number of species in a given area) and their relative abundances:

$$H' = -\sum_{i=1}^{S} p_i(\log_2 p_i)$$

Where p_i is the proportion of individuals belonging to species *i*, and *S* is the total number of species. This index corresponded well in the west fjords (Figure 18), but has a weaker relationship in the east fjords, especially between Very Good and Good statuses (Figure 19). This could indicate that there is difference in the levels of "normal" state of the index within the waterbodies. The sea temperature north and east of Iceland is generally colder than in the south and west that could affect the general species richness.

Simpson's Index (λ') is a measure of diversity and takes into account the number of species present, as well as the abundance of each species:

$$\lambda' = \sum_{i=1}^{S}\frac{n_i(n_i-1)}{N(N-1)}$$

where n_i is the abundance of species *i*, *N* is total abundance, and *S* is total number of species (Walvoort, 2019). Simpson's Index showed also a generally good but weak correspondence in the west fjords (Figure 20), with little relationship in the east fjords (Figure 21).

Species diversity *S* was calculated as the number of different species that are represented in a given community or site. Species diversity showed only vague correspondence with NQI1 status, depending on the fjord, in both the west fjords (Figure 22) and east fjords (Figure 23).

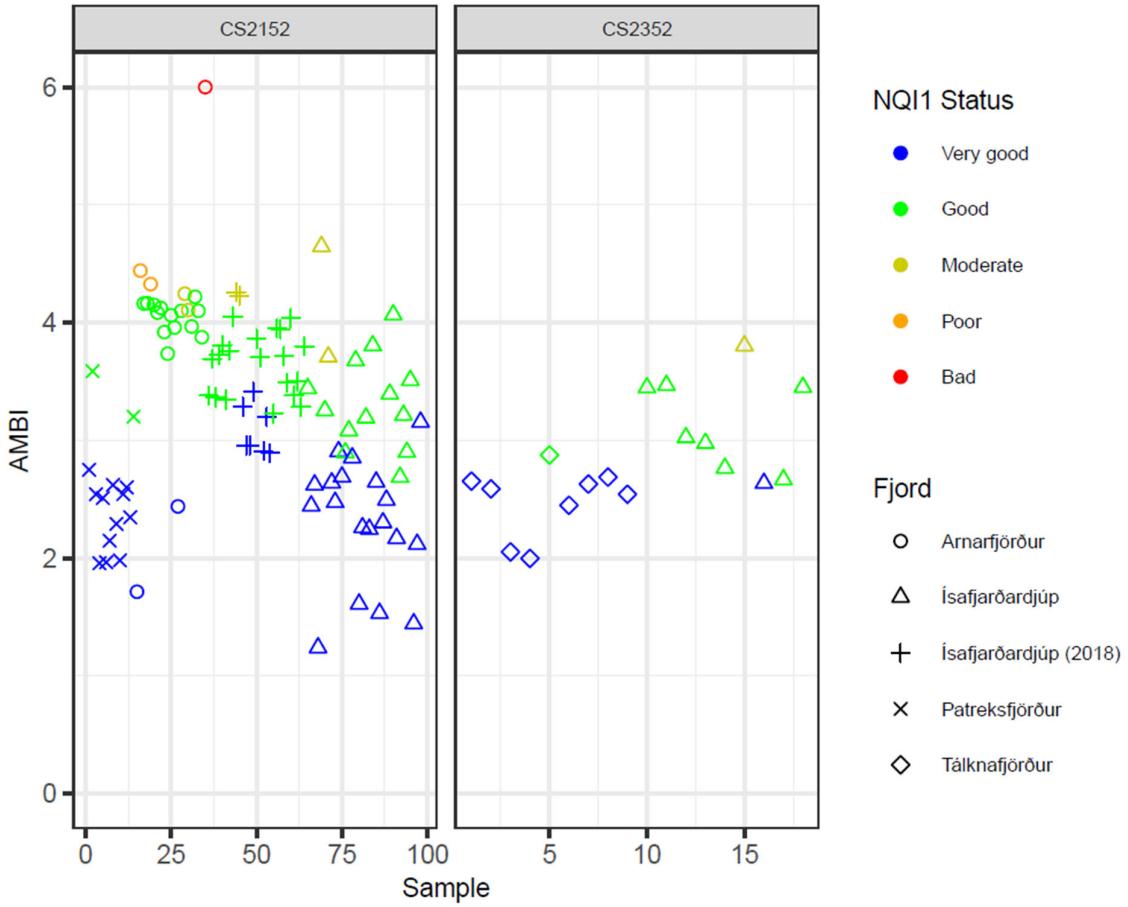

Figure 17. Sample AMBI values from the west fjords, coloured by sample NQI1 status according to the proposed boundaries.

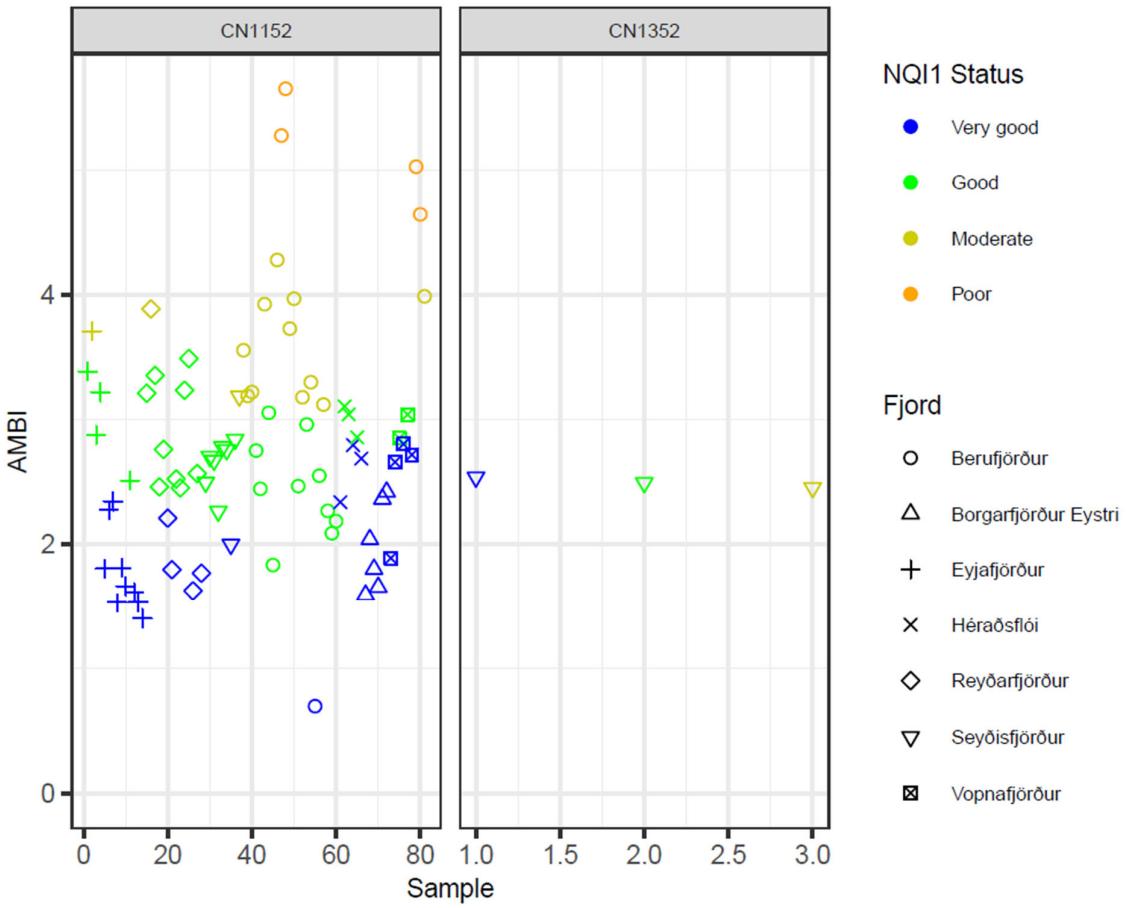

Figure 18. Sample AMBI values from the east fjords, coloured by sample NQI1 status according to the proposed boundaries.

