UKTAG Transitional and Coastal Water Assessment Method

Benthic Invertebrate Fauna

Infaunal Quality Index

by

Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG)

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Health and safety statement

WARNING. Working in or around water is inherently dangerous; persons using this standard should be familiar with normal laboratory and field practice. This published monitoring system does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate health and safety practices and to ensure compliance with any national regulatory guidelines.

It is also the responsibility of the user if seeking to practise the method outlined here, to gain appropriate permissions for access to water courses and their biological sampling.

UKTAG Guide to the Infaunal Quality Index
Water Framework Directive: Transitional and Coastal Waters

Purpose of document: To provide an overview of the Infaunal Quality Index (IQI) (version IV) to inform Practitioners of how to monitor, assess and classify suitable benthic invertebrate data according to Water Framework Directive (WFD) requirements in transitional and coastal waters.

Note: this document does not describe all aspects of the IQI development and its application; for this please refer to the full technical report (Phillips *et al*., 2014). A summary of key documents and references is provided within this document.

Introduction to WFD Terminology and Assessment: This guide describes a system for classifying in accordance with the requirements of Article 8; Section 1.3 of Annex II and Annex V of the WFD (2000/60/EC). Practitioners should recognise that the terminology used in this document is specific to the WFD and has a meaning defined by the directive.

To carry out a WFD biological assessment, each biological quality element (BQE, defined in the WFD) is required to give a statistically robust definition of the 'health' of that element in the sampled water body. The 'health' of a BQE is assessed by comparing the measured conditions (observed value) against that described for reference conditions (minimally disturbed). This is reported as an Ecological Quality Ratio (EQR). An EQR with a value of one represents reference conditions and a value of zero represents a severe impact. The EQR is divided into five ecological status classes (High, Good, Moderate, Poor and Bad) that are defined by the changes in the biological community in response to disturbance (Figure 1).

Alongside the EQR score and class status, any assessment must consider the certainty of the assessment (i.e. confidence in the assigned class).

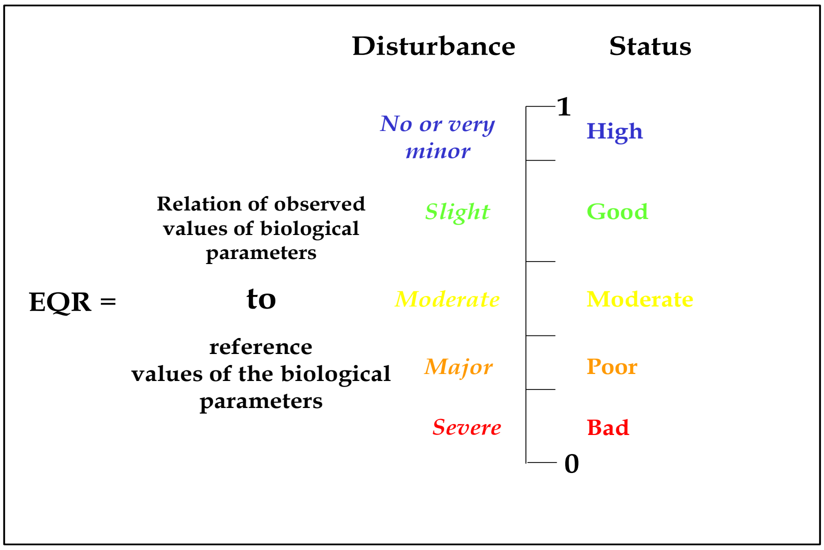

Figure 1: Illustration of the Ecological Quality Ratio and how it relates to the level of disturbance and ecological status during a classification. The class band widths relate to biological changes as a result of disturbance (WFD CIS Guidance Document No. 5, 2003).

1. Key Facts

1.1 Tool Overview: Infaunal Quality Index

The IQI enables an assessment of the ecological health of the biological quality element, "benthic invertebrate fauna" as listed in Tables 1.2.3 and 1.2.4 of Annex V to the WFD (2000/60/EC). The WFD requires that the assessment of the benthic invertebrate quality element considers abundance, diversity and the presence and/or absence of pollution-tolerant and disturbance-sensitive taxa.

