Ecological status assessment in Irish coastal and estuarine sedimentary habitats using the Water Framework Directive Ecological Quality Ratio Infaunal Quality Index

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Ecological Status Assessment in Irish Coastal and Estuarine Sedimentary Habitats Using the Water Framework Directive Ecological Quality Ratio Infaunal Quality Index

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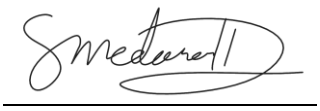
Declaration

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at the University of Galway or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of the University of Galway concerning plagiarism and intellectual property

Signed:

STORM MC DONALD



Abstract

The Infaunal Quality Index (IQI) is an Ecological Quality Ratio (EQR) developed for the purposes of monitoring coastal and polyhaline (estuarine) waterbodies to meet the requirements of the European directive Water Framework Directive WFD. In Ireland, from 2010 to 2023, IQI was calculated from benthic macrofaunal and sediment data using a custom Microsoft Excel software tool with embedded Visual Basic macros to determine suitable reference conditions for each station sampled. This involved the analysis of 4374 stations in 354 surveys of coastal and estuarine Assessment Groups (AG). The macrofaunal data was processed using a taxa library with a fixed taxonomy. An updated software tool, IQI V2 was released in 2024 including a substantially revised taxa library. Intercalibration between the IQI tools in coastal and estuarine surveys (Assessment Groups; AG) revealed that using all of the survey data, the agreement between the IQI was largely classified as "Very Good". This is less than the required classification of "Almost Perfect" required for intercalibration between different EQR under the requirements of the WFD. This was true of sample stations, AG and also in the classifications of the probabilities that the true mean EQR value for AG was above the critical Good/Moderate boundary in WFD ecological status assessment. The difference in output between the tools appears to be caused by differences in how the surveyed fauna taxonomy is entered into the tools, what taxonomic groups are included in calculating the EQR, and how taxa abundances are processed. When the stations dataset was reanalysed with the removal of 2033 stations where IQI V2 did not include >10% of the fauna in the EQR calculation, the agreement between the tools was greatly improved. The interpretation of IQI monitoring data from AG surveyed following the introduction of the IQI V2 tool should involve the recalculation of IQI from that waterbody in previous years to place subsequent findings in context.

The use of IQI in monitoring salmon aquaculture impacts in coastal waters was assessed using five years of survey data collected at three aquaculture sites on the west coast of Ireland (total 14 surveys). IQI was modelled as a function of distance from the sea pens, sediment organic content and the depth of the sediment apparent redox potential discontinuity (aRPD), including a random effect of aquaculture site. IQI decreased in response to increasing organic enrichment at the aquaculture sites (measured as increasing sediment organic content and decreasing aRPD). IQI was effective in mapping aquaculture impacts on coastal benthic communities. Adoption of IQI in aquaculture monitoring would facilitate improved management of coastal waterbodies by providing a common framework for aquaculture and other potential stressors to be assessed and managed in a cohesive network.

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List of Abbreviations

Abbreviation	Full Form
AMBI	AZTI Marine Biotic Index
AG	Assessment Group
aRPD	Apparent Redox Potential Discontinuity
BC	Biotic Coefficient
BHQ	Benthic Habitat Quality
BI	Biotic Index
BQE	Biological Quality Element
DAFF	Department of Agriculture, Fisheries and Food
EG	Ecological Group
EPA	Environmental Protection Agency
EQR	Ecological Quality Ratio
ES	Ecological Status
EU	European Union
GES	Good Ecological Status
IQI	Infaunal Quality Index
LOI	Loss on Ignition
NEAGIG	North East Atlantic Geographical Intercalibration Group
NMBAQC	National Marine Biological Analytical Quality Control Scheme
PC1	Principal Component 1
SEPA	Scottish Environmental Protection Agency
SI	Surface Index
SPI	Sediment Profile Imagery
UKTAG	United Kingdom Technical Advisory Group
VBH	Visual Benthic Health
WFD	Water Framework Dire ctive
WORMS	World Register of Marine Species

1. General Introduction

The Water Framework Directive (WFD) of the European Union, which went into effect in 2000, is a key instrument in the management of aquatic ecosystems in Europe (WFD, 2000). The directive's goal is to ensure that all member states' surface waters, such as rivers, lakes, transitional waters, and coastal waters, have a "Good" or "High" ecological and chemical status (WFD, 2000). This policy was developed to address concerns about how human activities, such as eutrophication, the removal of sensitive habitats, and chemical water pollution, were having detrimental effects on aquatic ecosystems (Smith et al., 1999). The WFD introduced the concept of Ecological Quality Ratios (EQR). EQR are a standardised way to measure how far an ecosystem is from its original or least disturbed state (Hering et al., 2006). The WFD's implementation depends on these ratios. EQRs are used to put water bodies into one of five ecological status categories (Bad, Poor, Moderate, Good or High). This provides a consistent means to assess and restore the health of aquatic ecosystems (WFD, 2000). The WFD has identified benthic ecosystems as habitats of concern. These are communities of species that live at the sediment-water interface (WFD, 2000).

Benthic communities play a key role in ecosystem functioning within waterbodies, including nutrient cycling, maintaining stable sediment types, and playing a key role in aquatic food webs (Gray et al., 2002). However, benthic communities are sensitive to anthropogenic disturbance such as eutrophication, sediment disturbance by trawling or dredging, and chemical pollution (Borja et al., 2000). The Water Framework Directive (WFD) requires that validated multimetric evaluation methods, such as the Infaunal Quality Index (IQI), are employed to monitor and manage the benthic component of ecosystems (WFD, 2000). The IQI EQR combines three biological indices, including the number of species, Simpson's Evenness Index (Simpson, 1949), and the AZTI Marine Biotic Index (AMBI) (Borja et al., 2000) to measure the ecological quality of benthic habitats (Borja et al., 2000; Muxika et al., 2007). This multifaceted approach ensures that the IQI can detect subtle changes in ecosystem health, making it a crucial component of WFD-compliant monitoring systems.

1.1. Historical Evolution of Benthic Monitoring

The observation of benthic ecosystems has historically been essential to marine ecological research and management (McCall, 2013; Snelgrove & Butman, 1994). The first attempts to assess benthic health were driven by the recognition of sedimentary environments as a source of ecological change. Rhoads & Boyer, (1982) highlighted the susceptibility of benthic organisms to physical disturbances, including sedimentation and dredging. Pearson & Rosenberg, (1977) introduced a

successional paradigm linking changes in benthic community structure to gradients of organic enrichment. This model suggested that rising levels of disturbance would cause a steady drop in sensitive taxa and a rise in opportunistic species. The initial frameworks laid the groundwork for modern benthic monitoring methodologies, emphasising the importance of incorporating biological responses in the context of the receiving environment. Subsequent studies (Grall & Glémarec, 1997) progressed the development of quantitative measures of benthic health using a successional paradigm. The AZTI Marine Biotic Index (Borja et al., 2000) was among the first tools to quantitatively classify benthic organisms based on their tolerance to organic enrichment. This enables the ratio of opportunistic, disturbance-tolerant species to climax, disturbance-sensitive species to be quantified. This index is a key part of the Infaunal Quality Index (Phillips et al., 2014), which uses AMBI along with other measures, including the number of species and evenness (Simpson, 1949), to give a more complete picture of ecological quality.

1.2. Development of the Infaunal Quality Index (IQI)

The IQI represents a significant advancement in benthic monitoring for the purposes of the WFD, combining multiple ecological indicators to generate a single EQR (Borja et al., 2000; Muxika et al., 2007)). Developed for use in the United Kingdom and Ireland, the IQI was designed to address the unique challenges posed by transitional and coastal waters (WFD-UKTAG, 2014). Unlike earlier indices, which often focused on single metrics, the IQI reflects a holistic understanding of benthic ecosystem dynamics, making it particularly effective for assessing complex and dynamic environments (WFD-UKTAG, 2014)

The IQI's utility has been demonstrated across various ecological contexts, from eutrophic estuaries to pristine coastal habitats (Borja et al., 2007; WFD-UKTAG, 2014). Its development was informed by extensive intercalibration exercises, which aligned IQI classifications with other assessment tools to ensure consistency and comparability (Borja et al., 2007; Phillips et al., 2014). However, the application of IQI is not without challenges. Variability in hydrodynamic conditions, sediment composition, and natural disturbance regimes necessitates site-specific calibration to provide accurate assessments of likely reference conditions against which to assess the EQR outputs (WFD-UKTAG, 2014). Furthermore, the incorporation of rapidly evolving taxonomic classifications remains a critical area for refinement, particularly as new species and ecological interactions are identified (Borja et al., 2000; Borja et al., 2008).

To align with the broader European framework under the WFD, Ireland has actively participated in intercalibration exercises to harmonise IQI assessments with those employed in other EU

member states (Poikane et al., 2014). These collaborative efforts involve standardising methodologies, reference conditions, and classification boundaries to ensure that ecological status classifications are consistent across member states in similar biogeographic regions (Borja et al., 2000; Borja et al., 2007; Poikane et al., 2014; WFD-UKTAG, 2014). This is particularly important in shared water bodies, such as the Irish Sea, where transboundary cooperation is crucial for effective management. Intercalibration plays a vital role in enabling Ireland to align with evolving European guidelines and contribute effectively to the broader objectives of the WFD (WFD-UKTAG, 2014). As the EU incorporates updated ecological metrics and climate adaptation strategies into its water management frameworks, Ireland's ability to integrate these advances will improve the comparability and relevance of its monitoring programs (European-Commission, 2023). This ongoing process provides consistency across member states while addressing region-specific challenges (Borja et al., 2007; WFD-UKTAG, 2014).

EQRs such as IQI usually require the development of a software tool to facilitate the processing of taxonomic and environmental data to set reference conditions for the component indices, and assess the ES of new survey data (Borja et al., 2007; Phillips et al., 2014). These software tools contain taxa libraries that include fields for, amongst other purposes, the Ecological Group (EG) assigned for AMBI calculation, whether taxa should be included in AMBI calculation, and how recorded abundance should be processed (as abundance, as presence/absence, etc.). Much of the data has been derived from expert judgement (Borja et al., 2007). IQI is calculated using a software tool developed as a Microsoft Excel workbook containing multiple Visual Basic macros (Phillips et al., 2014). Updating the taxonomic framework within the IQI is essential to maintain its relevance and accuracy (Phillips et al., 2014). Ongoing advances and improvements in the taxonomic classification of benthic taxa (WORMS, 2025) means that the fixed IQI taxa library requires regular updating to reflect the format of data produced by benthic survey in successive years (Korshunova et al., 2019). In practice, changes to the taxa library may alter the IQI EQR score assigned to individual samples, potentially changing the ES assigned to AG when the taxa library is updated. To ensure consistency in monitoring data, it has been standard practice in Ireland to treat the taxa library in the IQI tool as fixed and to make survey data collected in subsequent years compliant with the taxa library in the IQI V1 tool, dated 11/03/2014.

The IQI tool was updated in a release on the UK WFD Technical Advisory Group website in 2024, without accompanying development documentation equivalent to Phillips et al., (2014) and WFD-UKTAG, (2014). This update included a substantial revision, expansion and modernisation of the taxa library to be compliant with the WORMS taxonomic database (dated 14/12/2022). This

revised MS Excel tool is termed IQI V2. The IQI V2 tool contains instructions for the use of the Excel tool, including instructions on how to update the taxa library to maintain compliance with the WORMS database. This substantial revision of the IQI will greatly facilitate the inclusion of modern taxa by station matrices for IQI assessment, allowing for improved assessment of benthic biodiversity within the WFD framework. However, it raises the question of whether the IQI EQR scores produced by the two versions of the tool are directly comparable. This has potential management implications for WFD AG that have been assessed several times in the period 2010 to 2023.

1.3. Application of Infaunal Quality Index to Aquaculture Monitoring

Salmon pen aquaculture impacts potentially produce strong organic enrichment gradients on soft sediment benthic communities in coastal embayments (Lu & Wu, 1998; Pearson, 1978). These gradients are reflected in the distribution of benthic macrofaunal communities. Typical successional gradients consist of a dominance of opportunistic, stress-tolerant taxa under salmon net pens, with a gradual appearance of less opportunistic taxa with increasing distance from the pens (Macleod et al., 2002; Weston, 1990; D. J. Wildish et al., 2001). The monitoring protocol for aquaculture impacts in Irish coastal embayments includes the collection of benthic faunal samples, sediment type data and estimates of organic enrichment of the sediment from direct sediment organic content analysis (Dean, 1974; Hargrave et al., 2008; Hargrave et al., 1997; Pearson, 1978) or the use of Sediment Profile Imagery (Nilsson & Rosenberg, 2000; Pearson, 1978; Pearson & Rosenberg, 1977; Wildish et al., 2003). A planned update to the current aquaculture monitoring in Ireland protocols (DAFF, 2008) will include the calculation of IQI V2 from benthic stations located on transects from the edge of the sea pen, both in line with, and perpendicular to, the prevailing current. Benthic survey data collected using a similar spatial design has been collected at several aquaculture sites in Ireland. IQI has not previously been used to assess potential salmon pen aquaculture impacts in Irish waters. In Scotland, IQI has been previously used to assess aquaculture impacts on coastal waters (Fox et al., 2022; SEPA, 2019).

1.4. Objectives

The objectives of this study aim to elucidate the effectiveness of IQI in assessing anthropogenic impacts in soft seafloor macrofaunal communities for the purposes of effective environmental management. This study aims to produce comparable Ecological Status (ES) assessments in different habitats and in relation to a variety of potential environmental impacts.

Objective 1: To review and elucidate the development and functioning of the IQI tools. This objective aims to provide a clear account of the data processing approach taken by the IQI tools, including, where possible, an account of the differences between the IQI tools. This objective aims to place the potential impacts of the IQI software tool on the Ecological Status assigned to waterbodies in the context of ongoing management of WFD waterbodies.

Objective 2: To assess the intercalibration of the IQI V1 and IQI V2 tools using the approach previously reported in the literature for intercalibrating different EQR used by neighbouring member states in the same geographic area. All previously reported intercalibration exercises were focused on intercalibrating the EQR using station data collected along strong anthropogenic gradients. This study will also assess the agreement between the tools using long-term monitoring data at the Assessment Group (waterbody x year) level. It will examine both the mean IQI EQR and mean ES class returned by the two tools. It will also intercalibrate the agreement between the tools on the Confidence of Classification (the probability that the true mean EQR is above the Good/Moderate boundary).

Objective 3: To investigate the effectiveness of IQI V2 in detecting the impact of salmon net pen aquaculture in coastal embayments in Irish waters. The distribution of IQI EQR values and ES classes will be assessed on previously collected transects of macrobenthic samples from 3 aquaculture sites on the west coast of Ireland. The distribution of IQI EQR and ES values will be assessed in the context of supporting environmental sediment quality data, including Sediment Profile Imagery (SPI).

2. Evolution of the IQI: Comparing Version Updates in Assessing Benthic Ecosystems

2.1. Abstract

The Infaunal Quality Index (IQI) is an Ecological Quality Ratio (EQR) developed for the purposes of monitoring coastal and polyhaline (estuarine) waterbodies to meet the requirements of the European directive Water Framework Directive WFD. WFD EQR are multimetric indices that compare the diversity and abundance of invertebrate taxa and the proportion of disturbance-sensitive taxa when comparing sampled data to reference conditions. IQI is calculated as a decimal fraction between 0 and 1. The IQI EQR is classified into five Ecological Status classes (Bad, Poor, Moderate, Good, High). Waterbodies that are classified with a mean ES of Good or High are considered to have "passed" WFD assessment for the benthic element. The Good/Moderate boundary is a key criterion in comparing IQI EQR outputs. IQI is calculated using a software tool that processes macrofauna, associated sediment grain size data and waterbody salinity classification to compare the sampled data to reference conditions for the particular habitat of a given sample. IQI was developed by the Environment Agency in the United Kingdom (UK), using UK data to set reference conditions.

IQI has been used to annually monitor the benthic macrofaunal communities of designated Irish waterbodies, Assessment Groups (AG), from 2010 to 2025. In 2024, the software tool for IQI was updated from the previous version (IQI V1) to the current version (IQI V2). The update to the tool includes changes to the taxa library to update the taxonomy, changes to the taxa sensitivity to disturbance (Ecological Group) assigned to some of the taxa, and changes to whether or not some taxonomic groups are included in the calculation of IQI by the tool. Irish IQI data collected from 2010 to 2023 and processed using IQI V1 was intercalibrated with IQI V2 to verify that the ES assigned to AG is comparable between the two versions of the tool. Intercalibration was carried out using weighted Kappa analysis. This included 4,374 sample stations at 354 AG. The intercalibration was performed at sample station level, at AG level and also using a classification of the probability that the true mean IQI value for AG was above the Good/Moderate boundary. For each dataset, the intercalibration was carried out for all the data, and also using only the coastal and polyhaline portions of the data. The accepted standard for EQR intercalibration is Almost Perfect agreement.

Intercalibration results showed that for all stations the agreement between the tools was Very Good, while for coastal stations the agreement was Very Good and for polyhaline waters it was

Almost Perfect. The same analyses carried out at AG level showed Very Good agreement for all AG, and for the coastal and polyhaline AG considered separately. When the probability classes were intercalibrated, the probabilities for all AG and for coastal AG returned Very Good agreement, while polyhaline AG produced Almost Perfect agreement.

The suboptimal agreement for much of the data appears to be largely the result of changes in how taxa are assigned to Ecological Groups. For IQI V1, almost 100% of taxa are assigned an EG and included in the calculation of the EQR. For IQI V2, >10% of the fauna is not included in the calculation at 2,033 stations. Intercalibration with those stations removed returned Almost Perfect agreement at all stations and at polyhaline stations, while coastal stations agreement was classified as Very Good, but the Kappa statistic had improved and was barely below Almost Perfect agreement.

IQI V2 results are not directly comparable with IQI V1 results calculated in previous WFD assessments. When interpreting IQI V2 results in Irish waters, previous data, calculated in IQI V1, should be recalculated in IQI V2 to place new results in context for environmental management and WFD reporting requirements.

2.2. Introduction

This study addresses the potential impacts of the update of the software tool to assess seafloor habitat quality (Ecological Status) in soft sediments in Ireland using benthic infauna for the purposes of the Water Framework Directive (WFD). The measure used is the Infaunal Quality Index (Phillips et al., 2014), a multimeric index that incorporates measures of the level of diversity and abundance of invertebrate taxa, and the proportion of disturbance-sensitive taxa when determining the comparison of sampled data to reference conditions (Muxika et al., 2007; WFD, 2000). Calculation of IQI is carried out using an MS Excel software tool with embedded Visual Basic macros and species libraries that assign disturbance tolerance attributes to taxa recorded in samples. The tool also sets reference conditions for a given sample using the salinity and sediment characteristics of the station sampled. Changes to the species library and reference conditions have the potential to change the ES assigned to sample stations and waterbodies assessed for the purposes of the WFD. Version 1 of the IQI software tool was used to calculate IQI and assign ES to waterbodies from 2011 to 2023. Version 2 of the software tool was released in 2024.