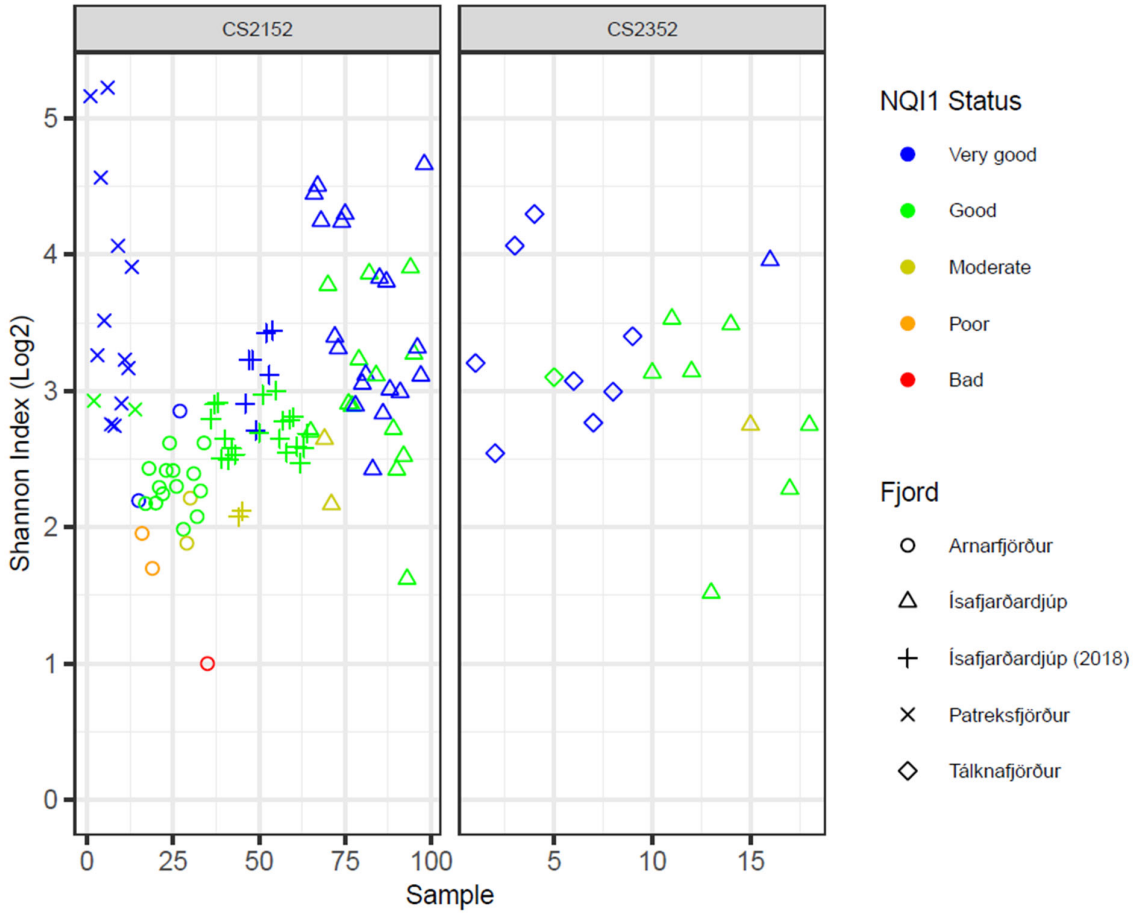

Figure 19. Sample Shannon-Wiener index values from the west fjords, coloured by sample NQI1 status according to the proposed boundaries.