The IQI is a multimetric index composed of three individual components known as metrics, these are the:

(i) AZTI Marine Biotic Index (AMBI)
(ii) Simpson's Evenness (1-λ')
(iii) number of taxa (S).

The individual metrics have been weighted and combined within the IQI in order to best describe the changes in the benthic invertebrate community in response to anthropogenic pressures. Each individual metric is normalised to a reference value, which is the expected value for that metric in the habitat type that is being assessed when there is minimal or no disturbance due to human activities. The $IQI_{v.\ IV}$ is the multimetric used for the WFD assessment of benthic invertebrates (and was used in the first River Basin Management Plans in 2009). The boundaries were optimised against methods used by Member States of the North East Atlantic Geographical Intercalibration Group.

The $IQI_{v.IV}$ is formulated as follows:

$$IQI_{v.IV} = \left(\left(\left(0.38 \times \left(\frac{1-(AMBI/7)}{1-(AMBI_{\text{Ref}}/7)} \right) \right) + \left(0.08 \times \left(\frac{1-\lambda'}{1-\lambda'_{\text{Ref}}} \right) \right) + \left(0.54 \times \left(\frac{S}{S_{\text{Ref}}} \right)^{0.1} \right) - 0.4 \right) \middle/ 0.6 \right)$$

The IQI operates over a range from 0 (a severe impact) to 1 (reference/minimally disturbed). The four class boundaries are:

- High/Good = 0.75
- Good/Moderate = 0.64
- Moderate/Poor = 0.44
- Poor/Bad = 0.24.

To calculate the IQI the following information is required:

(i) Abundance of benthic invertebrates (identified to lowest taxonomic level)
(ii) Characterisation of the habitat sampled (salinity and substratum)
(iii) Sampling methodology (e.g. sample method area and gear used)
(iv) Processing methodology (e.g. sieve mesh).

Reference condition metrics are derived for the specific habitat and sample method for calculation of the EQR.

1.2 Applicability

The IQI is applied at the sample level. Samples can be combined for assessments at different spatial scales depending on the aims of the survey. For WFD reporting the IQI is applied at the water body scale.

Where: The IQI can be applied to all UK coastal and transitional waters with soft sediment habitats. However, the IQI is not currently used for assessing saline lagoons due to the particular challenges in setting suitable reference conditions for these water bodies.

Note: the tool is habitat-specific and may not be suitable to assess the status of benthic invertebrates in all habitats in a water body (see Phillips *et al*., 2014). Reference conditions are estimated from existing data, and whilst no formal limit exists regarding the salinity and sediment characteristics that are currently suitable, the IQI workbook (see Section 1.3) highlights occurrences of low reliability in reference conditions where data falls outside the range of the majority of the data used for estimating the reference condition values.

When: The IQI has been developed to classify data from a single sampling event. Classification is possible using data collected throughout the year, however, the potential impact of seasonal bias on the classification must be considered if data are collected outside of the currently recommended sampling period (February to June, inclusive).

Response to pressure: The IQI has been shown to detect the impact of various pressures on benthic invertebrate assemblages. These pressures are hazardous substances, organic enrichment and general disturbance (e.g. smothering). For other pressures e.g. physical disturbance from fishing, the ability of the IQI to detect the response of benthic invertebrates is un-quantified so the IQI scores should be interpreted with caution.

1.3. Key Documents

The documents marked * will be hosted on the UK technical advisory group (UKTAG) website www.wfduk.org.

*Phillips, G. R, Miles, A. C., Prior, A., Martina, L. J., Brooks, L., & Anwar, A., (2014). Infaunal Quality Index: WFD classification scheme for marine benthic invertebrates. Environment Agency (UK), R&D Technical Report. – Research and Development report that documents the technical development of the IQI$_{\text{v IV}}$. *Includes (i) data treatment protocols (ii) classification tool development (iii) setting reference conditions (iv)setting ecological status boundaries (v) estimating ecological quality ratio variability (vi) estimating the risk of misclassification (vii) power analysis*