The WFD is part of the European Union's environmental policy, established to achieve “Good” or “High” Ecological Status (ES) and Chemical Status (CS) in all surface waters, including rivers, lakes, transitional waters, and coastal waters (WFD, 2000). It emphasises an integrated approach to water management, requiring member states to classify waterbodies based on their ecological and chemical quality and implement measures to restore and protect aquatic ecosystems (Copetti & Erba, 2024). Ecological Status (ES) is classified into one of five levels (High, Good, Moderate, Poor and Bad).

ES in a given waterbody is classified by sampling appropriate quality elements for that waterbody. The main quality elements for marine and estuarine waterbodies assessed for WFD ES classification were summarised as three types by (Devlin et al., 2007)

1. Physico-Chemical Quality Elements (PCQE) include salinity, water transparency, temperature, oxygen levels, salinity, nutrient concentrations, and some specific pollutants that are also considered in the directive.
2. Hydromorphological Quality Elements (HQE) include factors that may affect the condition of BQE, including: freshwater flow, substrate structure and extent, wave exposure characteristics and water depth distribution.

3. Biological Quality Elements (BQE) include: the composition and abundance of benthic invertebrates, fish, and attached aquatic flora and also the composition, abundance and biomass of phytoplankton.

Where human influence alters PCQE or HQE of a waterbody, this may impact the BQE of the waterbody, for example, where PCQE, such as nutrient concentrations in a waterbody are not in High or Good status, this may influence the structure of BQE, such as the benthic invertebrate community structure of subtidal sediments (Borja et al., 2004). Consequently, regular surveillance monitoring in WFD waterbodies is often focused on one or several BQE deemed appropriate for the waterbody under consideration (Devlin et al., 2007).

BQE are assessed by sampling biological communities in WFD waterbodies. The sampled data is used to calculate an Ecological Quality Ratio (EQR), which quantifies the degree to which a sample station deviates from reference conditions which represent the undisturbed or minimally disturbed state of the BQE (Borja et al., 2012). WFD EQR are required to generate a standardised score, ranging from 0 (severely degraded) to 1 (pristine conditions), by comparing the scores observed to reference conditions for the habitat type or ecosystem component sampled (WFD, 2000). Waterbodies are classified into one of the five ES classes using class boundaries for the EQR (Kennedy et al., 2011). Where a waterbody is classified as having ES lower than Good, the member state must take remedial action to restore the waterbody to Good Ecological Status (GES) (WFD, 2000). EQR may be calculated using biological, chemical, and hydro-morphological data, reflecting the multifaceted nature of aquatic ecosystems (Birk et al., 2012).

This study is focused on the Infaunal Quality Index (Phillips et al., 2014) an EQR used to assess the sediment benthic invertebrate BQE in Irish coastal and transitional WFD waterbodies. IQI was developed to assess ES in both United Kingdom and Irish waterbodies, using UK monitoring data to calibrate the reference conditions. Benthic EQRs are required to assess the proportion of disturbance-sensitive taxa, and the diversity and abundance of invertebrate taxa when comparing sampled data to reference conditions (Muxika et al., 2007; WFD, 2000).

IQI underwent several iterations in development as detailed in Philips et al (2014). The version of IQI that has been used for WFD monitoring in Ireland is the fourth iteration of the IQI equation (IQI_{IV}). The IQI equation includes three metrics: the AZTI Marine Biotic Index (AMBI), which categorises species based on their tolerance to disturbance (Borja et al., 2000), Simpson's Evenness Index ($1 - \lambda'$), which reflects species evenness (Simpson, 1949) and the total number of taxa (S). AMBI addresses the WFD criteria for the abundance of pollution-tolerant and

disturbance-sensitive taxa, and also the criteria for abundance. Simpson's Evenness Index complies with the WFD criteria for diversity and abundance. Number of taxa addresses the WFD criteria for diversity (WFD-UKTAG, 2014).

The AZTI Marine Biotic Index (AMBI, Borja et al., 2000) assesses the stress tolerance of the benthic faunal communities sampled by assigning each taxon identified to an Ecological Group (EG) using the classification approach described by (Grall & Glémarec, 1997) for organic enrichment gradients on the seafloor. This classifies benthic taxa into one of five EG based on their sensitivity to disturbance.

- EG I taxa are very sensitive to organic enrichment and present under unpolluted conditions. They include the specialist carnivores and some deposit-feeding tubicolous polychaetes.
- EG II taxa are less sensitive to organic enrichment. They are typically present in low densities with non-significant variations between undisturbed and moderately disturbed conditions. They include suspension feeders, less selective carnivores and scavengers.
- EG III taxa are tolerant of moderate organic matter enrichment. These taxa may occur under normal conditions, but their populations are stimulated by organic enrichment. They include surface deposit-feeding species, as tubicolous polychaetes of the family Spionidae.
- EG IV taxa are second-order opportunistic species that are prevalent under slight to pronounced organic enrichment. They are mainly small-sized polychaetes, including subsurface deposit-feeders, such as the Cirratulidae.
- EG V taxa are first-order opportunistic species, prevalent under pronounced organic enrichment. They are deposit-feeders, which proliferate in reduced sediments. They include tube-dwelling polychaetes of the family Capitellidae.

AMBI uses the distribution of these ecological groups to calculate a Biotic Index (BI) with eight levels, from 0 to 7 based on the approach of Hily, (1984) AMBI calculates a continuous index, the Biotic Coefficient (BC), using the percentage distribution of EG within a sample using Equation 1.

$$Biotic\ Coefficient = \frac{\{(0 \times \%EGI) + (1.5 \times \%EGII) + (3 \times \%EGIII) + (4.5 \times \%EGIV) + (6 \times \%EGV)\}}{100} \quad (1)$$

BC, as calculated, ranges from 0 when all taxa are in EG I, pristine conditions, to 6 when all taxa are in EG V, heavily polluted conditions. When no taxa are recorded (Azoic conditions), BC is assigned a value of 7. This provides a quantitative method of assigning seafloor samples to the BI levels of (Hily, 1984)

Simpson's Evenness (1-λ') is calculated by the method of (Simpson, 1949) using Equation 2:

$$1 - \lambda' = \frac{\sum_i \ N_i \times (N_i - 1)}{N \times (N - 1)} \qquad (2)$$

where: N_i is the number of individuals of species *i* in the sample

N is the total number of individual invertebrates identified in the sample

The IQI_{IV} EQR, a continuous variable between 0 and 1, is calculated using Equation 3 below, following the method outlined in Philips et al (2014).

$$IQI_{IV} = \frac{\left(\left(0.38 \times \left(\frac{(1 - AMBI/7)}{(1 - AMBI/7)_{ref}}\right)\right) + \left(0.08 \times \left(\frac{(1 - \lambda')}{(1 - \lambda')_{ref}}\right)\right) + \left(0.54 \times \left(\frac{S}{S_{ref}}\right)^{0.1}\right) - 0.4\right)}{0.6} \qquad (3)$$

Where: AMBI is the AZTI Marine Biotic Index (Borja et al., 2000)

1 – λ' is Simpson's evenness index (Simpson, 1949)

$S^{0.1}$ is the number of taxa (S) raised to the power 0.1

ref parameters are the reference values for the sample, which are assigned based on the salinity and sediment characteristics of the sample station

IQI_{IV} is calculated by dividing the observed score for each component index by the reference score for the station under consideration. Each quotient is multiplied by the corresponding weighting value. The sum of the products is IQI_{IV} (hereafter written as IQI). AMBI is input as (1-AMBI/7) so that the AMBI component of IQI ranges from 0 (severely disturbed) to 1 (pristine conditions). S was transformed to the power 0.1 to facilitate regression analyses to identify the weighting applied to the component indices (Philips et al., 2014).

Equation 3 shows the weighting assigned to the component indices (1-AMBI/7, 1 – λ', S). The weights were derived using fauna and pollutant data from strong pollution gradients associated

with a sewage sludge disposal site and a mine waste disposal site (Phillips et al. 2014). Multiple pollutants were recorded as quantitative data at each site. Pollutant data were combined into a principal component function that explained the highest fraction of variance in each dataset (PC1). At each site, regression analysis was carried out to maximise the variance in the PC1 explained by the component indices. The regression coefficients from both sites were weighted by the total variance explained by each regression equation and combined to give the weights assigned to each metric. The minimum possible value that could be obtained from the resulting equation was 0.4. Equation 3 shows that when calculating IQI, 0.4 is subtracted from each output, and the answer is divided by 0.6 to make the index compliant with the requirement to range from 0 to 1.

IQI is calculated using an MS Excel software tool that includes a series of Visual Basic macros for data processing (Phillips et al., 2014). The software tool has a lookup table of taxa that may be included in the IQI calculation. The V1 taxa lookup table was updated on 09/08/2013, while the V2 tool taxa lookup table was updated on 27/11/2020. Taxa names for each sample are made compliant with the World Registry of Marine Species (WORMS) database species list (WORMS, 2025). Taxa names and abundances are then pasted to a worksheet in the IQI workbook. Species abundance data are provided for most taxa, but some taxa are reported at a higher taxonomic level on a case-by-case basis. The taxa names are compared to the taxa lookup table and an AMBI (Borja et al., 2000) group is assigned to each taxon. Where the taxon name is not recognised by the tool, it is marked as unrecognised on the input worksheet. The user is given an opportunity to change the taxon name. This is a much more frequent occurrence when using the V1 tool than when using V2. It may involve changing the taxon name to the equivalent (usually older) name in the taxa lookup table, or by inputting the taxon name at a higher taxonomic level, such as genus, family, etc. This process often involves creating a list of alternative names for the taxa in question from the literature or the WORMS database and checking the list against the lookup table in the V1 tool. It may be a very time-consuming process, particularly for new or infrequent users of the V1 tool. In practice, many potential users of the IQI tool do not have a detailed knowledge of the taxonomy of all of the major benthic invertebrate groups, which can lead to uncertainty in assigning equivalent taxon names and AMBI groups to some taxa. Taxa which are not assigned to an equivalent in the taxa lookup table are not included in the IQI calculation.

Both IQI V1 and IQI V2 assign a truncation code to each taxon in the taxa table to determine how the abundance data for that taxon will be processed by the software tool (Phillips et al., 2014). This is to allow the software tool to incorporate taxa that are colonial or should not be included in the IQI assessment into the data workflow. These truncation codes are applied at the family level by

the IQI tools. The codes are listed in Table 2.1. IQI V2 applies different truncation codes to some taxa than IQI V1. This has the potential to change the EQR value and ES class assigned to some stations sampled.

TABLE 2. 1. FAMILY-LEVEL TRUNCATION CODES APPLIED TO TAXA IN THE INFAUNAL QUALITY INDEX V1 AND V2 SOFTWARE TOOLS

Truncation code	Data process
0	exclude from analysis
1	enumerate as one individual per sample
2	retain unaltered

When the taxa data has been made as compliant as possible with the taxa lookup table, the data is truncated into AMBI EG equivalents using a series of Visual Basic macros and an AMBI score is calculated for each sample. The number of taxa (S) included in the calculation of IQI is the number of valid IQI taxa, i.e. those taxa that have been assigned a truncation code of 1 or 2 in Table 2.1. Simpson's index ($1 - \lambda'$) is also calculated using the number of taxa and individuals from valid IQI taxa only.

For all three metrics, the sample score is divided by a reference score for the habitat that the sample was taken from (Phillips et al, 2014). The habitat is a combination of sediment type and salinity class. In coastal waters, benthic invertebrate community structure is strongly influenced by sediment type and water depth (Thorson, 1957). Assessing ecological quality in estuarine environments is complicated by the Estuarine Quality Paradox (Elliott & Quintino, 2007). Estuarine systems are dynamic and shaped by fluctuating salinity, turbidity, sediment composition, and tidal influences, all of which contribute to significant variability in benthic community structure (Dauvin, 2007). Natural estuarine benthic community distributions may closely resemble patterns associated with human induced disturbance, such as organic enrichment or pollution, making it difficult to evaluate ES accurately (Kennedy et al., 2011). The apparent estuarine stress generally leads to reference conditions being assigned to the component indices of IQI being reduced when compared to fully saline coastal waters, for a given sediment type. (WFD-UKTAG, 2014). Salinity data maybe be entered in one of three ways for each station:

1. As a binary classification, i.e. coastal or transitional (estuarine) waters
2. As classes of the Venice salinity classification (Anonymous, 1958). This approach recognises four classes, three of which occur within estuarine waters. In order of decreasing

mean salinity, the classes are Coastal, Polyhaline, Mesohaline and Oligohaline.. In order of decreasing mean salinity, the classes are Coastal, Polyhaline, Mesohaline and Oligohaline.

3. As a median salinity derived from field survey

Sediment data may be entered into the tool as either a percentage distribution from a series of whole phi sieve equivalents ranging from <63µm to >8mm, or as a qualitative sediment description in the form of a textural group *sensu* Folk (1954).

Reference conditions were derived from modelling the component indices of IQI using sediment grain size and salinity data collected in the field (Phillips et al, 2014). This approach allows appropriate reference conditions reflecting High status for each component metric to be applied to each sample assessed by the IQI tools.

The ecological heterogeneity across Europe has necessitated the development of different assessment methods (EQR) tailored to local conditions. There is a requirement to ensure that ecological status (ES) classifications are directly comparable between member states (WFD, 2000). Each member state is free to choose the appropriate EQR for a BQE in its territory. To address this, the WFD introduced an intercalibration process that aligns ES classes reported by different countries when classifying the same sample datasets using different EQR. This ensures that ES assessments reflect comparable levels of biological quality and anthropogenic impact, regardless of regional methodologies (Kelly et al., 2014; Poikane et al., 2014). This process is essential for maintaining consistency across regions while allowing for environmental variability. It aims to prevent discrepancies in classification that could otherwise lead to inconsistent water body management, uneven conservation priorities, or misallocation of resources (Borja et al., 2007). The intercalibration process involves establishing Geographical Intercalibration Groups (GIG), which cluster member states based on shared ecological and geographical conditions, such as climatic zones, hydrodynamic regimes, and dominant waterbody types. GIG provide a collaborative platform for harmonising assessment methods (Poikane et al., 2014). The intercalibration of EQR is central to achieving the WFD's objective of consistent and comparable ecological assessments across Europe. Ireland and the UK are members of the North East Atlantic Geographical Intercalibration Group (NEAGIG).

In the case of benthic EQR, intercalibration has been carried out within the NEAGIG using weighted Kappa analysis of agreement (Cohen, 1960) of the ES classes assigned to sample stations by different EQRs. Agreement between the EQR was also classified qualitatively using the

approach of Monserud & Leemans, (1992). Borja et al., (2007) intercalibrated M-AMBI with the Danish EQR (DKI), the Norwegian EQR (F3) and the second iteration of IQI (IQI_{II}), an earlier version of IQI than the one currently in use. They used data from strong environmental gradients from each member state to calculate all four of the EQR. The UK and Ireland were considered one dataset as they used the same EQR. By adjusting the class boundaries between the ES classes on the various EQR, they achieved agreement between the EQR ranging from 'very good' to 'almost perfect', as classified by the approach of Monserud & Leemans, (1992). The weighted Kappa value boundaries between the agreement classes used by Borja et al (2007) are listed in Table 2.2. In Ireland, Kennedy et al., (2011) used a similar approach to assess agreement between M-AMBI and IQI_{II} using a 26-year time series of benthic monitoring data from Kinsale Harbour in Ireland. They found 'almost perfect' agreement between the two EQR in coastal and polyhaline waters. Both Borja et al., (2007)and Kennedy et al. (2011) used a version of IQI that predates the release of the IQI V1 tool. It did not include salinity adjustment to reference conditions.

TABLE 2. 2. KAPPA VALUE BOUNDARIES BETWEEN AGREEMENT CLASSES USED IN THE INTERCALIBRATION OF BENTHIC ECOLOGICAL QUALITY RATIOS (EQR) BY BORJA ET AL., (2007).

Kappa Value	Agreement Class
1	Perfect
>0.85	Almost Perfect
>0.70	Very Good
>0.55	Good
>0.40	Moderate
>0.20	Low
>0.05	Very Low
≤0.05	Null

Following further development of the IQI and the EQR of other member states, the intercalibration exercise was repeated, using the same test data (Phillips et al., 2014). IQI was updated from IQI_{II} to IQI_{IV}, and the reference conditions were updated. The class boundaries between ES classes were updated for IQI. Similar development occurred with the EQR of other member states of the NEAGIG. The repeated intercalibration exercise included the addition of P-BAT, a Portuguese EQR not included in the assessment (Borja et al., 2007). Intercalibration between the five methods produced 'almost perfect' agreement in all cases. The final class boundaries for IQI, and the other

EQR, were accepted by the European Commission (European-Commission, 2008; Phillips et al., 2014). The ES class boundaries used in IQI are listed in Table 2.3

TABLE 2. 3. ECOLOGICAL STATUS CLASS BOUNDARIES FOR THE INFAUNAL QUALITY INDEX (IQI_{IV})

Ecological Status	EQR
High/Good	0.75
Good/Moderate	0.64
Moderate/Poor	0.44
Poor/Bad	0.24

For any given station sampled within a waterbody, the IQI EQR is calculated using Equation 3 and the appropriate reference conditions for that station. The Ecological Status (ES) is assigned to the sample by comparison to the class boundaries in Table 2.3. At the waterbody scale, ES assigned to the waterbody is determined by calculating the arithmetic mean of all samples from the waterbody, and comparing the value to the class boundaries (WFD-UKTAG, 2014).

The WFD (Annex V, Section 1.3) requires waterbody ES classifications to be accompanied by an estimate of uncertainty that the ES class designated to the waterbody using limited sample data is the same as the "true" ES class. Here, the "true" ES is the ES that would be assigned if the EQR value for the total population of possible samples from the waterbody were known without error. This uncertainty is referred to as the Risk of Misclassification (ROM). The associated statistical confidence that the ES assigned to the waterbody is in the correct class is known as the Confidence of Class (CofC). Data sampled from the waterbodies may contain variability from sources such as spatial change (in factors such as exposure or sediment type), temporal change (factors such as seasonality), random sampling error and measurement error (Phillips et al., 2014).