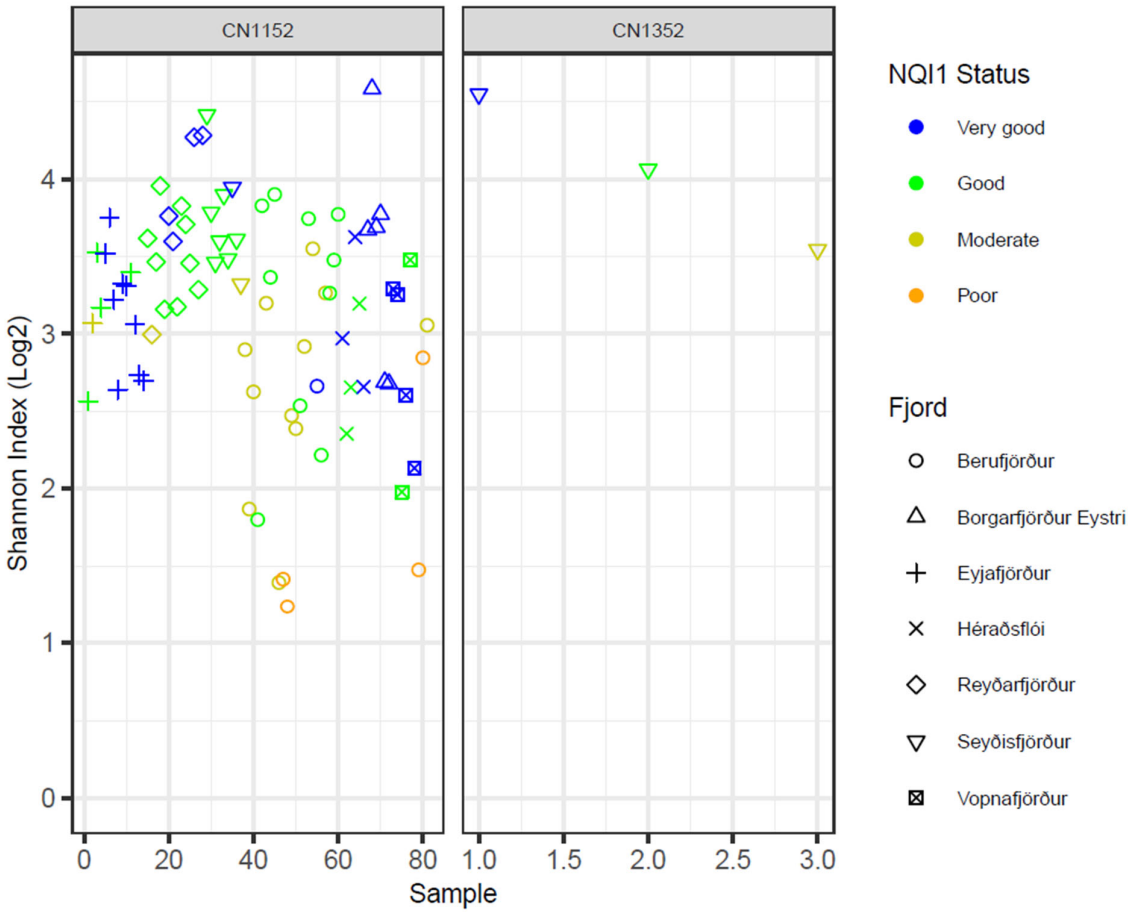

Figure 20. Sample Shannon-Wiener index values from the east fjords, coloured by sample NQI1 status according to the proposed boundaries.