Prior, A., Miles, A. C., Sparrow, A. J., & Price, N. (2004). Development of a classification scheme for the marine benthic invertebrate component, Water Framework Directive. Phases I & II – Transitional and coastal waters. Environment Agency (UK), R&D Interim Technical Report, E1-116, E1-132: 103 pp (+ appendix). – *Technical Research and development document outlining the early phases of development of the IQI*

*UKTAG Biological Status Methods: Coastal & Transitional Waters Benthic Invertebrate fauna – *High level non-technical summary*

*WFD Infaunal Quality Index workbook – *Excel standalone workbook that can be used for the calculation of the IQI.*

2. Background

2.1 Ecological principles

To establish a WFD compliant classification tool that is an indicator of disturbance, suitable metrics (a metric is a measure of the biota that changes in some predictable way with increased human influence) relating to the structure and functioning of benthic assemblages were combined to establish a single index, the IQI. The IQI classifies the ecological health of a benthic invertebrate assemblage according to the extent to which chosen measures of ecological health have departed from their expected state under minimal levels of anthropogenic disturbance (or reference conditions).

The biological metrics incorporated into the IQI are; number of taxa, the AZTI Marine Biotic Index (AMBI) and Simpson's evenness ($1-\lambda'$). The use of these three metrics in combination enables the IQI to reflect changes to multiple indicators of ecological health as described within the normative definitions of the WFD.

2.2 Normative definitions

In Annex V (1.2) of the WFD, normative definitions describe the aspects of the benthic invertebrate community that must be included in the ecological status assessment of a water body; these are:

- diversity
- abundance
- disturbance sensitive taxa
- taxa indicative of pollution.

To facilitate the development of a suitable assessment method the WFD normative definitions were interpreted into expanded normative definition (Table 1).

Table 1: Description of the characteristics of benthic invertebrate macrofaunal assemblages at each WFD status class in accordance with the normative definitions (WFD Annex V) and expanded normative definitions (detailed national interpretation).

	High	Good	Moderate	Poor	Bad
Normative definitions	The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All the disturbance-sensitive taxa associated with undisturbed conditions are present.	The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific communities are present.	The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with the type-specific conditions. Taxa indicative of pollution are present. Many of the sensitive taxa of the type-specific communities are absent.	Waters showing evidence of major alterations to the values of the biological quality elements for the surface water body type and in which the relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions, shall be classified as poor.	Waters showing evidence of severe alterations to the values of the biological quality elements for the surface water body type and in which large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent, shall be classified as bad.
Expanded normative definitions	Species richness and diversity high. Evenness high. Abundance ratio (Abundance/ No. of taxa) low. Taxonomic range high. Community Abundances (assessed by AMBI) – normal, unpolluted: Sensitive Taxa (EG I) of dominant abundance. Indifferent and Tolerant Taxa (EG II & III) absent or have sub-dominant abundance. Opportunistic Taxa (EG IV) absent or have negligible abundance. Indicator Taxa (EG V) absent or have negligible abundance.	Species richness and diversity slightly reduced. Evenness slightly reduced. Abundance ratio slightly elevated. Taxonomic range slightly reduced. Community Abundances (assessed by AMBI) – slightly unbalanced, slightly polluted: Sensitive Taxa (EG I) abundance may range from high sub-dominant to absent. Indifferent Taxa (EG II) have low, sub-dominant abundance. Tolerant Taxa (EG III) have dominant abundance. Opportunistic Taxa (EG IV) & Indicator Taxa (EG V) abundance may range from negligible or low to equi-abundance with Indifferent Taxa.	Species richness and diversity moderately reduced. Evenness moderately reduced. Abundance ratio moderately elevated. Taxonomic range moderately reduced. Community Abundances (assessed by AMBI) – transitional unbalanced to moderately polluted: Sensitive Taxa (EG I) have negligible abundance or absent. Indifferent Taxa (EG II) have low sub-dominant abundance. Tolerant Taxa (EG III), Opportunistic Taxa (EG IV) & Indicator Taxa (EG V) co-dominate the abundance.	Species richness and diversity shows major reduction. Evenness shows major reduction. Abundance ratio shows major elevation. Taxonomic range shows major reduction. Community Abundances (assessed by AMBI) – transitional moderately to heavily polluted: Sensitive and Indifferent Taxa (EGI & II) have negligible abundance or absent. Tolerant Taxa (EG III) have sub-dominant abundance. Opportunistic Taxa (EG IV) & Indicator Taxa (EG V) co-dominate the abundance.	Species richness and diversity shows severe reduction. Evenness shows severe reduction. Abundance ratio shows severe elevation. Taxonomic range severely reduced. Community Abundances (assessed by AMBI) – very heavily or extremely polluted: Azoic or if fauna present: Sensitive, Indifferent, & Tolerant Taxa (EG I, II, & III) absent. Opportunistic Taxa (EG IV) have sub-dominant abundance. Indicator Taxa (EG V) have dominant abundance.