The IQI workbooks (V1 and V2) implement the approach of (Ellis & Adriaenssens, 2006) in assessing uncertainty in ES classes assigned to macroinvertebrate data from WFD waterbodies. EQR values from individual samples are treated as random individuals from an approximately normal distribution of EQR values around the mean EQR for the waterbody. For each waterbody, this normal distribution is compared to the ES class boundaries using the Cumulative Distribution Function (CDF). The Confidence of Class (CofC) is calculated using Equations 4 to 6. This

comparison between the ES class boundaries and the waterbody mean and standard error for the IQI EQR value is repeated for each of the five ES classes. Data for the EQR values and class boundaries are log transformed before analysis to meet the assumptions of normality (Philips et al 2014).

$$\text{Confidence of Class (\%)} = 100 \times \left(1 - (\text{P:} > \text{Assigned Status} + \text{P:} < \text{Assigned Status})\right) \quad (4)$$

$$\text{P:} > \text{Assigned Status} = \text{NORMDIST}\left(\frac{\text{EQR} - \text{Upper boundary}}{\text{SE}}\right) \quad (5)$$

$$\text{P:} < \text{Assigned Status} = \text{NORMDIST}\left(\frac{\text{Lower boundary} - \text{EQR}}{\text{SE}}\right) \quad (6)$$

Where:

P:> Assigned Status is the probability that the true population mean is above the assigned status

P:< Assigned Status is the probability that the true population mean is below the assigned status

EQR is the mean value of the IQI EQR for the waterbody being assessed

SE is the standard error of the IQI EQR values from the waterbody

Upper and Lower boundaries are the Ecological Status class boundaries for the ES class being assessed.

NORMDIST is a Microsoft Excel function that calculates the probability that a value will be less than or equal to a specified value using the Cumulative Distribution Function (CDF) for the waterbody EQR population being assessed.

The Risk of Misclassification (ROM) is simply the CofC% subtracted from 100% (Equation 7).

$$\text{Risk of Misclassification (\%)} = 100 - \text{Confidence of Class (\%)} \quad (7)$$

A similar approach to the CofC assessment is repeated to assess the probability that the true population EQR lies above the Good/Moderate Boundary. This assessment addresses the pass / fail aspect of WFD monitoring within a waterbody. The WFD requires that member states investigate waterbodies when ES is classified below the Good/Moderate boundary, and implement

a Program of Measures (POM) to restore the waterbody to at least Good status where necessary (WFD-UKTAG, 2014). The probabilities that the true population mean EQR lies above and below the Good/Moderate boundary are calculated using Equations 8 and 9, respectively.

$$\mathrm{P{:}>G/M} = \mathrm{NORMDIST}\left(\frac{\mathrm{EQR-G/M\ boundary}}{\mathrm{SE}}\right) \qquad (8)$$

$$\mathrm{P{:}<G/M} = \mathrm{NORMDIST}\left(\frac{\mathrm{G/M\ boundary-EQR}}{SE}\right) \qquad (9)$$

The statistical confidence in the classification of ES is strongly influenced by the standard error (SE) used for the sample population. The IQI developers (Philips et al 2014) considered calculating the standard deviation (and SE) directly from the sampled population to not be the optimal approach. They considered coastal and estuarine seafloor sediment sample populations to be subject to pronounced spatial and random variability, particularly when sample sizes are small. They used unpublished data collected to monitor habitat quality in the Lower Tees estuary, in relation to changing industrial practices and the construction of a tidal barrier on the estuary, to assess the relationship between mean IQI EQR and its standard deviation under various ES classes. The stations sampled included both coastal and transitional (estuarine) waters. The data were collected over a seventeen-year period (1980 to 1997). Each station sampled within a year included three to five replicate samples. Over the course of the study, each station was sampled multiple times. The data were analysed using the Combines Appropriate Variance Estimates (CAVE) software tool developed for determining components of variation in biological community data collected for WFD purposes, described in (Ellis & Adriaenssens, 2006). This allowed the relationship between standard deviation and number of samples collected in a waterbody to be modelled under certain environmental conditions. The modelled standard error is calculated from the modelled standard deviation as per Equation 10.

$$\mathrm{SE} = \sqrt{\sum_{\mathrm{i=1}}^{\mathrm{n}}\left(\frac{\mathrm{SD_i^2}}{\mathrm{n_i}}\right)} \qquad (10)$$

Where:

SE is Standard Error

SD is the Standard Deviation

n is the number of samples

The default approach in the IQI V1 and V2 Excel tools is to use the modelled SE rather than the SE calculated directly from the sampled EQR data, where possible. For coastal sediments, this includes samples for macrofauna sieved on a 1mm mesh. The Tees estuary monitoring program collected macrofauna from transitional waters on a 0.5mm mesh. Consequently, the IQI tools only provide modelled SE for transitional waters samples that have been sieved on a 0.5mm mesh. It is possible to manually calculate SE for a waterbody within the IQI tools if the sampling procedure does not comply to the expectations of the tool. The Irish WFD monitoring program employs a 1mm sieve to sample macrofauna from estuarine and transitional waters. In the case of Irish WFD data, the modelled SE are used for coastal waters, while SE calculated directly from the EQR within a waterbody are used in transitional waters.

Monitoring of the subtidal sediments invertebrate BQE for the purposes of the WFD began during the 2009 to 2015 River Basin Management Plan (WFD Reporting Cycle) in both the UK and Ireland. In Ireland, a subset of a total of 69 waterbodies has been sampled annually from 2010 to 2025. This study focuses on the data collected from 2010 to 2023. During that time period, IQI ES was assessed using IQI V1. Following the release of IQI V2 in 2024, ES has been assessed in Ireland using the IQI V2 software. Both versions of the IQI tool are designed to facilitate Ecological Status assessment in coastal and transitional waters by processing complex datasets that incorporate multivariate faunal community data and supporting environmental data in relation station specific reference conditions. The tools produce both ES assessments and estimates of the probability that the assigned ES class is correct, notably with reference to the Good/Moderate boundary. The IQI V2 tool updates the taxonomic library and Ecological Groups assigned to taxa to be compliant with the latest available data. In addition, the V2 tool incorporates changes to how species data are processed and reference conditions are set based on the experience of processing IQI data from UK WFD waterbodies. However, transitioning from V1 to V2 introduces the need for intercalibration to ensure consistency between sampling years (Borja et al., 2007) and continuity in long-term ecological monitoring programs.

The objectives of this intercalibration process are to:

- Standardise all fauna data to a single taxonomic reference point, ensuring consistency across datasets (WORMS, 2025)
- Update GIS data to support spatially explicit management of WFD waterbodies in Ireland (WFD, 2000)

- Intercalibrate IQI V1 and V2 using all data explicitly collected for WFD monitoring between 2010 and 2023 to determine if transitioning to IQI V2 will introduce a change in the ES assigned to waterbodies over time. This will facilitate consistent assessment of Ecological Status and ensure that historical assessments remain comparable with current and future monitoring efforts, providing continuity and reliability in evaluating benthic ecosystem health
- Identify waterbodies that consistently fail to achieve “Good” Ecological Status, providing a foundation for targeted management and remediation efforts

2.3. Methods

2.3.1. Sample Collection

This study focused on 69 Water Framework Directive (WFD) waterbodies in Ireland, comprising of 39 coastal waterbodies and 30 polyhaline transitional waterbodies (Figure 2.1). These data have been used to determine the benthic ecological status of Irish WFD waterbodies using IQI V1. They span a variety of environmental conditions, including differences in salinity, sediment type, exposure and water depth. Sampling took place annually between 2010 and 2023. The main sampling effort in each year was a dedicated WFD monitoring survey that took place onboard a Marine Institute survey vessel in January of each year, known as the Winter Environmental Survey (WES). The survey route was from Dublin to Galway each year. The survey route alternated between years. In one year, the sampling campaign progressed northward around the Irish coastline, while in the following year, the survey covered waterbodies south of a line drawn between Dublin and Galway. This rotation ensured comprehensive coverage of Irish coastal and transitional waterbodies over multiple years. Waterbodies not sampled during the main survey were sampled by environmental consultancies using the same sampling protocols as used by the Marine Institute (MI) surveyors from small inshore vessels. In most cases, these waterbodies included enclosed or shallow areas that were not accessible by the MI vessel. Each year, relevant waterbodies were identified after the WES, ensuring that any gaps in the sampling program were addressed promptly. This approach maintained the integrity of the annual monitoring effort, ensuring that data from all designated waterbodies were collected consistently and reliably.

Grab sampling was conducted using a Day Grab with a 0.1 m^2 sampling area, with weights adjusted based on sediment type to ensure adequate sample retrieval. The winching process was carefully controlled, with slow deployment and recovery rates (<1 m/sec, <0.5 m/sec near the seabed) to minimise disturbance and preserve sediment integrity. Samples were rejected if sediment thickness was less than 7 cm in muddy soft sediments or less than 5 cm in compacted sand (Cusack et al., 2008). Field observations included sediment colour, smell (e.g., hydrogen sulfide), and surface features such as fauna or algae. Photographs of both the grab and sieved fractions were taken for sample validation purposes. Two grab samples were collected per station: one for faunal analysis and another for sediment physico-chemical properties, including Particle Size Analysis (PSA) and organic carbon content. All sediment physico-chemical samples were frozen immediately at <-18°C and stored in sealed labelled containers (Cusack et al., 2008). Faunal samples were sieved using a 1 mm mesh, with careful handling to avoid damage to fragile species. Stones and large shells were removed to prevent mechanical damage to fauna. The sample residue and fauna

retained on the sieve were fixed in buffered 4% formaldehyde for at least 24 hours. Samples were transferred from formaldehyde to 70% Industrial Methylated Spirit (IMS) for long-term storage. Sediments were entirely covered with the fixative to ensure long-term preservation. Careful labelling and detailed log sheets were maintained to track all samples (Cusack et al., 2008).

All samples collected in WES were analysed by environmental consultancies using standard sample processing protocols, as specified by the Marine Institute. Participating laboratories demonstrated compliance with quality control measures and/or were active participants in the NE Atlantic Marine Biological Analytical Quality Control (NMBAQC) Scheme. This scheme covers both faunal sample processing, faunal identification and sediment grain size analysis.

2.3.3. Sediment Processing

Sediment grain size analysis was carried out using the wet and dry sieving technique described in (Bale & Kenny, 2005) or a combination of wet and dry sieving and laser particle size analysis (Forde et al., 2012). Sediment organic content was estimated by loss on ignition using a modification of the method of (Dean, 1974).

2.3.4. Faunal Processing

Faunal samples were sieved on a 1mm mesh. Fauna picked from the mesh were identified using the best available taxonomic keys at the time of processing, to the lowest practical taxonomic level in accordance with the Marine Institute specifications and NMBAQC scheme guidelines. The published taxonomic keys recommended by the NMBAQC scheme are listed in Worsfold & Hall, (2025) available from the NMBAQC website (NMBAQC, 2025). Unpublished keys developed by the NMBAQC scheme are available from the same source. All taxa were enumerated. Taxa identified from the samples processed in each year were made compliant with the World Registry of Marine Species (WORMS) (WORMS, 2025) taxon list at the time of reporting.

2.3.5. Data Collation

Sediment data was returned by contractors in a standard format that was compliant with entry into both the IQI V1 and IQI V2 tools. This study collated and standardised the data collected by the Marine Institute and by contractors. Macrofaunal data from all survey years were collated into a single dataset, and the taxonomy was standardised into a common format using the Match Taxa tool on the WORMS website. This tool assigns the latest accepted taxonomic names to all taxa in a dataset. Duplicate occurrences of taxon names within a survey year were aggregated to a single taxon in the taxon by station matrix. This WORMS-compliant dataset was entered into the IQI V2

tool without further adjustment, as the taxon lookup table in that tool was largely WORMS-compliant.

For the IQI V1 tool, a conversion table was made to facilitate the use of WORMS-compliant data from 2024 with an Excel tool that contained a taxon lookup table that was updated in 2013. The taxon list from the taxon lookup table in the IQI V1 tool was updated to the WORMS-compliant format on 28/07/24. The survey data were converted to the taxonomy of the IQI V1 taxon lookup table, including, where necessary, the assignment of higher taxonomic levels. Data was entered into the IQI V1 tool using the original taxonomy of the tool as assigned in 2013.

2.3.6. IQI Calculation

The two versions of the macrofaunal data were input to their corresponding versions of the IQI tool workbook. In all cases, survey data from a single year were input into each workbook. Both the IQI V1 and V2 tools can process a maximum of 250 samples in a single workbook. For some years, two or three workbooks were necessary to process all of the survey data collected. Data was input in accordance with the instructions of Philips et al. (2014) and the tool instructions listed on the second worksheet of the IQI tools Excel workbook. The faunal data were validated, and the component indices of the IQI (1-AMBI/7, 1 – λ', S) were calculated as described above using a series of Visual Basic macros. Taxa that were not assigned an AMBI group were not included in the calculation of the component indices for either version of the tool. IQI V1 and IQI V2 differed in which taxa were not assigned. The percentage of taxa not included in the calculation of IQI was recorded for each sample.

Reference conditions for each component index were set by entering sediment data and salinity class for each station. Sediment data was entered as whole phi sieve equivalents where possible, but in a small number of stations, sediment was entered into the IQI tools as a textural group (Folk, 1954). Salinity classification was derived from a GIS shapefile of the WFD waterbodies provided by the Environmental Protection Agency (EPA). Each station from a waterbody surveyed was assigned the same salinity class in this manner. Because the salinity classification in the EPA shapefile classified the estuarine waterbodies as being "Polyhaline or Mesohaline", all estuarine waterbodies were classified as polyhaline for the purposes of this assessment. The same sediment and salinity data were entered for each station into both the IQI V1 and IQI V2 tools.

Following the assignment of reference conditions, IQI was calculated for each station using Equation 3, and the ES class for that station was assigned. This process was the same for IQI V1 and IQI V2. Each station was assigned to an Assessment Group by entering a waterbody name

into the relevant field in the IQI tools. The mean Assessment Group IQI EQR value and ES class was assigned by the IQI tools. In the case of coastal waters, the modelled Standard Error (SE) of the IQI EQR was calculated using Equation 10. It was used to estimate the probability that the true mean EQR occurred in each of the five ES classes (Confidence of Class; CofC) using Equation 4. The probability that the true mean IQI EQR value was greater than the Good/Moderate Boundary for each Assessment Group was calculated using Equation 8. The probability that the true mean IQI EQR value was less than the Good/Moderate Boundary for each Assessment Group was calculated using Equation 9. For transitional waterbodies, the IQI tools cannot model the expected SE for samples processed on a 1mm sieve. The SE was manually calculated for each Assessment Group and input into a column in the Excel workbook that allows manually calculated SE to override modelled SE in calculating the probabilities (Phillips et al., 2014)

2.3.7. Intercalibration of IQI V1 and IQI V2

The results of the IQI V1 and IQI V2 tools were intercalibrated using the method of Borja et al., (2007). This analysis was applied to the datasets in several ways:

In the first approach to intercalibration, the IQI tools were intercalibrated at the level of per-station ES. Weighted Kappa analysis (Cohen, 1960; Fleiss & Cohen, 1973) was carried out between IQI V1 and IQI V2 for all stations sampled in all years. The percentage mismatch between the two versions of the IQI was calculated, and agreement between the IQI tools was classified using the scheme of Monserud & Leemans, (1992). This analysis was repeated for the coastal stations only and for the polyhaline stations only to assess the agreement between the IQI V1 and IQI V2 tools in the different salinity classes.

In the second approach, ES was intercalibrated between the IQI tools at the Assessment Group level. Weighted Kappa analysis was calculated between the mean EQR values calculated for each Assessment Group. The analysis was also carried out using only the coastal Assessment Groups, and only the polyhaline Assessment Groups. The percentage mismatch and agreement between the tools were determined in the same manner as for the stations data.

The third approach to intercalibration was applied at the Assessment Group level, but included Kappa analysis on the probability that the true mean IQI value was greater than the Good/Moderate boundary. The probabilities were assigned to five classes, analogous to the ES classes, using the boundaries shown in Table 2.4. As for the previous analyses, this intercalibration was carried out

for all Assessment Groups, and also for the coastal and polyhaline Assessment Groups separately. The percentage mismatch and agreement between the tools were assessed.

TABLE 2. 4. CLASS BOUNDARIES FOR THE PROBABILITIES INFAUNAL QUALITY INDEX ECOLOGICAL STATUS IS ABOVE THE GOOD/MODERATE BOUNDARY FOR ASSESSMENT GROUPS

Probability Class	Probability
High/Good	0.80
Good/Moderate	0.60
Moderate/Poor	0.40
Poor/Bad	0.20

The fourth approach to intercalibration was carried out using the ES assigned to stations, but in this case, any station where IQI V2 had classified more than 10% of the fauna as unassigned was removed from the data before the weighted Kappa analysis was performed. This involved the removal of 2,033 of the 4374 stations sampled in all of the Assessment Groups. This Kappa analysis was carried out using 2,341 stations. As with the previous analyses, this analysis was carried out for all stations, and also for the coastal stations and polyhaline stations separately.

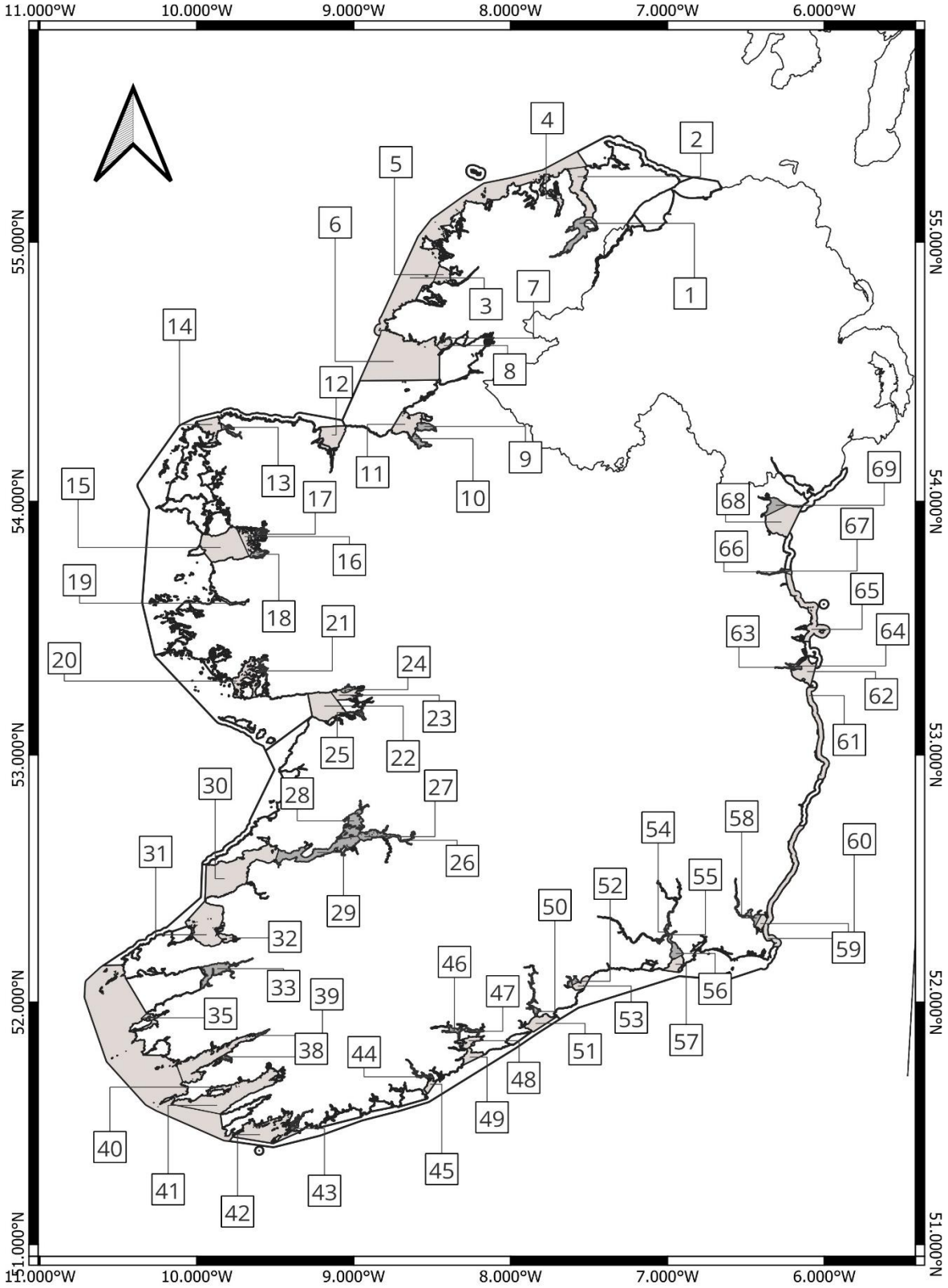

FIGURE 2. 1. MAP SHOWING THE LOCATION OF WATERBODIES SAMPLED FROM 2010-2023, NUMBERED 1 TO 69. THE CORRESPONDING WATER BODY NAMES ARE AS FOLLOWS IN THE TABLE BELOW.