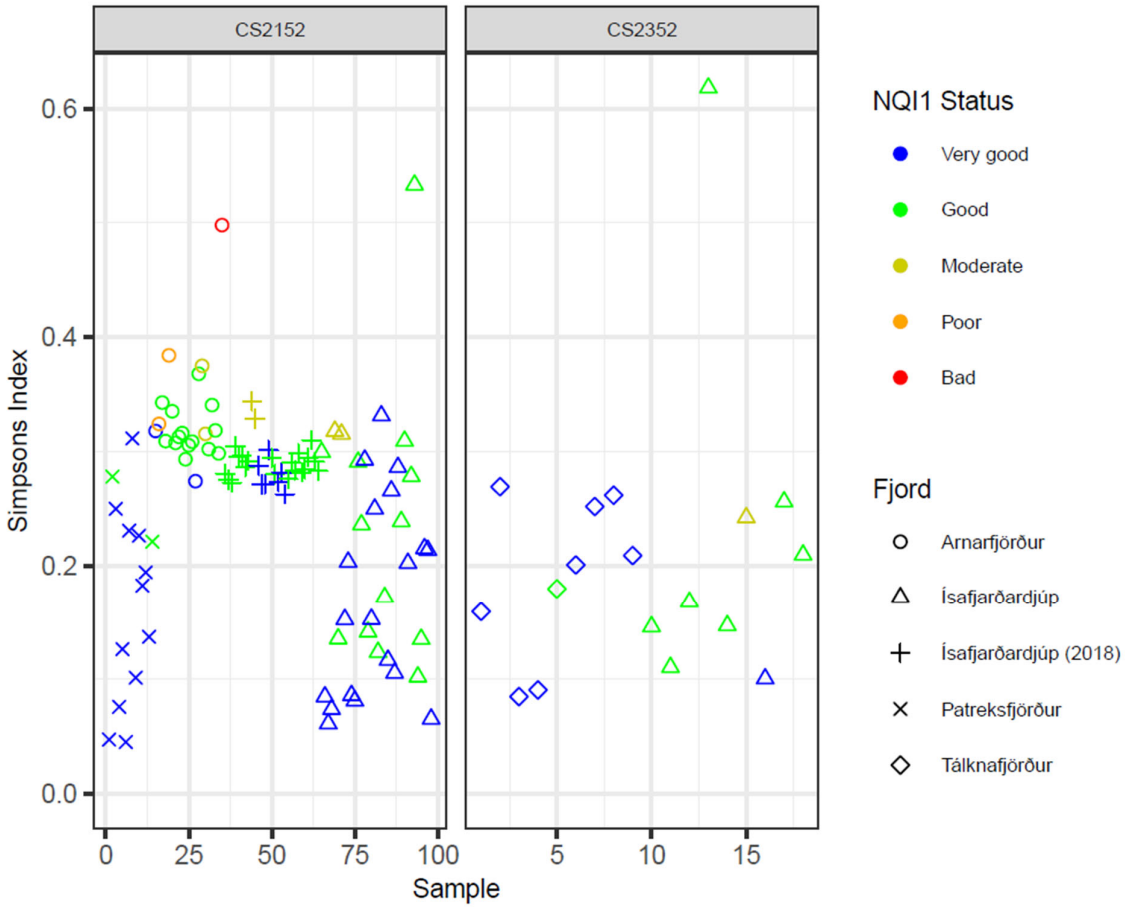

Figure 21. Simpsons index values from the west fjords, coloured by sample NQI1 status according to the proposed boundaries.