2.3 Development of the Index

The IQI combines suitable metrics to establish a single index describing the ecological status of the benthic invertebrate community. Benthic invertebrate abundance datasets with quantifiable pressure data (organic enrichment gradient from a sewage sludge disposal site and the effects of physical smothering from a mine waste disposal ground with associated hazardous substances), were used to formulate the IQI (Prior *et al.*, 2004).

Changes in abundance of different taxa were demonstrated to correlate to changes in the levels of anthropogenic pressure/s (measured contaminants). A subset of contaminants were then selected that held the strongest correlation to the overall variance in the biota. The first principal component of the contaminant data (PC1) was used to test against the biological data. A range of metrics, compliant with the normative definitions, that could be used to describe benthic invertebrate communities in terms of abundance, diversity and disturbance sensitive taxa, were then calculated and analysed to show correlations to the contaminants within the pressure gradient data. Metrics were also investigated to check that they display monotonic (linear/curvilinear) relationship with pressure gradients.

A sub-set of metrics were selected that were demonstrated to encompass the majority of the variability within the full range of metrics considered through the UK Clean Seas Environmental Monitoring Programme (CSEMP) data. The metrics selected were number of taxa, AMBI (Borja *et al.*, 2000) and Simpson's evenness (Simpson, 1949).

The selected metrics were weighted in the IQI, using regression analysis to derive the linear combination that provided the closest correlation to the quantitative contaminant data from the pressure gradients. The linear combination of taxa number, AMBI and Simpsons eveness from the two regression analyses were normalised to operate between 0 and 1. The resulting index was then tested against the range of quantified disturbance gradients.

Details describing the full development process of the $IQI_{v.IV}$ can be found in Phillips *et al*. (2014).

Understanding the individual metrics within the IQI:

AZTI Marine Biotic Index (AMBI) (WFD criteria compliance – presence/absence of pollution-tolerant/ disturbance-sensitive taxa, abundance)

The AMBI (Borja *et al.,* 2000) describes the sensitivity of a macrobenthic assemblage to pressure in terms of its weighted average of five ecological sensitivity groups, and has been demonstrated to respond to pressures, both anthropogenic and natural in origin. The five ecological groups are related to the sensitivity or tolerance of taxa to environmental disturbance (AMBI was initially developed against a gradient of organic enrichment) (Figure 2).

Within the AMBI, each taxa within a sample is assigned an ecological group (EG) from EG I to EG V, EG I being taxa sensitive to organic enrichment and EG V being first order opportunistic taxa. Intermediate to these two extremes are EG II, EG III and EG IV, which describe indifferent, tolerant and second order opportunistic taxa, respectively. The proportions of taxa in each these groups is used to calculate the AMBI biotic coefficient (BC) for a sample:

$$AMBI = \{(0x\%EG\ I) + (1.5x\%EG\ II) + (3x\%EG\ III) + (4.5x\%EG\ IV) + (6x\%EG\ V)\}/100$$

The AMBI BC operates between 0 (high status) and 6 (bad status) with azoic conditions being allocated a seven (Figure 2).

Within the IQI, the AMBI BC has been normalised and inverted to give a value between 0 and 1 (to align with the WFD EQR scale) where 0 indicates the extreme end of bad status (azoic conditions) and 1 indicates the top end of high status (1 – (AMBI BC/7)).
The master list of taxa and their assigned ecological group is maintained by the AZTI group (Basque Region, Spain); the list is available through the AZTI web site (www.azti.es).