No.	Water Body Name	No.	Water Body Name
1	Swilly Estuary	36	Portmagee Channel
2	Lough Swilly	37	Outer Kenmare River
3	Northwestern Atlantic Seaboard (HAs 37;38)	38	Kilmakilloge Harbour
4	Mulroy Bay Broadwater	39	Inner Kenmare River
5	Gweebarra Bay	40	Berehaven
6	Donegal Bay Northern	41	Outer Bantry Bay
7	Killybegs Harbour	42	Roaring Water Bay
8	McSwines Bay	43	Ilen Estuary
9	Garavoge Estuary	44	Lower Bandon Estuary
10	Ballysadare Estuary	45	Kinsale Harbour
11	Sligo Bay	46	Lough Mahon
12	Killala Bay	47	North Channel Great Island
13	Sruwaddacon Bay	48	Cork Harbour
14	Broadhaven	49	Outer Cork Harbour
15	Clew Bay	50	Lower Blackwater M Estuary / Youghal Harbour
16	Inner Clew Bay	51	Youghal Bay
17	Newport Bay	52	Colligan Estuary
18	Westport Bay	53	Dungarvan Harbour
19	Killary Harbour	54	New Ross Port
20	Kilkieran Bay	55	Lower Suir Estuary (Little Island - Cheekpoint)
21	Camus Bay	56	Barrow Suir Nore Estuary

22	Outer Galway Bay	57	Waterford Harbour
23	Inner Galway Bay North	58	Lower Slaney Estuary
24	Corrib Estuary	59	Wexford Harbour
25	Kinvarra Bay	60	Southwestern Irish Sea (HAs 11;12)
26	Limerick Dock	61	Southwestern Irish Sea - Killiney Bay (HA10)
27	Upper Shannon Estuary	62	Dublin Bay
28	Fergus Estuary	63	Liffey Estuary Lower
29	Lower Shannon Estuary	64	Tolka Estuary
30	Mouth of the Shannon (HAs 23;27)	65	Northwestern Irish Sea (HA 08)
31	Outer Tralee Bay	66	Boyne Estuary Plume Zone
32	Inner Tralee Bay	67	Boyne Estuary
33	Castlemaine Harbour	68	Outer Dundalk Bay
34	Southwestern Atlantic Seaboard (HAs 21;22)	69	Inner Dundalk Bay
35	Valencia Harbour		

2.4. Results

Summary results for the Ecological Status (ES) class assigned to each sampling station from all Assessment Groups in the 2010 to 2023 surveys are shown in Table 2.5. When the total dataset is considered (Table 2.5.a) without incorporating station identity, the IQI V1 and IQI V2 tools produced very similar results for each ES class in terms of how many stations were assigned to each class. In the case of each ES class, the difference in the number of stations assigned was less than 1% of the total number of stations in the dataset. The total difference in ES class assigned at all five classes was 2.01%. The %Difference between the tools was also low (0.39%) when the stations were considered at the Good/Moderate boundary. The agreement between the tools was similar (all differences <1%) when only the coastal stations were considered (Table 2.5.b). The percentage differences in ES class assigned were lower than for the total dataset for each ES class, including the agreement about how many stations were above and below the Good/Moderate boundary (0.21%). The total difference in ES class assigned at all five classes was 0.48% for the coastal stations. For the polyhaline stations (Table 2.5.c), IQI V1 and IQI V2 disagreed at 2.18% of stations at the High ES class, and at 1.43% of cases at the Good ES class. For the other ES classes, the differences between the tools were <1%. For polyhaline stations, the total difference in ES class assigned at all five classes was 5.31%. IQI V1 assigned 32 more stations as High than IQI V2, while IQI V2 assigned 21 more stations as Good than IQI V1. When considered at the Good/Moderate boundary, the difference between the tools for polyhaline stations was <1%. All percentage differences for the polyhaline stations were larger than the corresponding percentage differences for the coastal stations, and for all stations combined.

At the Assessment Group (AG) level, summary results are shown in Table 2.6. Table 2.6.a shows the results for mean ES class at all AG surveyed. The difference between the tools was <1% for the High, Poor and Bad mean ES classes. For both the Good and Moderate ES classes, the difference between the tools in the number of AG assigned to each class was 3.95%. The total difference in ES class assigned at all five classes was 9.04%. At the Good/Moderate boundary, the tools differed in assigning the “pass/fail” criteria in 4.52% of cases. IQI V1 assigned 16 AG more than IQI V2 as above the Good/Moderate boundary. For the coastal AG (Table 2.6.b), the agreement between the tools was higher. For the Bad, Poor and High ES classes, the percentage difference was <1%. For both the Good and Moderate ES classes, the percentage difference was 1.46%. The total difference in ES class assigned at all five classes was 3.9%. At the Good/Moderate boundary, the percentage difference was 1.95%. For the polyhaline AG (Table 2.6.c), the percentage differences were higher for each ES class (with the exception of Bad) than for all AG and coastal AG. There were no differences between the tools in the assignment of the

single Bad AG. IQI V1 and IQI V2 had a percentage difference of 0.67% in the cases of both High and Poor ES. For both the Good and Moderate ES classes, the tools differed by 7.38% of the total number of assessment groups. The total difference in ES class assigned at all five classes was 16.11%. At the Good/Moderate boundary, the tools differed by 8.05%. For both coastal and polyhaline AG, IQI V2 classified mean ES as being below the Good/Moderate boundary in more cases than IQI V1.

Table 2.7. shows probabilities that the true mean AG Ecological Status is above the Good/Moderate boundary, classified into five classes that are analogous to the ES classes. For all AG (Table 2.7.a), there was no difference in the number of AG assigned a Bad probability, where the true mean ES was above the Good/Moderate boundary. The difference in AG assigned a High probability was <1%. IQI V2 assigned Good and Moderate probabilities to fewer AG than IQI V1 (3.11% and 1.98% respectively), while assigning a Poor probability to more AG than IQI V1 (5.65%). The total difference in probability class assigned at all five classes was 11.3%. When aggregated to Good or better (>Good) versus less than Good (<Good) probabilities, IQI V2 assigned 3.67% less of the AG as Good or better than IQI V1. For the coastal AG (Table 2.7.b) IQI V2 assigned High probabilities to 3.90% less AG than IQI V1, and Good probabilities to 3.41% more AG than IQI V1. IQI V2 assigned 3.90% less stations with Moderate probability and 1.46% less stations with Bad probability than IQI V1. IQI V2 assigned 5.85% more AG with Moderate probability than IQI V1. The total difference in probability class assigned at all five classes was 18.54%. When considered at the >Good/<Good level, agreement between the tools was good. They disagreed in only one case (0.49%). For the polyhaline AG (Table 2.7.c) IQI V2 assigned High probability in 9.40% less cases than IQI V1, and Good probability in 1.34% more cases. IQI V2 assigned more AG to Moderate, Poor and Bad probabilities than IQI V1 (0.67%, 5.37% and 2.01% respectively). The total difference in probability class assigned at all five classes was 18.79%. IQI V2 assigned >Good probability to 8.05% fewer AG than IQI V1.

Table 2.8. shows the differences in ES class assigned when sample station and AG identity are incorporated. The differences between the outputs of the IQI tools increases as the identity of the samples is given more influence. When sampling station identity is considered, Table 2.8.a, the percentage of all stations at which there is disagreement between the five ES classes assigned by the two versions of the IQI tool is an order of magnitude larger (15.96%) than when the stations are compared as a single group (Table 2.5). Disagreement between the IQI tools is greater for polyhaline stations (19.74%) than for coastal stations (14.05%). Where the tools disagreed, for both coastal (52.45%) and polyhaline (58.28%) stations, IQI V1 classified marginally more of the stations with a higher ES than IQI V2.

Table 2.8.b shows the mismatch at the Good/Moderate boundary between IQI V1 and IQI V2 at the station level. The overall mismatch between all stations was 8.34%. Similar levels of mismatch occurred at coastal (8.47%) and polyhaline (8.1%) stations. Where the tools disagreed, IQI V1 assigned slightly more stations as above the Good/Moderate boundary for both coastal (51.22%) and polyhaline (54.62%) stations.

Table 2.8.c shows the mismatch between the tools at the Good/Moderate boundary for AG. Overall mismatch for all AG was 9.6%. This was higher for polyhaline AG (12.08%) than for coastal AG (7.8%). Where a mismatch occurred, IQI V1 assigned more of the AG as above the Good/Moderate boundary than IQI V2. For all groups, IQI V1 assigned 73.53% of the mismatched AG as above the Good/Moderate boundary. This figure was higher for polyhaline AG (83.3%) than for coastal AG (62.5%).

Mismatch between the tools about the Confidence of Classification (CofC) probability class assigned to each AG is shown in Table 2.8.d. The table shows where the tools disagreed about whether the CofC class assigned differed in classifying the probability as either less than Good, or Good/High. For All AG, the mismatch was 9.89%. The mismatch was higher for polyhaline AG (12.75%) than for coastal AG (7.8%). Where the tools disagreed, IQI V1 assigned a higher CofC than IQI V2 for both coastal (62.5%) and polyhaline (73.68%).

TABLE 2. 5. AGREEMENT IN HOW MANY STATIONS WERE ASSIGNED TO EACH ECOLOGICAL STATUS (ES) CLASS, AND AS ABOVE AND BELOW THE GOOD/MODERATE BOUNDARY, BY IQI V1 AND IQI V2 USING (A) ALL STATIONS SAMPLED, (B) COASTAL STATIONS ONLY AND (C) POLYHALINE STATIONS ONLY.

(a) All Stations

ES Class	IQI V1	IQI V2	% Difference
High	884	848	0.82%
Good	2053	2072	0.43%
Moderate	1299	1291	0.18%
Poor	92	109	0.39%
Bad	45	53	0.18%
Total Difference at ES Class			2.01%
>G/M	2937	2920	0.39%
<G/M	1436	1453	0.39%

(b) Coastal Stations

ES Class	IQI V1	IQI V2	% Difference
High	286	282	0.14%
Good	1658	1656	0.07%
Moderate	919	918	0.03%
Poor	33	38	0.17%
Bad	8	10	0.07%
Total Difference at ES Class			0.48%
>G/M	1944	1938	0.21%
<G/M	960	966	0.21%

(c) Polyhaline Stations

ES Class	IQI V1	IQI V2	% Difference
High	598	566	2.18%
Good	395	416	1.43%
Moderate	380	373	0.48%
Poor	59	71	0.82%
Bad	37	43	0.41%
Total Difference at ES Class			5.31%
>G/M	993	982	0.75%
<G/M	476	487	0.75%

TABLE 2. 6. AGREEMENT IN HOW MANY ASSESSMENT GROUPS (AG) WERE ASSIGNED TO EACH ECOLOGICAL STATUS (ES) CLASS, AND AS ABOVE AND BELOW THE GOOD/MODERATE BOUNDARY, BY IQI V1 AND IQI V2 USING (A) ALL AG SAMPLED, (B) COASTAL AG ONLY AND (C) POLYHALINE AG ONLY.

(a) All Assessment Groups			
ES Class	IQI V1	IQI V2	% Difference
High	58	56	0.56%
Good	191	177	3.95%
Moderate	97	111	3.95%
Poor	7	9	0.56%
Bad	1	1	0.00%
Total Difference at ES Class			9.04%
>G/M	249	233	4.52%
<G/M	105	121	4.52%

(b) Coastal Assessment Groups			
ES Class	IQI V1	IQI V2	% Difference
High	7	6	0.49%
Good	134	131	1.46%
Moderate	63	66	1.46%
Poor	1	2	0.49%
Bad	0	0	0.00%
Total Difference at ES Class			3.90%
>G/M	141	137	1.95%
<G/M	64	68	1.95%

(c) Polyhaline Assessment Groups			
ES Class	IQI V1	IQI V2	% Difference
High	51	50	0.67%
Good	57	46	7.38%
Moderate	34	45	7.38%
Poor	6	7	0.67%
Bad	1	1	0.00%
Total Difference at ES Class			16.11%
>G/M	108	96	8.05%
<G/M	41	53	8.05%

TABLE 2. 7. AGREEMENT IN HOW MANY ASSESSMENT GROUPS (AG) WERE ASSIGNED TO EACH PROBABILITY CLASS WITH REFERENCE TO WHETHER THE TRUE MEAN ECOLOGICAL STATUS WAS ABOVE OR BELOW THE GOOD/MODERATE BOUNDARY, BY IQI V1 AND IQI V2 USING (A) ALL AG SAMPLED, (B) COASTAL AG ONLY AND (C) POLYHALINE AG ONLY.

(a) All Assessment Groups Probability Class	IQI V1	IQI V2	% Difference
High	177	175	0.56%
Good	53	42	3.11%
Moderate	39	32	1.98%
Poor	25	45	5.65%
Bad	60	60	0.00%
Total Difference at Probability Class			11.30%
>Good	230	217	3.67%
<Good	124	137	3.67%

(b) Coastal Assessment Groups Probability Class	IQI V1	IQI V2	% Difference
High	40	32	3.90%
Good	59	66	3.41%
Moderate	58	50	3.90%
Poor	18	30	5.85%
Bad	30	27	1.46%
Total Difference at Probability Class			18.54%
>Good	99	98	0.49%
<Good	106	107	0.49%

(c) Polyhaline Assessment Groups Probability Class	IQI V1	IQI V2	% Difference
High	52	38	9.40%
Good	19	21	1.34%
Moderate	41	42	0.67%
Poor	7	15	5.37%
Bad	30	33	2.01%
Total Difference at Probability Class			18.79%
>Good	71	59	8.05%
<Good	78	90	8.05%

TABLE 2. 8. DIFFERENCE IN ES CLASS ASSIGNED WHEN SAMPLE STATION AND AG IDENTITY ARE INCORPORATED.

(a) Difference of Ecological Status incorporating station identity

	No. Stations	Difference	% Difference	V1>V2	V1>V2%	V2>V1	V2>V1%
All Stations	4373	698	15.96%	383	54.87%	315	45.13%
Coastal Stations	2904	408	14.05%	214	52.45%	194	47.55%
Polyhaline Stations	1469	290	19.74%	169	58.28%	121	41.72%

(b) Mismatch at Good/Moderate boundary incorporating station identity

	No. Stations	Mismatch	% Mismatch	V1>V2	V1>V2%	V2>V1	V2>V1%
All Stations	4373	365	8.34%	191	52.33%	174	47.67%
Coastal Stations	2904	246	8.47%	126	51.22%	120	48.78%
Polyhaline Stations	1469	119	8.10%	65	54.62%	54	45.38%

(c) Mismatch at Good/Moderate boundary incorporating AG identity

	No. AG	Mismatch	% Mismatch	V1>V2	V1>V2%	V2>V1	V2>V1%
All AG	354	34	9.60%	25	73.53%	9	26.47%
Coastal AG	205	16	7.80%	10	62.50%	6	37.50%
Polyhaline AG	149	18	12.08%	15	83.33%	3	16.67%

(d) Mismatch of CofC at the Good/Moderate probability level incorporating AG identity

	No. AG	Mismatch	% Mismatch	V1>V2	V1>V2%	V2>V1	V2>V1%
All AG	354	35	9.89%	24	68.57%	11	31.43%
Coastal AG	205	16	7.80%	10	62.50%	6	37.50%
Polyhaline AG	149	19	12.75%	14	73.68%	5	26.32%

AG: Assessment Groups
CofC: Confidence of Classification; probability that the true mean ES > Good/Moderate boundary
V1>V2: IQI V1 classified ES as higher than IQI V2

The results of the intercalibration between IQI V1 and IQI V2 by weighted Kappa analysis are shown in Table 2.9. For Ecological Status (ES) calculated at the sampling station level for all Assessment Groups (Table 2.9.a), the Kappa value was 0.837, the % Mismatch between the IQI tools was 8.34% and the Agreement was classified as Very Good. For the coastal Assessment Groups, the Kappa value was 0.824, the % Mismatch between the IQI tools was 8.47% and the Agreement was classified as Very Good. For the polyhaline Assessment Groups, the Kappa value

was 0.853, the % Mismatch between the IQI tools was 8.10% and the Agreement was classified as Almost Perfect.

For ES calculated at the waterbody level for all Assessment Groups (Table 2.9.b), the Kappa value was 0.826, the % Mismatch between the IQI tools was 9.60% and the Agreement was classified as Very Good. For the coastal Assessment Groups, the Kappa value was 0.827, the % Mismatch between the IQI tools was 7.80% and the Agreement was classified as Very Good. For the polyhaline Assessment Groups, the Kappa value was 0.824, the % Mismatch between the IQI tools was 12.08% and the Agreement was classified as Very Good.

For Confidence of Classification for all Assessment Groups (Table 2.9.c) the Kappa value was 0.826, the % Mismatch between the IQI tools was 9.89% and the Agreement was classified as Very Good. For the coastal probabilities, the Kappa value was 0.854, the % Mismatch was 7.80% and the Agreement was Almost Perfect. For the polyhaline probabilities, the Kappa value was 0.802, the % Mismatch was 12.75% and the Agreement was Very Good.