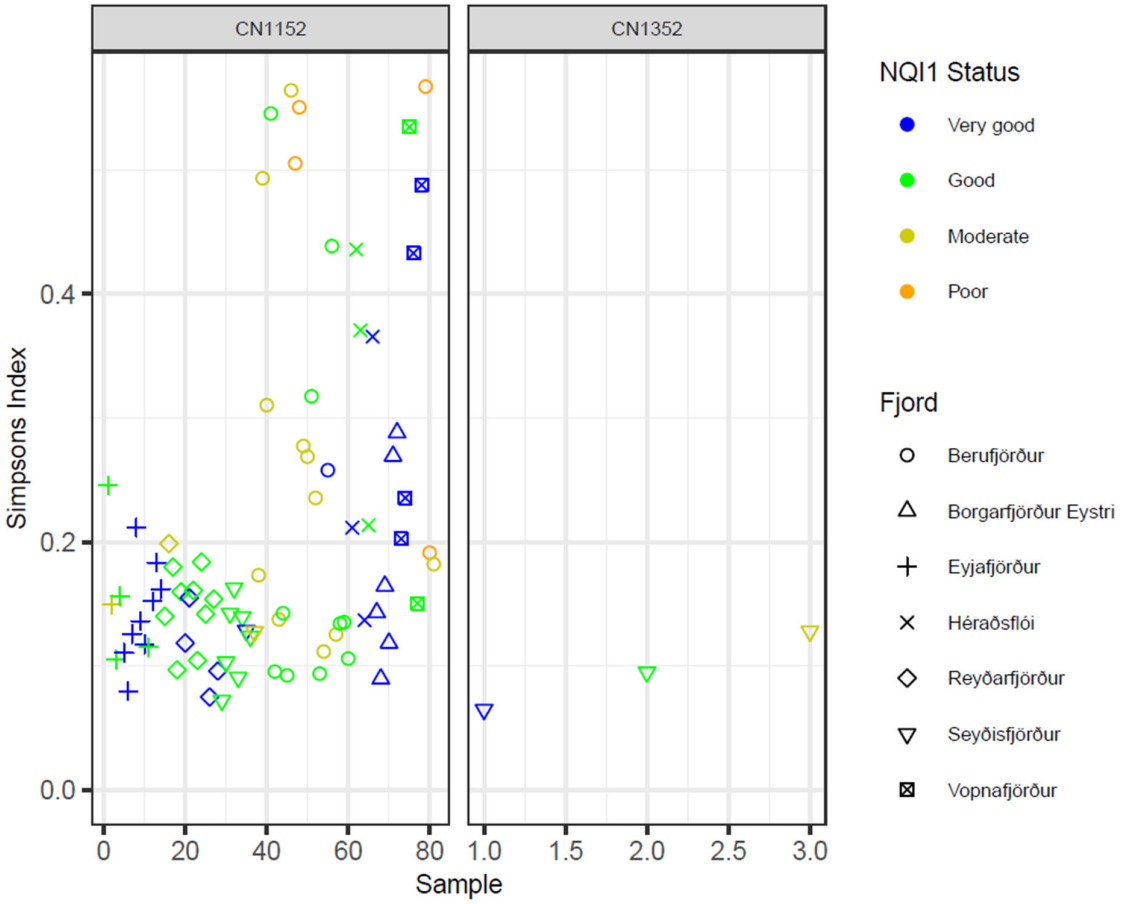

Figure 22. Simpsons index values from the east fjords, coloured by sample NQI1 status according to the proposed boundaries.