The analysis for ES calculated at the sampling station level when all stations where IQI V2 did not assign 10% or more of the taxa to an AMBI Ecological Group were removed from the dataset is shown in Table 2.9.d. The Kappa value for all Assessment Groups was 0.863, the % Mismatch between the IQI tools was 8.15% and the Agreement was classified as Almost Perfect. For the coastal Assessment Groups, the Kappa value was 0.845, the % Mismatch was 8.22% and the Agreement was Very Good. For the polyhaline Assessment Groups, the Kappa value was 0.882, the % Mismatch was 8.05% and the Agreement was Almost Perfect.

TABLE 2. 9. INTERCALIBRATION RESULTS BETWEEN IQI V1 AND IQI V2 CALCULATED FROM BENTHIC WATER FRAMEWORK DIRECTIVE MONITORING IN COASTAL AND TRANSITIONAL WATERS IN IRELAND, 2010 TO 2023.

(a) Ecological Status by Station

	Kappa	% Mismatch	Agreement
All Stations	0.837	8.34%	Very Good
Coastal Stations	0.824	8.47%	Very Good
Polyhaline Stations	0.853	8.10%	Almost perfect

(b) Ecological Status by Assessment Group

	Kappa	% Mismatch	Agreement
All Assessment Groups	0.826	9.60%	Very Good
Coastal Assessment Groups	0.827	7.80%	Very Good
Polyhaline Assessment Groups	0.824	12.08%	Very Good

(c) Confidence of Classification

	Kappa	% Mismatch	Agreement
All Assessment Group Probablities	0.826	9.89%	Very Good
Coastal Assessment Group Probablities	0.854	7.80%	Almost perfect
Polyhaline Assessment Group Probablities	0.802	12.75%	Very Good

(d) Ecological Status by Station (>10% fauna unassigned stations removed)

	Kappa	% Mismatch	Agreement
All Stations	0.863	8.15%	Almost perfect
Coastal Stations	0.845	8.22%	Very Good
Polyhaline Stations	0.882	8.05%	Almost perfect

2.5. Discussion

Monitoring benthic sediment habitats for the purpose of the Water Framework Directive (WFD) is an important component of assessing potential anthropogenic impacts in coastal and estuarine waters (Zettler et al., 2007). The nature of the receiving environment is an important consideration in assessing whether seafloor communities have changed with respect to the expected level associated with natural variability (Muxika et al., 2007). This had led to the development of local Ecological Quality Ratios (EQR) within the North East Atlantic Geographical Intercalibration Group (NEAGIG). These EQR are designed to optimise the assessment of Ecological Status (ES) within the waters of an EU member state. This is achieved by choosing component indices of the EQR that respond to anthropogenic impact in a predictable manner, and weighting the component indices appropriately (Muxika et al., 2007; Phillips et al., 2014). In the case of the Infaunal Quality Index (Phillips et al., 2014) reference conditions for the component indices are set for a given sample in relation to the local sediment type and salinity characteristics. The complexity of processing multivariate macrofaunal data, multivariate sediment sieve class data and salinity characteristics necessitated the development of a bespoke software tool to ensure consistent data processing and ES results between operators and Assessment Groups (AG). An update to the IQI tool requires assessment of the outputs of the IQI V1 and IQI V2 tools to ensure that the results are comparable, particularly in terms of the ES classes assigned to AG.

The agreement between the findings of the two tools is generally good when viewed at the population level of all stations, though the disagreement is considerably higher for the polyhaline stations than for the coastal. As the identity of the stations and the AG is incorporated, the %Difference in agreement increases. While the two tools return very similar results for the population of all stations sampled, the results are very different at the level of the mean ES class assigned to the individual stations and AG. This is also the case with the probability that the mean ES of the AG lies above the Good/Moderate boundary (Confidence of Classification). Incorporating AG identity is very important in making an appropriate assessment of the mean ES class assigned to the AG, particularly with reference to the Good/Moderate boundary.

Kappa analysis (Cohen, 1960; Fleiss & Cohen, 1973) has been used extensively to address this issue in intercalibrating EQR developed for the WFD, in addition to the intercalibration for the benthic element of the North East Atlantic Geographical Intercalibration Group (Borja et al., 2007; Phillips et al., 2014). For example, Simboura & Reizopoulou (2008) used weighted Kappa analysis to intercalibrate the use of other benthic EQR in Greek waters, which are part of the

Mediterranean GIG. The indices assessed were AMBI, M-AMBI and BENTIX (Simboura & Zenetos, 2002). Guinda et al., (2008) used Kappa analysis to intercalibrate two EQR that address ES in rocky shore macroalgal communities in the NEAGIG. These were the Reduced Species List (Wells et al., 2007) and the quality of rocky bottoms index (CFR) of Juanes et al. (2008). Lepage et al., (2016) intercalibrated seven EQR used in the NEAGIG to assess ES based on the distribution and abundance of fish species in transitional waters. Kappa analysis allows the intercalibration of EQR based on any Biological Quality Element (BQE). It was the method used to gain approval of the European Commission (European-Commission, 2008) for the intercalibration of the benthic BQE in the NEAGIG. This is because Kappa analysis allows the identity of the stations and AG sampled to be incorporated into a well-structured assessment of agreement that scales the degree of mismatch to penalise cases where disagreement between observations is more than one ES class. Using the Agreement aspect of Kappa allows the agreement between EQR to be classified in a manner that facilitates the assessment of the correspondence of their outputs in a meaningful way.

Kappa analysis is also flexible enough to allow the analyst to set appropriate standards in the level of matching required between the output classes to satisfy the requirements of the assessment being undertaken. The intercalibration approach of Borja et al., (2007) and this study, sets the Kappa statistic classified as Almost Perfect agreement as 0.85. In many applications of the Kappa test in the literature, this boundary is set as 0.80 (McHugh, 2012) as originally suggested by Cohen, (1960). By setting the boundary between Good and Almost Perfect agreement at 0.85 in this study, the requirement for the EQR to agree regarding ES class assigned and at the G/M boundary is set at a high level. This is appropriate for ascertaining agreement between metrics that will be used to determine the extent of anthropogenic impacts in different areas and member states within GIG. In a similar manner, this study has intercalibrated the two versions of the IQI tool that have been used to assess ES for the purposes of WFD classification at a high standard of Agreement.

The Kappa findings at the sampling station level (with station identity incorporated) classified agreement as Very Good for all the stations and for the coastal stations. The agreement between the polyhaline stations was classified as Almost Perfect. For the purposes of intercalibration, the members of the NEAGIG considered Very Good agreement between the methods as reported in Borja et al., (2007) as insufficient to meet the requirements of the WFD. The EQR from the member states were extensively redeveloped. When submitted to the EC, each EQR in the NEAGIG had achieved Almost Perfect agreement with all of the other EQR (European-

Commission, 2008; Phillips et al., 2014). At the station level, using all of the stations sampled, the agreement between IQI V1 and IQI V2 is not sufficient to consider the outputs directly comparable.

Similar findings were returned for the AG when assessed using all of the stations sampled, aggregated to AG. In this case, the agreement was classified as Very Good for all AG, and also for the coastal and polyhaline AG considered separately. There are no published accounts of the use of Kappa analysis to intercalibrate between mean ES assigned to AG using different EQR. The intercalibration studies cited above all use Kappa to intercalibrate between stations from smaller datasets than the one considered here. Most of these studies were performed before the commencement of WFD monitoring in 2009. They do not contain data in the AG structure associated with WFD monitoring. For the purposes of this study, where two versions of the same EQR are being assessed, the same requirement for Almost Perfect agreement between IQI V1 and IQI V2 is assumed to be required.

When the probabilities that the true mean ES for each AG lies above the Good/Moderate boundary were assessed by Kappa analysis, the agreement for all of the AG was Very Good. The agreement for coastal AG was Almost Perfect, while for polyhaline AG, the agreement was Very Good. As for the mean AG ES above, there are no published intercalibration studies that compare the probabilities derived from annual WFD monitoring of multiple AG. This study assumes that agreement for these probabilities between two versions of the same EQR should be Almost Perfect.

Overall, the Kappa analyses at the sample station, AG and probability levels do not return sufficient agreement classifications to meet the criteria for successful intercalibration (European-Commission, 2008; Phillips et al., 2014) This points to there being substantial and significant differences in ES classification between the two IQI tools when applied to the entire dataset.

Philips et al (2014) is a substantive account of the development of the IQI V1 tool, incorporating detailed guidance on the use of the Excel tool. Much of this information is of necessity and specific to the IQI V1 tool. While the IQI V2 tool includes instructions on the use of the tool in a worksheet within the V2 workbook, the Philips et al (2014) manual for the development and use of the tool has not been updated for V2 at the time of writing. In the absence of updated guidance, the guidance in Philips et al. (2014) has been used to investigate the mismatch between the IQI V1 and IQI V2 outputs.

The most apparent difference in the outputs of the V1 and V2 tools is in the percentage of taxa that were not assigned to an AMBI Ecological Group (EG). For the IQI V1 tool, (Phillips et al., 2014) recommends that any sample station at which more than 10% of the fauna are unassigned to an EG should be interpreted with caution, and ideally removed from the assessment of the waterbody. When using the WFD monitoring data with the faunal taxonomy aligned with the V1 taxonomic library, none of the stations had >10% of the fauna unassigned in the IQI V1 dataset. When using the V2 tool, the fauna was classified using the currently valid taxonomic classification obtained from (WORMS, 2025). All taxa input to the V2 tool were recognised by the taxonomic library within the V2 tool. Of the 4673 stations in the overall dataset, 46.31% of the sample stations had >10% of the taxa unassigned. The guidance for IQI V1 was applied to this dataset, and stations with >10% fauna unassigned were removed, and the Kappa analysis was repeated. The agreement in the resulting Kappa analysis showed a good deal of improvement. For all stations and for polyhaline stations, the agreement was classified as Almost Perfect. For coastal stations, the agreement remained at Very Good, but in this case, the Kappa statistic calculated was 0.845, approaching the 0.85 boundary for Almost Perfect agreement.

Without a detailed account of the update process in the development of IQI V2, a detailed investigation into all possible causes of the differences in the IQI tool outputs lies outside the remit of this study. It appears that differences in the process of assigning EG to the taxa is a substantial cause of the differences between the tool outputs. Phillips et al., (2014) provide an account of how the criteria for determining which taxa should be included for IQI V1 were decided, often by expert judgement. This information is not currently available for IQI V2. Further guidance is required. While the greatly improved agreement achieved when the stations with a high proportion of unassigned taxa are removed is encouraging, removing >2000 stations from the overall dataset must substantially change the overall pattern of the dataset and its power to detect significant anthropogenic disturbance.

IQI is an EQR that has been reported relatively little in the scientific literature when compared to similar EQRs such as M-AMBI (Muxika et al., 2007). All available sources from subtidal sediments in the published literature at the time of writing refer to IQI V1 rather than the use of IQI V2. In the Irish setting, for subtidal sediments, Kennedy et al. (2011) intercalibrated the use of IQIII (which predates IQIV V1) and M-AMBI in long-term benthic monitoring in Kinsale Harbour, and found that the agreement between the EQR was Almost Perfect. Both M-AMBI and IQI have been substantially updated in terms of the taxa libraries and the EG assigned to those taxa in the intervening period. There is a need for a revised intercalibration of the benthic

EQR used to assess the Benthic Quality Element (BQE) in the NEAGIG. This revised intercalibration could include elements of long-term monitoring for the purpose of the WFD from multiple member states to determine the relationship between the EQR when used for the purposes of the WFD, rather than simply comparing the EQR on short, very strong disturbance gradients consisting of relatively small datasets. For long-term monitoring, it is clear that any assessment of current ES within a particular AG using IQI V2 needs to be compared to previous data from that AG calculated using the IQI V2 tool. This is the most appropriate approach to determine if a significant change has occurred within the AG in the intervening period. The outputs of the two tools cannot be viewed as equivalent at this point. IQI V2 has been adopted by the relevant competent authorities as the current tool for determining ES in WFD waterbodies in Ireland and the UK.

Future work in this area should include an updated intercalibration of IQI V2 with the other EQR used within the NEAGIG. Additional future work should include time series assessment of ES determined using IQI V2 at all individual waterbodies monitored for the BQE. This may reveal additional local factors, such as the accuracy of local waterbody salinity classification, that may affect the ES assigned by the IQI V2 tool.

3. Monitoring benthic impacts of salmon pen aquaculture on the west coast of Ireland using Infaunal Quality Index and Sediment Profile Imagery.

3.1. Abstract

Two methods of benthic Ecological Status (ES) assessment were applied to monitoring data collected at three salmon aquaculture sites on the west coast of Ireland over a five year period. Fourteen surveys were analysed. Macrofaunal data from grab samples were used to calculate Infaunal Quality Index, an Ecological Quality Ratio (EQR) designed for Water Framework Directive (WFD) assessment. Sediment organic content (LOI) was measured from sediment samples. Sediment Profile imagery (SPI) data were used to estimate the depth of the apparent redox potential discontinuity (aRPD) and calculate Visual Benthic Health (VBH), an SPI index optimised for aquaculture monitoring. aRPD is used to estimate the oxidation state of the surficial sediments. Faunal and sediment samples were taken by SCUBA diver under the cages and using a grab sampler at other stations. SPI were collected by SCUBA at all stations.

Individual farm surveys used a sampling design of perpendicular transects: Transect 1 in line with the prevailing current, Transect 2 at right angles to Transect 1. For IQI, 5 of the 14 farms failed the assessment criteria (Farm A in 2017, Farm B in 2021, Farm C in 2017, 2018, 2019). These farms were assigned an ES of Moderate or worse at the end of a sampling transect. For VBH, 4 of the same farms failed the assessment criteria (Farm B in 2021, Farm C in 2017, 2018, 2019). No surveys failed the assessment criteria for LOI, defined as the station exceeding a value double that of the reference station at the end of a transect. For aRPD, the assessment criterion is that a sample at the end of a transect should have an aRPD of 3cm or 50% that of the reference station, whichever is the lesser value. 8 of the 14 surveys failed the aRPD assessment criteria.

IQI V2 and VBH were both analysed using a mixed model with distance from the sea cage centre and transect direction as fixed effects, and Survey (farm x year) as a random effect. A similar mixed modelling approach was used to assess the relationship between IQI, sediment organic content and the depth of the aRPD. Both IQI and VBH increased with increasing distance from the centre of the sea cage, and were lower on the transect in line with the prevailing current. IQI responded to increasing organic enrichment by decreasing both when LOI increased and when aRPD decreased.

Intercalibration of the ES classes assigned to sampling stations by IQI and VBH showed only a moderate level of agreement between the indices. The indices agreed in 7 of the 14 cases about

whether the Survey passed the assessment criteria. In the five cases where IQI assessment produced a fail, VBH agreed in only two cases. The VBH index shows potential as a rapid assessment tool to support the use of IQI in aquaculture monitoring. It responds in the same general manner to aquaculture impacts as IQI. The agreement between IQI and VBH may be improved if replicated SPI are taken at each sampling station. A lander-mounted SPI camera should be deployed from the survey vessel at the same sampling occupancy as the grab sampler rather than the diver-operated system used in this study.

IQI was effective in mapping aquaculture impacts on coastal benthic communities. Adoption of IQI in aquaculture monitoring would facilitate improved management of coastal waterbodies by providing a common framework for aquaculture and other potential stressors to be assessed and managed in a cohesive network. In addition to WFD coastal and estuarine waters, this may include marine protected areas and Natura 2000 sites, which frequently overlap with aquaculture sites on the west coast of Ireland.

3.2. Introduction

Marine benthic macrofaunal communities have long been used to monitor anthropogenic impacts on the soft seafloor in response to physical disturbance (Rhoads & Boyer, 1982) or organic enrichment (Pearson, 1978). Successional paradigms (Pearson, 1978) of change in community structure predict the removal of disturbance-sensitive taxa and their replacement with disturbance-tolerant taxa. This trend continues with increasing disturbance. The community becomes less diverse with increasing disturbance as a small number of opportunistic, disturbance-tolerant taxa dominate the community. In the case of severe disturbance, all taxa may be removed from the sediment. This successional model is suitable for monitoring disturbance and recovery from point source impacts over time and spatial recovery with distance from chronic disturbance (Borja et al., 2007).

The effects of organic enrichment on the seafloor in relation to marine salmonid pen culture have been extensively studied. The accumulation of organic material under and around fish cages may result in the complete removal of macrofauna to create an Azoic zone in the case of severe impact (Lu & Wu, 2007). Opportunistic small-bodied taxa, often tube-dwelling, tolerant of organic enrichment dominate the area adjacent to the Azoic zone. This is followed by a transitional zone in which opportunistic species are increasingly outcompeted for space by taxa characteristic of undisturbed conditions, as distance from the fish cage increases. With sufficient distance, the benthic community returns to an undisturbed state. This successional approach to monitoring fish

farm disturbance to benthic communities has been validated using univariate and multivariate approaches (Tomassetti et al., 2009). Fish farms in a broad geographic range have been monitored using this approach (Brooks et al., 2003; Brown et al., 1987; Cheshire et al., 1996; Findlay et al., 1995; Hargrave et al., 1997; Holmer & Kristensen, 1992; Macleod et al., 2002; Ritz et al., 1989; Weston, 1990).

Organic enrichment changes the geochemistry of the affected sediments, notably increasing the labile organic content of the sediment and reducing the depth of the redox potential discontinuity (RPD) (Hargrave et al., 1997; Pearson, 1978). Many studies have reported a statistically significant relationship between these parameters and the distribution of infaunal communities in relation to aquaculture disturbance (Brown et al., 1987; Cheshire et al., 1996; Findlay et al., 1995; Hargrave et al., 1997; Holmer & Kristensen, 1992; Macleod et al., 2002; Weston, 1990; D. Wildish et al., 2001). Infaunal benthic macrofauna modify the geochemistry of the sediments they occupy by mixing sediments and increasing the exchange of porewater with the overlying water column, a process termed bioturbation (Aller, 1994). Organic enrichment from aquaculture alters benthic community structure, sediment biogeochemical functioning and the interaction between them (Bannister et al., 2014; Edgar et al., 2010; Kutti et al., 2007; Sanchis et al., 2021; Tomassetti et al., 2016; Tomassetti & Porrello, 2005).

Macrofaunal community structure and bulk sediment geochemistry currently form the basis for the assessment of organic enrichment by coastal salmonid aquaculture in fish pens or cages in Ireland (DAFF 2008). This program stipulates monitoring requirements at two levels of intensity determined by the tonnage of fish being held at a licensed fish farm site and the prevailing current speed.

Level 1 monitoring involves the measurement of sediment organic content and the depth of the RPD at each station sampled. It is common practice in Ireland that Sediment Profile Imagery (Rhoads & Boyer, 1982; Rhoads & Germano, 1986), as described below, is used to estimate the depth of the RPD (O'Connor et al., 1989). Infaunal community status is estimated from photographic and/or video surveys of the sediment surface along two transects taken under the cage or array of cages. The visual survey also records the extent of coverage of the sediment surface by bacterial mats, waste feed pellets, faecal material and the presence of sediment outgassing. The first transect is taken from under the cage in the direction of the prevailing current (or seaward). It includes stations directly under the cage, at the cage edge and distances of 10m, 20m, 50m and 100m from the edge of the cage. The second transect is taken perpendicular to the prevailing current at the same intervals up to 50m, but not at 100m. A representative control station

at a distance of 500m from the cage is sampled in the same manner. Level 2 monitoring includes the samples taken at Level 1 in addition to macrofaunal samples collected by diver cores or grab samples at each of the stations.

Sediment Profile Imagery (SPI) is a rapid assessment tool for visualising and assessing benthic habitat quality on the soft seafloor (Gibson et al., 2011; Rhoads & Boyer, 1982; Rhoads & Germano, 1986). A sediment profile camera consists of an inverted periscope pushed into the surficial sediment to a depth of approximately 15cm either by weights under hydraulic control on a lander or by manual insertion by a diver. Images obtained of the surficial sediments in profile are analysed for the presence of visible infauna or tubes, burrows or feeding structures in the sediment and layers of pelletised sediment that indicate active deposit feeding by infauna. The Redox Potential Discontinuity (RPD) may be estimated from a colour or luminosity change of the sediment associated with the oxidation state of metals coating sediment grains. Oxidised sediments tend to be reflective and yellow in colour. Reduced sediments tend to be non-reflective and dark grey to black in colour. The apparent RPD (aRPD) depth is estimated by threshold analysis in an appropriate image analysis program to determine the area of oxidised surficial sediment. SPI images are usually between 13cm and 15cm in width. aRPD is calculated by dividing the oxidised area by the image width. In fish farm settings, aRPD as determined by SPI, has been shown to be a reliable predictor of RPD as measured by redox electrode profiling of field cores (Mulsow et al., 2006; Simone & Grant, 2017).

SPI analysis for environmental monitoring has often involved the recording and measuring of specific features in images to calculate an index score for each image. Images are then grouped into successional stages based on boundary points in the index scores Nilsson & Rosenberg, (2000) and Rhoads & Germano, (1986). **(**SPI has been used to characterise the successional stages of organic enrichment gradients in many studies of disturbance gradients on the seafloor (Nilsson & Rosenberg, 2000; Pearson & Rosenberg, 1977; Wildish et al., 2003). SPI has been shown to be cost-effective for detecting and monitoring organic enrichment around fish farms, compared to conventional macrofaunal sampling and analysis (Wildish et al., 2001).

The Water Framework Directive (WFD; 2000/60/EC) was designed to improve and protect all transitional and coastal waters within the EU. The WFD requires that EU member states monitor waterbodies in their territorial areas and measure habitat quality by calculating Ecological Quality Ratios (EQR) using a biodiversity component suitable for each broad habitat type and putative impact. EQR values are classified into one of five Ecological Status (ES) classes (Bad, Poor, Moderate, Good and High). Member states are obliged to take action to ensure that ES is of Good

or High status. In Ireland and the United Kingdom, the Infaunal Quality Index (IQI; Philips et al. 2014) is the EQR used for determining the ES of the macrobenthic communities of subtidal soft sediments (Forde et al., 2015; Walls et al., 2017; WFD, 2000). The IQI has proven effective in comparing anthropogenic impacts across a range of locations and sediment types (Walls et al., 2017). IQI uses three metrics to calculate the ES of benthic habitats that include AMBI (Borja et al 2000), Simpson's evenness index (Simpson, 1949) and the number of invertebrate taxa. IQI is used as the measure of benthic impact in relation to the deposition of particulate fish farm waste in Scotland (SEPA, 2019; Fox et al, 2022). IQI ES is used to determine if benthic impacts from farm cages are limited to a mixing zone area that is equivalent to 100m in all directions from the pens. At dispersive sites, the mixing zone may extend to more than 100m in an elliptical shape, provided that the total area does not exceed the symmetrical area equivalent to 100m in all directions. There is an additional requirement that benthic biological processes are functioning to breakdown assimilated waste at the pen edge, which is in part determined using IQI ES.

Rosenberg et al., (2004) optimised the Benthic Habitat Quality (BHQ) index of Nilsson & Rosenberg (1997) for the purposes of classifying seafloor habitats to one of the five ES classes of the WFD using SPI. Simone and Grant (2020) modified the general BHQ index for specific use in proximity to salmonid cages at sea, adding some parameters typically observed near salmonid cages. This SPI index for monitoring aquaculture impacts is the Visual Benthic Health (VBH) index. Simone & Grant (2020) added parameters that include cage falloff of mussel shells or algae, the coverage of bacterial mats on the sediment surface and the concept of Farm Sediment (Karakassis et al., 2002), which is an accumulation of fish pellets, faeces and casts under or near to fish cages. Farm Sediment may be variable in appearance depending on the time since its deposition and local environmental conditions. For SPI analysis, it is treated as a spoil layer and considered to be incorporated into the sediment profile. Simone and Grant (2020) classified the index values into five stages using correspondence to the benthic organic enrichment groups of Hargrave et al. (2008). This provides an SPI analysis framework easily adapted for WFD monitoring in relation to salmonid cages at sea. A subset of VBH parameters without the SPI called the Surface Index (SI, Simone and Grant 2020) was calculated using diver photographs of each station.

In this study, the feasibility of using IQI to determine the impact of salmon cage aquaculture on the soft seafloor in terms of ES is assessed using data collected at three farms on the west coast of Ireland over a 5-year period (2017-2021) using the Level 2 monitoring protocol described above. The effectiveness of the VBH as a rapid assessment tool in classifying ES in relation to aquaculture

impacts using SPI is assessed. The agreement between IQI, VBH and SI assessment in determining Ecological Status (ES) at a sampling location is determined using a Kappa analysis, applying the method presented in Borja et al., (2007). The distribution of IQI and VBH as a function of distance from the cage and transect orientation is assessed using a mixed modelling approach, with Survey as a random effect. A similar mixed model is used to determine the relationship between IQI, and the sediment parameters LOI and aRPD. A Kappa analysis of the agreement of IQI, VBH, SI, aRPD and LOI regarding whether the sample stations pass or fail the relative criteria is carried out.

3.3. Methods

3.3.1. General description of the survey

Farm surveys were carried out using the regulatory monitoring protocol for Level 2 surveys described in (DAFF, 2008). Level 2 surveys included grab sampling for benthic invertebrates and sediment organic content and measurement of the depth of the redox potential discontinuity in the surficial sediments. Surface photographs with an area of approximately $1m^2$ were taken to identify sediment colour and texture, fauna and algae, bacterial mats, outgassing and farm waste. Sediment profile images (SPI; Rhoads & Cande, 1971) were taken with a diver-operated sampler at each station. Two sampling transects were taken in each survey. One transect was taken in line with the prevailing current. The second transect was taken perpendicular to the prevailing current. In the first transect, samples were taken directly under the cage, at the edge of the cage, and at distances of 10m, 20m, 50m and 100m from the cage edge. In the second transect, samples were taken at the edge of the cage and at 10m, 20m and 50m from the cage edge. A single reference station was located within 500m of the cage at a location chosen to represent the ambient sediment type. In all cases the reference station was at least 150m from the cage edge.

Surveys were carried out at three farm sites; see Figure 3.1 for locations. Farm A was in Inver Bay in the northwest of Ireland, Farms B and C were in Bantry Bay in the southwest. Sampling was carried out annually between 2017 and 2021 at each site, with the exception of Farm A in 2020. In 2020, Farm A was classified as a Level 1 farm based on fish tonnage. No grab samples for macrofauna were taken that year, but all other data were collected.

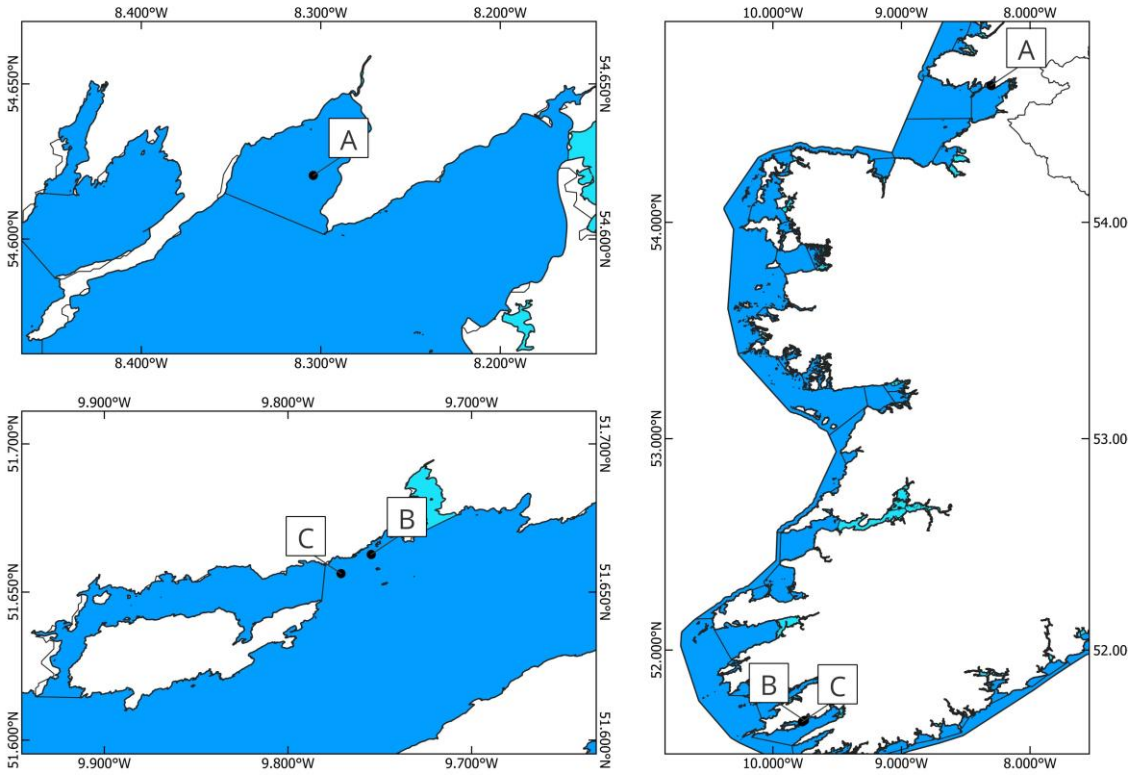

FIGURE 3. 1. MAP OF FISH FARM LOCATIONS IN 2017-2021

At each station between the cage edge and the transect ends two replicate grab samples for macrofauna and a single grab sample for sediments were collected using a 0.025m^2 van Veen grab sampler deployed from a boat. Under the cage, bottom samples were taken by SCUBA divers using 0.018m^2 cores. The faunal abundances from the replicate samples were combined to produce a single abundance dataset for the station and converted to abundance per 0.1m^2 before data processing.

Faunal samples were sieved on a 1mm mesh. Retained fauna were fixed in buffered 4% formaldehyde, stained with rhodamine dye and preserved in 70% methanol. Fauna was picked from the sediment residue using a stereo microscope at x10 magnification. They were identified to species or the lowest possible taxonomic level and enumerated.

The species list was made compliant with the World Registry of Marine Species (WORMS, 2025) species list and then reclassified to match the taxa list in the Infaunal Quality Index (IQI) MS Excel tool Version 2 (Phillips et al., 2014).

The IQI Ecological Quality Ratio (EQR), a continuous variable between 0 and 1, was calculated using Equation 1 below, following the method outlined in the tool instructions (Phillips et al., 2014).

$$IQI = \frac{\left(\left(0.38\times\left(\frac{(1-AMBI/7)}{(1-AMBI/7)_{ref}}\right)\right)+\left(0.08\times\left(\frac{(1-\lambda')}{(1-\lambda')_{ref}}\right)\right)+\left(0.54\times\left(\frac{S}{S_{ref}}\right)^{0.1}\right)-0.4\right)}{0.6} \quad (1)$$

Where:

- AMBI is the AZTI Marine Biotic Index (Borja et al., 2000),
- 1 – λ' is Simpson's evenness index (Simpson, 1949)
- $S^{0.1}$ is the number of taxa (S) raised to the power 0.1,
- ref parameters are the reference values for the sample.

AMBI is a continuous variable based on the proportions of five Ecological Groups allocated to benthic species (Borja et al., 2000). AMBI was calculated within the IQI tool using scores assigned in the taxa list. Within the IQI tool, an algorithm was used to adjust reference conditions for component metrics based on local sediment and salinity environmental conditions. Salinity and sediment parameters were entered into the IQI tool as categorical variables. The salinity classifications were derived from the shapefiles for coastal and transitional waters in Ireland used for regulatory monitoring by the government of Ireland. All stations were classified as coastal (fully marine). Sediment classification by the scheme of (Folk, 1954) were assigned by surveyors in the field by visual assessment of sediment samples and by visual assessment of surface and sediment profile images as described below. For the purposes of analysis in the IQI tool, sediments were all assigned to the broad class Muddy Sand/Sandy Mud.

IQI EQR values were converted to Ecological Status (ES) classes within the tool used the class boundary values in Table 3.2. Sediment samples for organic carbon analyses were frozen at -20C prior to analysis using the Loss on Ignition (LOI) technique of (Dean, 1974). This method involves oven drying the sediment to a constant weight at 100 °C before placing a pre-weighed aliquot in an oven at 450 °C for a period of 6 hours and calculating the percentage loss of mass.

At each station, a digital SLR camera fitted with a wide-angle lens and two strobes was used by a SCUBA diver to photograph the epibenthos and seafloor features. One photograph was taken per station.

A diver-operated SPI camera was used for photographing sediment profiles of the seafloor and for calculating apparent redox discontinuity (aRPD) measurements (Rhoads & Germano, 1986). This unit used a digital single lens reflex (SLR) camera in a water-tight pressure vessel mounted above a prism penetrating the upper 25 cm of sediment (Solan & Kennedy, 2002). The sediment profile was viewed through a plexiglass window.

Mean aRPD depth was measured in SPI using Image-J (Schneider et al., 2012). Images were corrected for uneven illumination using a mask. Apparent RPD depth was measured using colour thresholding to estimate the area of oxidised sediment in contact with the sediment-water interface. Mean aRPD was calculated by dividing this area by the image width (O'Reilly et al., 2006). aRPD was set to zero in the presence of significant amounts of Farm Sediment (Simone & Grant, 2020). Farm sediment is a spoil layer of uneaten fish food, fish casts and faecal matter. This layer may appear pale and yellow or orange, reflecting the colour of the fish food or fish waste. The occurrence of this colour change does not fit the assumptions of the successional SPI model as described in Rhoads & Boyer, (1982) and Nilsson & Rosenberg, (1997). Farm Sediment is likely to be highly reduced.

SPI analyses were carried out to determine the successional status of each station sampled using the Visual Benthic Health index (VBH; Simone & Grant, 2020). A subset of the VBH parameters was used to calculate a disturbance metric based only on features visible in surface photography, the Surface Index (SI; Simone & Grant, 2020) at each station. VBH is a modification of the BHQ (Nilsson & Rosenberg, 1997) optimised to determine aquaculture impacts in coastal waters (Simone & Grant, 2020). Calculation of the VBH and SI are shown in Table 3.1. For the SI, the presence or absence of tubes, feeding pits/mounds and burrows was identified from surface photography. The presence of epifauna in the taxa echinoderms, benthic fish and epibenthic crustaceans was recorded. The presence of outgassing at the sediment surface was recorded. The percentage cover of *Beggiatoa* bacterial mats was classified into levels of cover as in Table 3.1. For VBH, the parameters used for SI were measured from SPI where present. Mean aRPD was assigned a score based on depth. Cage falloff of shell debris and organic debris was assigned a negative score (Table 3.1).

TABLE 3. 1. CALCULATION OF THE VISUAL BENTHIC HEALTH (VBH) AND SURFACE INDEX (SI)

	Feature	Value
A Infuana	Tubes	2
	Feeding pit/mound	2
	Burrows	2
B Epifauna	Echinoderms	1
	Benthic fish	1
	Epibenthic crustaceans	1
C Mean aRPD	0	0
	< 1	1
	1 < x < 2	2
	2 < x < 3	3
	3 < x < 5	4
	>5	5
D Cage Falloff	Shell debris	-1
	Organic debris	-1
E Outgassing	Gas bubbles	-2
F Beggiatoa (% cover)	0	0
	1-29	-0.5
	30-59	-1
	60+	-2
$VBH = \Sigma A + \Sigma B + C + \Sigma D + E + F$ $SI = \Sigma A + \Sigma B + E + F$		

VBH index outputs range from -7 to 14, while SI outputs range from -5 to 9. In this study, the outputs of VBH and SI indices were standardised to be a decimal fraction between 0 and 1, where 0 is the lowest possible score that can be achieved in the index, and 1 is the maximum value that can be achieved. Class boundaries for VBH and SI are shown in Table 3.2. Simone and Grant (2020) classified the outputs of VBH and SI into five benthic organic enrichment classes using the scheme of Hargrave et al. (2008). Hargrave et al. (2008) classified sediments impacted by fish farms as anoxic to oxic A, with three of the classes being anoxic or hypoxic and two of the classes being oxic. This is broadly equivalent to the WFD classification of three classes below the

Good/Moderate boundary and two classes above the G/M boundary. Correspondence between WFD ES and the classes of VBH / SI are shown in Table 3.2. In this study, these classes were treated as equivalent to the five Ecological Status classes of the WFD.

TABLE 3.2. ECOLOGICAL STATUS CLASS BOUNDARIES FOR THE ECOLOGICAL QUALITY RATIO INFAUNAL QUALITY INDEX (IQI) AND CORRESPONDING CLASS BOUNDARIES FOR THE VISUAL BENTHIC HEALTH (VBH) AND SURFACE INDEX (SI), AND CORRESPONDING ORGANIC ENRICHMENT BOUNDARIES OF HARGRAVE ET AL. (2008).

WFD	IQI	VBH	SI	(Hargrave et al. 2008)
Good-High	0.75	0.67	0.79	Oxic B-Oxic A
Moderate-Good	0.64	0.52	0.36	Hypoxic A-Oxic B
Poor-Moderate	0.44	0.43	0.50	Hypoxic B-Hypoxic A
Bad-Poor	0.24	0.24	0.21	Anoxic-Hypoxic B

Aquaculture monitoring protocols allow a farm to have an impact footprint up to 100m from the cage in line with the prevailing current, and up to 50m perpendicular to the prevailing current (DAFF, 2008). The status of each survey was assessed for each EQR based on the distance from the cages at which the IQI was classified as being above the Good / Moderate (G/M) boundary for the remainder of the survey transects. Where a station at the end of a Transect was classified below the G/M boundary, the Survey was deemed to have failed the assessment for that EQR, even if an intermediate station had passed the assessment.

The agreement in classification between methods using different sample processing techniques was determined using a Kappa analysis (Cohen, 1960; Landis & Koch, 1977) as previously applied to intercalibrate WFD EQR (Borja et al., 2007) and Kennedy et al., (2011). This methodology applies weighting to misclassifications to down-weight the importance of misclassification between adjacent classes, while misclassifications between non-adjacent classes were assigned larger weighting.