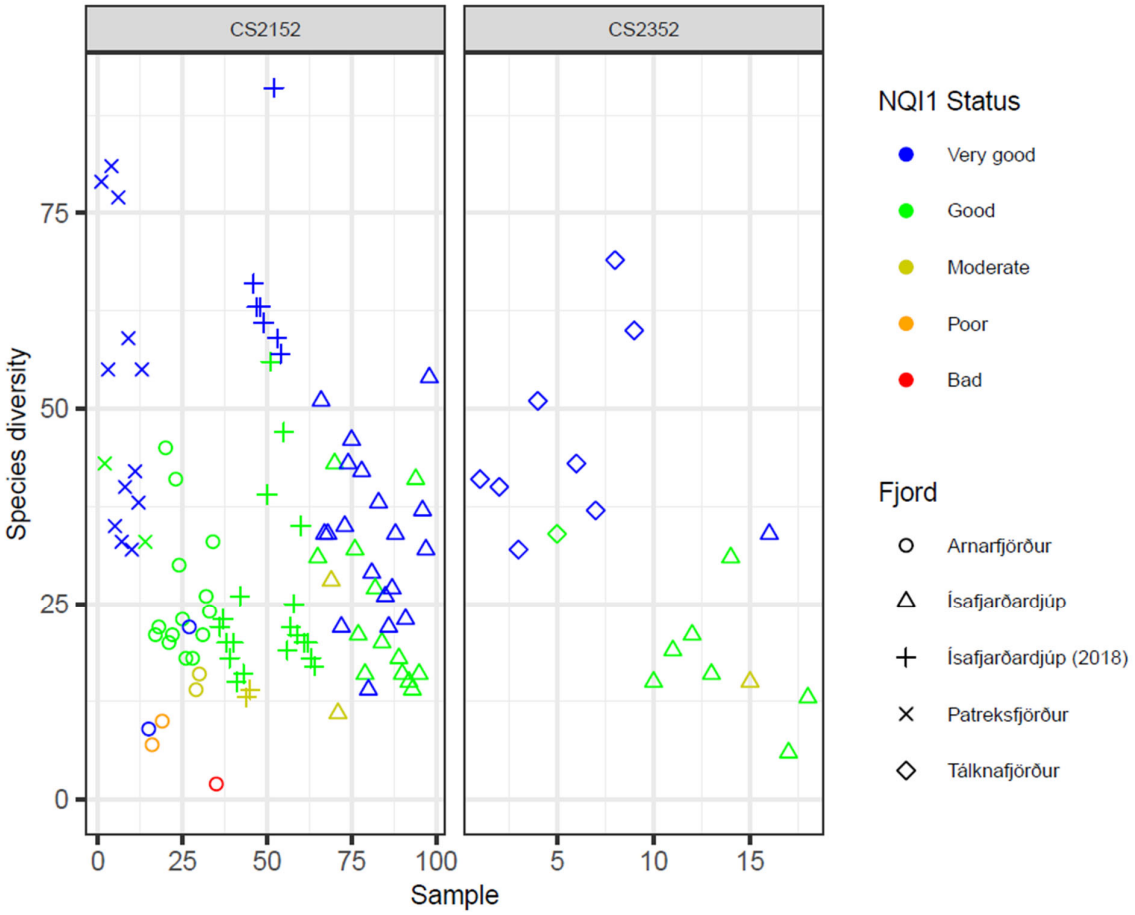

Figure 23. Sample species diversity values from the west fjords, coloured by sample NQI1 status according to the proposed boundaries.