The distribution of IQI in relation to distance from the cage and transect orientation was determined using a linear mixed effects model fitted using a restricted maximum likelihood test (REML). The IQI EQR was the response variable. Survey was included as a random categorical effect with fourteen levels, for each survey analysed. Distance and Transect were fixed effects. Optimum model fitting was carried out by ANOVA between the outputs of the full model and nested models. The p-value was estimated using a likelihood ratio test (Zuur et al., 2007). When carrying out the regression, the Reference station was used as the reference level for Distance,

Transect 2 was used as the reference level for Transect. A similar mixed model analysis was carried out using VBH as the response variable. The relationship between IQI, aRPD and LOI was investigated using a mixed effects model with IQI as the response, LOI and aRPD as covariate predictors and random categorical effects for Survey and Transect.

3.4. Results

All EQR scores from IQI, VBH and SI are listed in and available on request. At the reference stations, IQI ranged from 0.66 to .84 (Good to High). Stations under the cages ranged between 0.15 and 0.54 (Bad to Moderate). Stations between the cage edge and the end of the transect ranged from 0.22 to .83 (Bad to High) on Transect 1 and from 0.10 to 0.86 (Bad to High) on Transect 2. For VBH, the reference stations ranged between 0.38 and 0.86 (Poor to High). Stations under the cages ranged between 0.29 and 0.57 (Poor to Good). Stations between the cage edge and the end of the transect ranged from 0.14 to .76 (Bad to High) on Transect 1 and from 0.33 to 0.90 (Poor to High) on Transect 2. For SI, the reference stations ranged from 0.36 to 0.79 (Moderate to High). Stations under the cages ranged between 0.36 and .71 (Moderate to Good). Stations between the cage edge and the end of the transect ranged between 0.21 and .79 (Poor to High) on Transect 1, and between 0.21 to 0.86 (Poor to High) on Transect 2.

Table 3.3 shows the distance from the cages at which the IQI was classified as being above the Good / Moderate (G/M) boundary for the remainder of the survey transect as a Pass/Fail measure. The reference station ES classes relative to the G/M boundary are also included in Table 3.3. For IQI, all reference stations were classified as being above the G/M boundary. At Farm A, Transect 1 failed the IQI assessment in 2017. Transect 1 scored above the G/M boundary within 100m of the cage in all other years. Transect 2 was above the G/M boundary within 50m of the cage in all years. At Farm B, Transect 1 failed the IQI assessment in 2021. Transect 1 scored above the G/M boundary within 100m of the cage in all other years. Transect 2 was above the G/M boundary within 50m of the cage in all years. At Farm C, Transect 1 and Transect 2 failed IQI assessments in 2017, 2018 and 2019. In 2020 and 2021, both transects scored above the G/M boundary within 10m of the cage.

All data extracted from SPI and surface imagery data to calculate the VBH and SI are available on request. Table 3.3 shows the distance from the cages at which the SI and VBH were classified as being above the Good / Moderate (G/M) boundary for each survey transect. For VBH, Farm A passed the ES assessment in all years on Transect 1. Farm A failed the ES assessment on Transect 2 in 2019 and passed in all other years. Farm A passed the ES assessment in other years on

Transects 1 and 2. All of the reference stations passed the assessment. At Farm B, all reference stations passed the ES assessment. Transect 1 failed the ES assessment in 2018 and 2021, passing in the other years. Transect 2 failed the ES assessment in 2017, passing in all other years. At Farm C, the reference station failed the ES assessment in 2017, passing in all other years. Transect 1 failed in 2017 and 2021, passing in the other years. Transect 2 failed in 2017, passing in the other years. For SI at Farm A, the reference station failed the ES assessment in 2017, passing in all other years. Transects 1 and 2 both passed the ES assessment in all years. At Farm B, the reference station failed the ES assessment in 2018, passing in all other years. Transect 1 failed in 2020, passing all other years. Transect 2 passed the assessment in all years. The reference station failed at Farm C in 2019 and 2020, passing in the other years. Transects 1 and 2 both passed the ES assessment in all years at Farm C.

For all the surveys, sediment Loss on Ignition (LOI) and the mean depth of the apparent Redox Potential Discontinuity (aRPD) are available on request. For LOI, the reference stations ranged between 4.6 and 14.0%. Stations under the cages ranged between 2.6 and 16.5%. Stations between the cage edge and the end of the transect ranged from 3.4 to 21.5% on Transect 1 and from 3.1 to 15.3% on Transect 2. For aRPD, the reference stations ranged between 4.0 and 6.9cm. Stations under the cages ranged between 0 and 1.6cm. Stations between the cage edge and the end of the transect ranged from 0 to 6.0cm on Transect 1 and from 0 to 7.5cm on Transect 2. For LOI, all reference stations passed the assessment in all years. At Farm A and Farm C, both Transect 1 and 2 passed the assessment in all years. At Farm B, transect 1 failed in 2019, passing in all other years. Transect 2 passed the assessment in all years. For aRPD, all of the Reference stations passed the assessment. Farm A failed the assessment on Transect 1 in 2019 and 2020, passing in the other years. Transect 2 failed the assessment in 2018 and 2019, passing in the other years. At Farm B, both Transect 1 and Transect 2 failed in 2021. Transect 1 and 2 both passed in the other years. At Farm C, transect 1 failed in 2017 and 2021, passing in the other years. On Transect 2, 2017, 2018 and 2019 failed the assessment, while 2020 and 2021 passed the assessment.

The results of the Kappa analysis to intercalibrate the Ecological Status (ES) assessments using the IQI, VBH and SI are shown in Table 3.4. When using the five ES classes (Table 3.4a), the agreement between IQI and VBH (0.52) was Moderate. There was null agreement between SI and the other two indices. Table 3.4b shows the agreement between IQI and VBH at the G/M, and aRPD and LOI at the pass/fail criteria for sediment measures. The agreement between IQI and VBH is Low, while SI produced null agreement with both IQI and VBH. IQI had low agreement with aRPD, and null agreement with LOI. VBH had low agreement with aRPD and null agreement

with LOI. SI had null agreement with aRPD and with LOI. There was null agreement between aRPD and LOI.

TABLE 3.3. DISTANCE FROM THE SEA CAGE FROM WHICH STATIONS ACHIEVED GOOD ECOLOGICAL STATUS USING INFAUNAL QUALITY INDEX (IQI), VISUAL BENTHIC HEALTH (VBH)AND SURFACE INDEX (SI), AND PASS/FAIL FOR SEDIMENT ORGANIC CONTENT (LOI) AND APPARENT REDOX POTENTIAL DISCONTINUITY (aRPD) AT SALMON AQUACULTURE SITES A, B AND C IN 2017 – 2021.

IQI A	T1	T2	Ref	IQI B	T1	T2	Ref	IQI C	T1	T2	Ref
G/M -17	FAIL	50	PASS	G/M -17	50	20	PASS	G/M -17	FAIL	FAIL	PASS
G/M -18	20	Edge	PASS	G/M -18	20	Edge	PASS	G/M -18	FAIL	FAIL	PASS
G/M -19	100	50	PASS	G/M -19	50	20	PASS	G/M -19	FAIL	FAIL	PASS
G/M -20				G/M -20	100	50	PASS	G/M -20	10	10	PASS
G/M -21	100	20	PASS	G/M -21	FAIL	20	PASS	G/M -21	Edge	10	PASS
VBH A	T1	T2	Ref	VBH B	T1	T2	Ref	VBH C	T1	T2	Ref
G/M -17	Edge	20	PASS	G/M -17	100	Fail	PASS	G/M -17	FAIL	FAIL	FAIL
G/M -18	50	50	PASS	G/M -18	Fail	Edge	PASS	G/M -18	100	20	PASS
G/M -19	10	Fail	PASS	G/M -19	50	20	PASS	G/M -19	50	50	PASS
G/M -20	Edge	20	PASS	G/M -20	50	10	PASS	G/M -20	50	20	PASS
G/M -21	50	50	PASS	G/M -21	FAIL	50	PASS	G/M -21	FAIL	10	PASS
SI A	T1	T2	Ref	SI B	T1	T2	Ref	SI C	T1	T2	Ref
G/M -17	10	Edge	FAIL	G/M -17	Edge	Edge	PASS	G/M -17	Edge	Edge	PASS
G/M -18	Edge	Edge	PASS	G/M -18	Edge	Edge	FAIL	G/M -18	Edge	Edge	PASS
G/M -19	Edge	Edge	PASS	G/M -19	Edge	Edge	PASS	G/M -19	Edge	Edge	FAIL
G/M -20	Edge	Edge	PASS	G/M -20	FAIL	Edge	PASS	G/M -20	Edge	Edge	FAIL
G/M -21	Edge	Edge	PASS	G/M -21	10	10	PASS	G/M -21	Edge	Edge	PASS
LOI A	T1	T2	Ref	LOI B	T1	T2	Ref	LOI C	T1	T2	Ref
G/M -17	Edge	Edge	Pass	G/M -17	Edge	Edge	Pass	G/M -17	Edge	Edge	Pass
G/M -18	Edge	Edge	Pass	G/M -18	Edge	Edge	Pass	G/M -18	Edge	Edge	Pass
G/M -19	Edge	Edge	Pass	G/M -19	Fail	Edge	Pass	G/M -19	Edge	Edge	Pass
G/M -20	Edge	Edge	Pass	G/M -20	20	Edge	Pass	G/M -20	Edge	Edge	Pass
G/M -21	Edge	Edge	Pass	G/M -21	Edge	Edge	Pass	G/M -21	Edge	Edge	Pass
aRPD A	T1	T2	Ref	aRPD B	T1	T2	Ref	aRPD C	T1	T2	Ref
G/M -17	50	50	Pass	G/M -17	100	50	Pass	G/M -17	Fail	Fail	Pass
G/M -18	100	Fail	Pass	G/M -18	Edge	Edge	Pass	G/M -18	100	Fail	Pass
G/M -19	Fail	Fail	Pass	G/M -19	50	50	Pass	G/M -19	100	Fail	Pass
G/M -20	Fail	50	Pass	G/M -20	50	20	Pass	G/M -20	100	50	Pass
G/M -21	100	50	Pass	G/M -21	Fail	Fail	Pass	G/M -21	Fail	10	Pass

Ref = Reference station

Fail = no station on the transect passed the assessment, or the reference station failed the assessment.

TABLE 3. 4. KAPPA ANALYSIS OF AGREEMENT IN (TABLE 3.4.A.) ECOLOGICAL STATUS (ES) BETWEEN INFAUNAL QUALITY INDEX (IQI), VISUAL BENTHIC HEALTH (VBH), SURFACE INDEX (SI) AT SALMON AQUACULTURE SITES A, B AND C 2017 – 2021 WITH RESPECT TO THE FIVE ES CLASSES AND (TABLE 3.4.B.) PASS/FAIL AT THE GOOD / MODERATE BOUNDARY BETWEEN IQI, VBH, SI, SEDIMENT ORGANIC CARBON (LOI) AND APPARENT REDOX POTENTIAL DISCONTINUITY (ARPD).

(Table 3.4.A.) Five Ecological Status classes

	Kappa	% Mismatch	Agreement
	IQI		
VBH	0.52	22.52	Moderate
SI	0.00	57.62	Null
	VBH		
SI	0.00	65.24	Null

(Table 3.4.B.) Pass/Fail at the Good/Moderate Boundary

	Kappa	% Mismatch	Agreement
	IQI		
VBH	0.36	23.18	Low
SI	-0.03	55.63	Null
aRPD	0.28	33.77	Low
LOI	0.00	64.9	Null
	VBH		
SI	-0.06	57.93	Null
aRPD	0.22	37.80	Low
LOI	-0.02	58.54	Null
	SI		
aRPD	-0.01	56.71	Null
LOI	0.02	21.34	Null
	aRPD		
LOI	0.00	59.76	Null

Results of the mixed model of the IQI EQR using Distance from the sea cage and transect as fixed factors and Survey as a categorical random factor are shown in Table 3.5. The random effect of Survey was statistically significant ($p < 0.05$), accounting for 50.1% of the response variance. Distance and Transect were both highly significant ($p < 0.001$) predictors of IQI. The adjusted r^2 for the model was 0.725. The Constant value for IQI was 0.452. Stations under the cage, at the cage edge and at 10m from the cage are modelled as having lower IQI, while stations at 20m, 50m and 100m are modelled as having higher IQI. Stations on Transect 1 are modelled as having lower IQI than those on Transect 2. The predicted average IQI values and the corresponding Ecological Status (ES) classes are shown in Table 3.6, without the random effect of Survey. On Transect 1, in line with the prevailing current, IQI ES is Bad under the cage, Poor at the cage edge and 10m from the cage, Moderate at 20m and 50m, and Good at 100m from the cage. At Transect 2, perpendicular to the current, IQI ES is Poor under the cage and at the cage edge, Moderate at 10m and at 20m, and Good at 50m.

Table 3.7. shows the outputs of the mixed model of VBH using Distance from the sea cage and transect fixed factors and Survey as a categorical random factor. The random effect of Survey was statistically significant ($p < 0.05$), accounting for 21.9% of the response variance. Distance and Transect were both highly significant ($p < 0.001$) predictors of VBH. The adjusted r^2 for the model was 0.502. Stations from under the cage to 20m from the cage are modelled as having lower VBH, while stations at 50m and 100m are modelled as having higher VBH. Stations on Transect 1 are modelled as having lower VBH than those on Transect 2. Table 3.8. shows the predicted average VBH values and the corresponding Ecological Status (ES) classes, without the random effect of Survey. On Transect 1, in line with the prevailing current, VBH ES is Poor under the cage, at the cage edge and 10m from the cage, Moderate at 20m, and Good at 50m and 100m from the cage. At Transect 2, perpendicular to the current, VBH ES is Moderate from under the cage to 20m, and Good at 50m.

Table 3.9. shows the mixed effects model results using IQI EQR as the response, LOI and aRPD as covariate predictors, and a random categorical effect for Survey. The random effect of Survey was significant ($p < 0.05$), accounting for 27.94% of the response variance. The Adjusted r^2 for the mixed model is 0.535. This model produced a significant effect of aRPD and LOI. The predicted values of IQI increase with increasing aRPD and with decreasing LOI.

TABLE 3.5. MIXED EFFECTS MODEL OF INFAUNAL QUALITY INDEX (IQI) USING SURVEY AS A RANDOM FACTOR AND DISTANCE AND TRANSECT AS FIXED FACTORS, AT SALMON AQUACULTURE SITES A, B AND C, 2017 – 2021.

Marginal Fit Equation

IQI EQR V2 = 0.4521 - 0.2231 Under - 0.1281 Edge - 0.0534 10m+ 0.0315 20m + 0.1503 50m + 0.2228 100m - 0.0517 Transcet_1 + 0.0517 Transcet_2

Variance Components

Source	Var	% of Total	SE Var	Z-Value	P-Value
Survey	0.01443	50.07	0.006193	2.329957	0.010
Error	0.014392	49.93	0.001778	8.092483	0.000
Total	0.028823				

-2 Log likelihood = -142.323901

Tests of Fixed Effects

Term	DF Num	DF Den	F-Value	P-Value
Distance	5	131.03	42.52	0.000
Transect	1	131.01	25.59	0.000

Model Summary

S	R-sq	R-sq(adj)	AICc	BIC
0.119968	0.7361	0.7251	-138.24	-132.38

Coefficients

Term	Coef	SE Coef	DF	T-Value	P-Value
Constant	0.452081	0.033718	13.22	13.40787	0.000
Distance					
0	-0.22308	0.021185	131.00	-10.5298	0.000
30	-0.12812	0.021185	131.00	-6.04757	0.000
40	-0.05341	0.021185	131.00	-2.52118	0.013
50	0.031512	0.021546	131.08	1.462535	0.146
80	0.150312	0.021517	131.01	6.9859	0.000
Transect					
1	-0.05171	0.010222	131.01	-5.05834	0.000

TABLE 3. 6. PREDICTED OUTPUTS FOR THE AVERAGE INFAUNAL QUALITY INDEX (IQI) AND ECOLOGICAL STATUS (ES) IN THE 100M MIXING ZONE AT SALMON AQUACULTURE SITES A, B AND C, 2017 – 2021, FROM THE MIXED MODEL SHOWN IN TABLE 3.5.

	Transect 1		Transect 2	
Distance	**IQI EQR**	**IQI ES**	**IQI EQR**	**IQI ES**
Under	0.177	Bad	0.281	Poor
Edge	0.272	Poor	0.376	Poor
10	0.347	Poor	0.450	Moderate
20	0.432	Moderate	0.535	Moderate
50	0.551	Moderate	0.654	Good
100	0.661	Good		

TABLE 3. 7. MIXED EFFECTS MODEL OF VISUAL BENTHIC HEALTH (VBH) USING SURVEY AS A RANDOM FACTOR AND DISTANCE AND TRANSECT AS FIXED FACTORS AT SALMON AQUACULTURE SITES A, B AND C, 2017 – 2021.

Marginal Fit Equation

VBH EQR = 0.4919 - 0.0871 Under - 0.0744 Edge - 0.0429 10m- 0.0208 20m + 0.0696 50m + 0.1557 100m - 0.02869 Transcet_1 + 0.02869 Transcet_2

Variance Components

Source	Var	% of Total	SE Var	Z-Value	P-Value
Survey	0.002421	21.85	0.001262	1.918074	0.028
Error	0.008656	78.15	0.001069	8.097566	0.000
Total	0.011076				

-2 Log likelihood = -229.559203

Tests of Fixed Effects

Term	DF Num	DF Den	F-Value	P-Value
Distance	5	131.29	18.57	0.000
Transect	1	131.24	13.1	0.000

Model Summary

S	R-sq	R-sq(adj)	AICc	BIC
0.093037	0.522	0.5021	-225.47	-219.62

Coefficients

Term	Coef	SE Coef	DF	T-Value	P-Value
Constant	0.491901	0.015385	13.85	31.97309	0.000
Distance					
0	-0.08714	0.016428	131.22	-5.30422	0.000
30	-0.07438	0.016428	131.22	-4.52781	0.000
40	-0.04292	0.016428	131.22	-2.61267	0.010
50	-0.02079	0.016705	131.43	-1.24442	0.216
80	0.069566	0.016685	131.23	4.169381	0.000
Transect					
1	-0.02869	0.007926	131.24	-3.61927	0.000

TABLE 3. 8. PREDICTED OUTPUTS FOR THE AVERAGE VISUAL BENTHIC HEALTH (VBH) AND ECOLOGICAL STATUS (ES) IN THE 100M MIXING ZONE AT SALMON AQUACULTURE SITES A, B AND C, 2017 – 2021, FROM THE MIXED MODEL SHOWN IN TABLE 3.7.