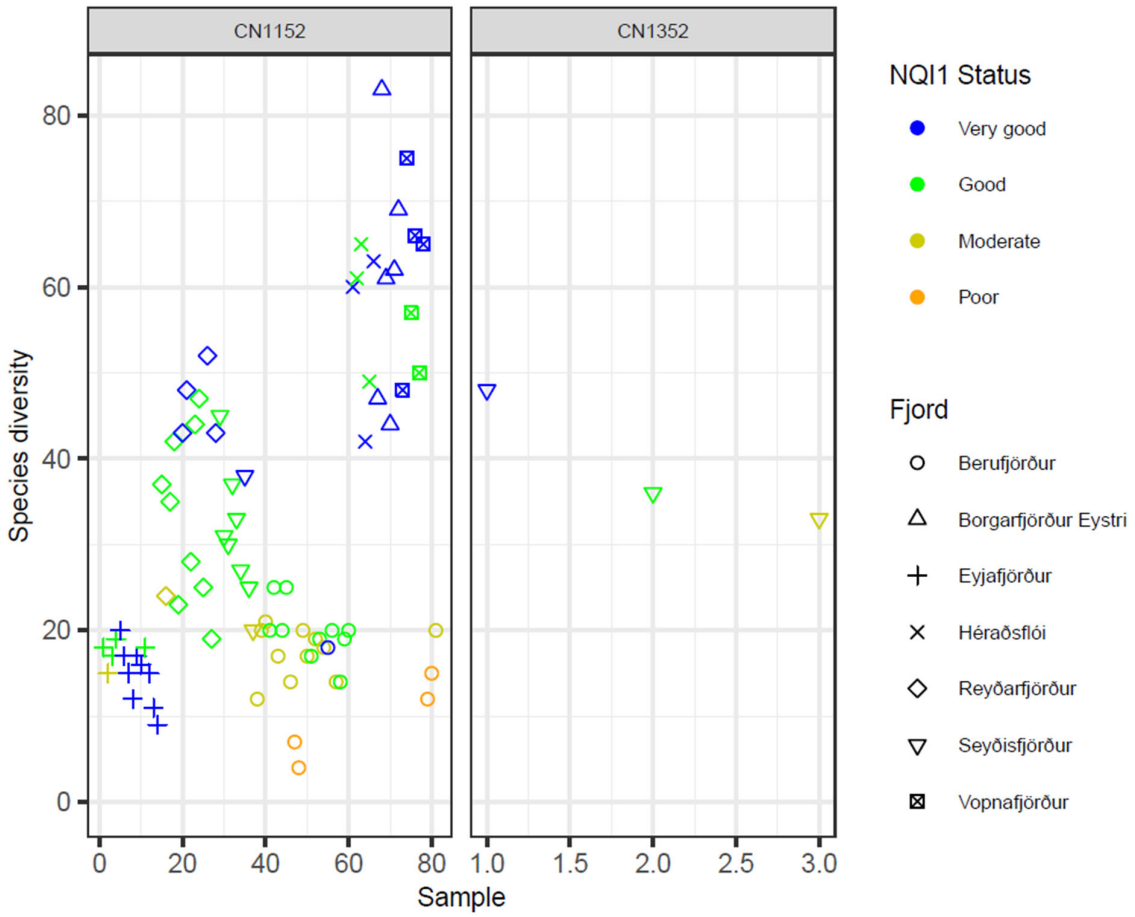

Figure 24. Sample species diversity values from the east fjords, coloured by sample NQI1 status according to the proposed boundaries.

4. Conclusion

This study suggests that, given data limitations on benthic invertebrates in Iceland as well as a focus on collection of samples from mainly undisturbed conditions, boundaries among ecological statuses should be derived mainly from how spatial and temporal patterns in diversity indices correspond with expert judgement. Many of the diversity indices considered have been intercalibrated and used in support of compliance with the Water Framework Directive in other locations, and therefore have a high likelihood of being responsive to environmental pressures within Iceland. NQI1 was chosen as the main benthic invertebrate quality index upon which EQR should be monitored in the short-term, due to its good performance in relation to expert judgement. However, the boundaries proposed here should only be taken as the first step in setting the framework for monitoring of coastal waters to be in compliance with the Water Framework Directive. Future work should include 1) the collection and analysis of samples designed for monitoring so that boundaries can be revised and made more precise in accordance with a defined sampling regime, 2) an analysis of how diversity indices respond to gradients of anthropogenic pressures, 3) the distinction of boundaries between open and sheltered water body types (if deemed necessary from future samples), and 4) evaluation of whether multiple informative quality indices should be used for evaluation by first normalizing and then combining EQR values.

5. Acknowledgments

We would especially like to thank Eydís Salome Eiríksdóttir and Sólveig Rósa Ólafsdóttir for direction in preparing these analyses to be applicable to the evaluation of coastal waters under the Water Framework Directive.

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HAFRANNSÓKNASTOFNUN

Rannsókna- og ráðgjafarstofnun hafs og vatna