	Transect 1		Transect 2	
Distance	**VBH EQR**	**VBH ES**	**VBH EQR**	**VBH ES**
Under	0.376	Poor	0.433	Moderate
Edge	0.389	Poor	0.446	Moderate
10	0.420	Poor	0.478	Moderate
20	0.442	Moderate	0.500	Moderate
50	0.533	Good	0.590	Good
100	0.619	Good		

TABLE 3. 9. MIXED EFFECTS MODEL OF INFAUNAL QUALITY INDEX (IQI) USING SURVEY AS A RANDOM FACTOR AND SEDIMENT ORGANIC CONTENT (LOI) AND APPARENT REDOX DISCONTINUITY (ARPD) AS FIXED FACTORS, AT SALMON AQUACULTURE SITES A, B AND C, 2017 – 2021.

Marginal Fit Equation

IQI EQR V2 = 0.4480 + 0.06769 aRPD Depth - 0.01411 LOI %

Variance Components

Source	Var	% of Total	SE Var	Z-Value	P-Value
FarmYear	0.009313	27.94	0.004619	2.01627	0.022
Error	0.024013	72.06	0.002925	8.210458	0.000
Total	0.033326				

-2 Log likelihood = -93.117327

Tests of Fixed Effects

Term	DF Num	DF Den	F-Value	P-Value
aRPD Depth	1	147.91	56.26	0.000
LOI %	1	146.44	5.96	0.016

Model Summary

S	R-sq	R-sq(adj)	AICc	BIC
0.154962	0.5412	0.535	-89.03	-83.12

Coefficients

Term	Coef	SE Coef	DF	T-Value	P-Value
Constant	0.448044	0.054106	84.09	8.280884	0.000
aRPD Depth	0.067693	0.009025	147.91	7.500697	0.000
LOI %	-0.01411	0.005782	146.44	-2.4403	0.016

3.5. General Discussion of the thesis

In this study, the utility of IQI as a monitoring tool for potential salmonid aquaculture impacts in Irish coastal waters is assessed using data collected under a monitoring protocol designed for a different data analysis process (DAFF 2008). The data were analysed to determine if IQI detected the benthic impacts of sea cage aquaculture using the spatial survey design, and if IQI was related to the sediment redox and organic content parameters measured in the expected manner.

IQI assessment of the fourteen surveys at the three farms produced a range of results. Farms A and B failed the assessment in one year each, while Farm C failed the assessment in three years. There was substantial change in the findings of surveys at individual farms over the duration of the study. The farms surveyed were under various levels of impact and surveyed in different seasons and years. Including a random effect of Survey was necessary to delineate the effect of station location relative to the sea cage in this dataset (Zuur et al., 2007). The mixed model results were highly significant, while the random factor for Survey accounted for a large proportion of the variance in the predicted IQI values. The results of the mixed model of IQI in relation to survey sampling design show that IQI is an effective Ecological Quality Ratio (EQR) for monitoring subtidal benthic communities in relation to salmon pen aquaculture. The IQI EQR value consistently decreased with greater proximity to the cages. IQI was also consistently lower at stations in line with the prevailing current compared to stations on a transect perpendicular to the prevailing current.

IQI has been successfully employed in a similar survey design for salmon pen aquaculture monitoring in Scotland (SEPA, 2019). In those studies, IQI provided a robust measure of benthic ES to support management decisions in the licensing of aquaculture installations. Bloodworth et al., (2019) used IQI to inform an assessment of the effects of sea lice therapeutant emamectin benzoate at low concentrations on benthic communities adjacent to salmon aquaculture installations. Fox et al., (2022) used IQI as a measure of benthic ES to assess the efficacy of models of the dispersal of solid farm waste under various current regimes and hydrographic settings. SEPA, (2019) provides an overview of the use of IQI to create a calculated ellipse of the area of the benthic community negatively affected by a salmon cage installation for licensing and monitoring purposes.

The relationship between IQI, aRPD and LOI determined by the mixed model was as expected: IQI increased with increasing aRPD depth and decreased with increasing LOI. There was a significant effect of Survey, which accounted for 27.9% of the variance in the predicted IQI values.

There were highly significant effects of aRPD and LOI, but the adjusted r^2 was moderate at 0.535. The relationship between organic carbon addition to sediments and macrobenthic succession has proven difficult to model in relation to aquaculture impacts (Fox et al., 2022) and may vary based on case-specific factors. Environmental factors may include the current regime (Cromey et al., 2002; Findlay et al., 1995; Keeley et al., 2013), exposure (Macleod et al., (2014)and seabed type (Kalantzi & Karakassis, 2006). The nature of the benthic community in the receiving area and the surrounding seafloor is linked to these factors. Seasonality in the community is also linked to environmental factors and may affect the assimilative capacity of the benthic communities (Brown et al., 1987; Keeley et al., 2013). Farm management factors affecting benthic communities may include the species being cultured (Nickell et al., 2009), the feeding regimen (Cromey et al., 2002) and the fallowing cycle (Macleod et al., 2006; Macleod et al., 2007). Data for many of these factors are not available for this study. Notably, aRPD and sediment grain size were derived from SPI analysis rather than sediment samples taken concurrently with the macrofaunal samples. The SPI-derived aRPD measures were not taken from the same sampling occupancies as the macrofaunal and LOI samples, which were taken from grab samples at the same time. The relationship may be improved by measuring RPD directly from sediment cores or SPI taken at anchor while grab sampling at aquaculture sites or by taking multiple replicate samples for macrofauna, LOI and RPD. The varying nature of the environmental gradients in the various surveys included in this study may partially explain the significant influence of Survey on the relationship. SPI tends to produce more similar patterns to macrofaunal data in strong gradients than in weak gradients (Rumohr & Karakassis, 1999).

IQI mapped the spatial footprint of the aquaculture impact effectively in each survey and was effective at delineating change in seafloor communities on a spatial scale appropriate for monitoring purposes at aquaculture sites. Adopting the IQI for aquaculture monitoring has the potential to develop a cohesive monitoring program for WFD and Natura 2000 water bodies. Aquaculture impacts in Natura 2000 sites require Appropriate Assessment (European Commission, 2019). Aligning aquaculture monitoring with the regulatory monitoring carried out under the Water Framework Directive and Habitats Directive (Council Directive 92/43/EEC) can potentially optimise monitoring and licensing in coastal waters. Any potential impacts can be assessed using broad-scale monitoring of similar waterbodies, which may prove particularly useful in the context of climate change. This study is the first account of the use of the Infaunal Quality Index (IQI) in monitoring salmon cage aquaculture effects in Irish waters. In previous studies in Ireland, IQI has been effective in delineating aquaculture effects caused by intertidal oyster

aquaculture (Forde et al., 2012) and the interaction between oyster aquaculture impacts and storm disturbance (O'Carroll et al., 2017). (Walls et al., 2017) applied IQI in a before after control impact survey to monitor floating algal aquaculture impacts on the subtidal benthos. Kennedy et al., (2011) found that IQI was significantly related to storm disturbance in the shallow subtidal benthic communities of transitional and coastal waters in Kinsale Harbour, Ireland, in a 26-year time series study.

For VBH the mixed model results in relation to survey sampling design were highly significant, while the random factor for Survey accounted for a small proportion of the variance in the predicted VBH values. The adjusted r2 of the model (0.502) is smaller than that of the corresponding model using IQI as the response. VBH did show potential as an EQR for monitoring subtidal benthic communities in relation to salmon pen aquaculture. The VBH values decreased with greater proximity to the cages and was lower at stations in line with the prevailing current compared to stations on a transect perpendicular to the prevailing current.

The VBH produced a Kappa agreement to IQI that was Moderate for the five ES classes and Low with respect to the G/M. VBH classified some Reference stations as below the G/M, while IQI classified all reference stations above the G/M. When compared to IQI, the VBH mismatches on the farm transects did not display a consistent spatial pattern. In some cases, VBH identified the G/M as nearer to the salmon cage and, in some cases, further away. When compared to Pass/Fail criteria for LOI and aRPD, both IQI and VBH had Low agreement with aRPD, and both had Null agreement with LOI. The low level of agreement between IQI and VBH may be associated with the varying strengths of the disturbance gradients on each survey (Rumohr & Karakassis, 1999). It may also be partly caused by the SPI and grab surveys not sampling precisely the same conditions on the seafloor. Greater replication of sampling using both techniques and deploying both samplers concurrently at a fixed station may improve the agreement between IQI and VBH in future surveys (O'Reilly et al., 2006)

SPI has been employed extensively to rapidly assess changing benthic community structure in relation to aquaculture (Karakassis et al., 2002; Mulsow et al., 2006; O'Connor et al., 1989; Simone & Grant, 2020). These studies have generally concluded that SPI provides a useful rapid assessment approach to monitoring aquaculture impacts that may support or replace monitoring using benthic macrofauna. This is particularly the case where the underlying deposit is homogeneous soft sediment, and the potential for spatial dispersal of farm waste by currents is relatively low (Karakassis et al., 2002). In higher energy environments or locations where the substrate is variable, there is more potential for the dispersal of farm waste (Fox et al., 2022), and

the background sediment and macrofaunal faunal community distribution may be more variable. Temporal change in macrofaunal multivariate community structure concerning aquaculture disturbance is less correlated with SPI monitoring than spatial change (Karakassis et al., 2002; Rumohr & Karakassis, 1999). IQI has been developed to produce a response (EQR value) that is directly comparable between locations with differing sedimentary and hydrographic conditions and can be used to monitor for temporal change in Ecological Status (ES). SPI may provide useful supporting information at lower costs and on timescales far shorter than macrobenthic survey (Karakassis et al., 2002), but IQI is likely to be a more robust measure than VBH in assessing aquaculture impacts on broader spatial scales and in time series analyses.

The SI is a subset of VBH (without the sediment profile image) that can be used to determine ES. In this study, SI showed no Kappa agreement with the other EQRs. Likely, the single surface image taken at each station was insufficient to characterise ES (Simone & Grant, 2020). Greater replication may improve the utility of SI for monitoring aquaculture impacts. It holds potential for rapid, low-cost assessment of ES when targeted sampling is called for, or when impacts are likely to be minimal. If SI is employed in future assessments, it should be derived from georeferenced video of sufficient resolution to identify and enumerate individual organisms, tubes etc. (DAFF, 2008), collected using a sampler design that incorporates a means of calibrating the size of objects in the imagers collected (O'Carroll et al., 2017).

3.6. Conclusions

IQI proved effective in mapping aquaculture impacts on subtidal soft sediments in relation to aquaculture on the west coast of Ireland. The index delineated the spatial footprint of the aquaculture impact in various settings, responding to differing levels of impact. It detected changes in Ecological Status on spatial scales (10 to 100m from the cages) appropriate for monitoring aquaculture impacts. IQI responded to increases in sediment organic carbon and changes in the sediment redox depth by decreasing in value in response to increasing organic enrichment. This relationship was consistent across the dataset but was improved when each survey was considered separately, highlighting the importance of local conditions in determining the extent of the impacts of aquaculture. SPI analysis using the VBH index, shows potential for a rapid assessment tool to support the use of IQI in aquaculture monitoring.

4. General Discussion

Water Framework Directive monitoring aims to assess Ecological Status (ES) across multiple Biological Quality Elements (BQE) that pertain to different components of aquatic ecosystems (Devlin et al., 2007). Competent authorities must assess the nature and setting of particular waterbodies and choose the most appropriate BQE to assess ES in the way most relevant to potential anthropogenic impacts and the sensitivities of the waterbody (WFD, 2000). In many cases, the benthic BQE is chosen to monitor potential impacts because benthic communities integrate ecological quality into the multivariate distribution of species in coastal and estuarine sediments (Pearson, 1978). A broad range of species have known or agreed sensitivities to anthropogenic disturbance, often agreed on using a combination of published literature and expert judgement as is the case with M-AMBI (Borja et al., 2008) and IQI (Phillips et al., 2014) Deriving an appropriate Ecological Quality Ratio (EQR) for a particular region requires that the local level of variability is considered in setting appropriate reference conditions (Muxika et al., 2007) for the receiving environment. The complexity of processing data to produce component index scores for each index within an EQR, and adjusting these indices within appropriate reference conditions, usually requires that software tools (Borja et al. 2008; Philips et al 2104) are deployed to all users of a macrobenthic EQR to ensure consistency between operators and between the results obtained for different Assessment Groups (AG). An update to the IQI tool requires assessment of the outputs of the IQI V1 and IQI V2 tools to ensure that the results are comparable, particularly in terms of the ES classes assigned to AG.

Weighted Kappa analysis (Cohen, 1960; Fleiss & Cohen, 1973) is the method most frequently used to intercalibrate EQR in the literature. It has been used successfully to intercalibrate EQR in a diverse range of BQE that assessed the subtidal sediment benthos (Simboura & Zenetos, 2002), intertidal rocky shore (Guinda et al., 2008), and fish in transitional waters (Lepage et al., 2016). Kappa analysis may be used to intercalibrate EQR that addresses any (BQE). It was used to secure the approval of the European Commission (European-Commission, 2008) for the intercalibration of the benthic BQE in the NEAGIG. This study has used Kappa analysis to intercalibrate the two versions of the IQI tool that have been used to assess ES for the purposes of WFD classification using the method originally applied to intercalibrate IQI with the other EQR in the NEAGIG (Borja et al 2007, (European-Commission, 2008).

When all of the WFD monitoring sample stations were included, the Kappa findings at sampling station level (with station identity incorporated) classified agreement as Very Good for all the stations and for the coastal stations. The agreement between the polyhaline stations was classified

as Almost Perfect. The agreement standard applied by the NEAGIG intercalibration group for satisfactory agreement was a classification of Almost Perfect (Borja et al 2007, (European-Commission, 2008). At the station level, using all of the stations sampled, the agreement between IQI V1 and IQI V2 is not sufficient to consider the outputs directly comparable.

This study is the only account of intercalibration of WFD monitoring that includes Kappa analysis at the AG level. The agreement was classified as Very Good for all AG, and also for the coastal and polyhaline AG, considered separately. For the purposes of this study, where two versions of the same EQR are being assessed, the same requirement for Almost Perfect agreement between IQI V1 and IQI V2 is assumed to be required. The results of the two versions of the IQI tool are not directly comparable. This study is also the only account of intercalibration of WFD monitoring that includes Kappa analysis at the agreement level for Confidence of classification (CofC). In this case, the agreement for all of the AG and for polyhaline AG was Very Good, while agreement for coastal AG was Almost Perfect. The outputs of the two tools are not directly comparable. Overall, the Kappa analyses at the sample station, AG and probability levels do not return sufficient agreement classifications to meet the criteria for successful intercalibration (European-Commission, 2008; Phillips et al., 2014) This points to there being substantial and significant differences in ES classification between the two IQI tools when applied to the entire dataset.

The most apparent difference in the outputs of the V1 and V2 tools is in the percentage of taxa that were not assigned to an AMBI Ecological Group (EG). All of the taxa information input to the IQI v1 tool was recognised by the taxa library. For IQI V2, which received the taxa information in an updated format, more in line with current classification on the WORMS website, >2000 sampling stations had >10% of taxa unassigned to an Ecological Group. The Philips et al (2014) description of the development and use of the IQI V1 tool. While the updated IQI V2 workbook does contain sufficient information to use the IQI V2 tool, Philips et al (2014) have not been updated. There is no guidance on how the faunal data should be processed in terms of, for example, what level of unassigned taxa represents a sample that should be removed from the assessment matrix. When the guidance for IQI V1 was applied to the IQI V2 dataset, approximately 46% of the sampling stations were removed from the dataset. The agreement between the tools improved markedly, with all stations and polyhaline stations achieving Almost Perfect agreement. Coastal stations were classified as having Very Good agreement, but the Kappa value bordered the level necessary to achieve Almost Perfect agreement.

While it is encouraging that such a high level of agreement can be achieved when the stations with a large fraction of unassigned taxa are removed, this is a problematic step in assessing long-

term monitoring data at active WFD sites. Removing 46% of the sample stations is likely to greatly effect the ES classifications applied to the AG. In any case, most of the survey data have already been used to assess AG and report on AG ES to the European Union, as per the requirements of the WFD. Matching to a greatly reduced subset of the data is not likely to be a useful management tool for competent authorities. An updated version of the Philips et al (2014) guidance on the use of IQI V2 is necessary to place this issue in context and advise on the appropriate course of action.

At the time of writing, the recommendation from this study is that any data collected at AG using IQI V2 should be considered in the context of previous data from that waterbody that has been analysed using IQI V2 for the purposes of determining a significant change in ES class within the AG. The outputs of the two tools cannot be viewed as equivalent at this point. IQI V2 has been adopted by the relevant competent authorities as the current tool for determining ES in WFD waterbodies in Ireland and the UK.

This study is the first account of the use of the Infaunal Quality Index (IQI) in monitoring salmon cage aquaculture effects in Irish waters. IQI V2 proved to be very effective in monitoring salmon aquaculture impacts in Irish coastal waters using the spatial survey approach of DAFF (2008). Using the mixed model approach with a random factor for Survey showed that the IQI EQR decreased at stations that were near to the farm cages and increased with increasing distance from the farm cages. IQI was also significantly lower at stations that were positioned on the transect that was in line with the prevailing current than on stations that were at the same distance from the cage on the perpendicular transect. This is despite the historic data collected from these farm sites not being collected in accordance with the recommendations of Philips et al (2014). Notably, the data were a mix of diver cores, and van Veen grabs rather than the standard $0.1m^2$ Day grabs recommended for WFD monitoring. IQI analyses also produced this consistent spatial pattern despite the 14 farm surveys sampling sites that were under various levels of impact in different seasons of different years. IQI mapped the spatial footprint of the aquaculture impact effectively in each survey and was effective at delineating change in seafloor communities on a spatial scale appropriate for monitoring purposes at aquaculture sites. IQI has been successfully employed in a similar survey design for salmon pen aquaculture monitoring in Scotland (Bloodworth et al., 2019; Fox et al., 2022; SEPA, 2019). IQI provided a robust measure of benthic ES to support management decisions in the licensing of aquaculture installations in Scottish waters.

The utility of IQI in monitoring aquaculture impacts points to the potential to develop a single unified assessment regime for benthic habitats for the monitoring requirements of all monitoring

programs. For example, aquaculture impacts in Natura 2000 sites require Appropriate Assessment (European- & Commission, 2019). Developing a single assessment regime for benthic habitats for aquaculture monitoring, and for monitoring for the purposes of the WFD and the Habitats Directive (Council Directive 92/43/EEC) would strengthen the power of both monitoring programs to detect anthropogenic impacts, and to ascertain the scale of natural variability in similar habitats, improving the efficacy of both programs.

IQI V2 has great potential to improve benthic monitoring in Irish coastal and transitional waters by updating the taxa library to make it compliant with the format of WORMS-compliant data as currently produced by survey work. Mismatch issues with legacy IQI V1 data need to be resolved by the production of guidance by the tool developers.

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Appendix

Data Availability:

Supplementary data and tables associated with this thesis are available upon reasonable request.

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