The AMBI is currently considered as a component of the benthic invertebrate assessment by several Member States within the North East Atlantic.

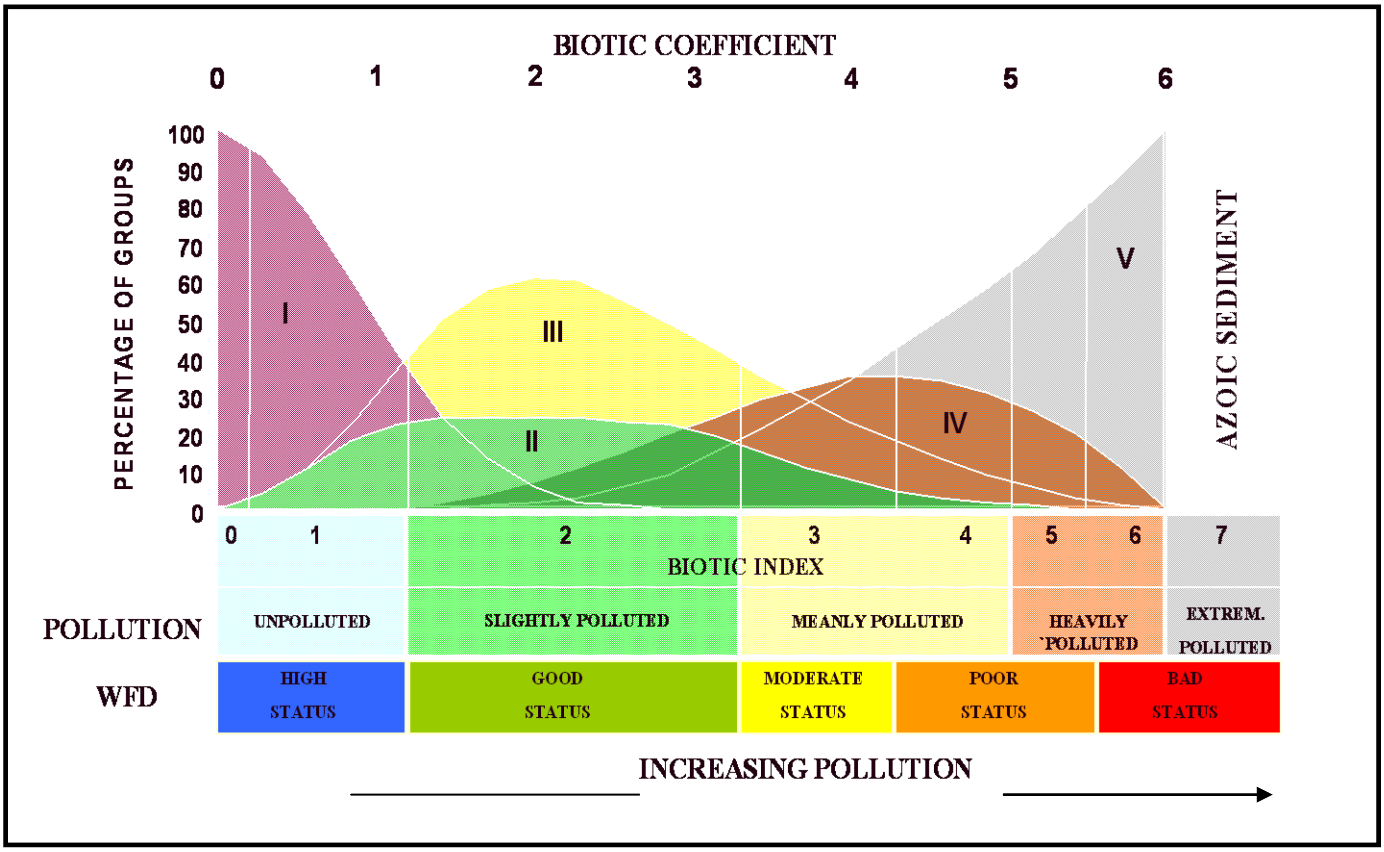

Figure 2: The AMBI biotic coefficient, relating the ecological groups present in a sample to an assessment of the benthic invertebrate community (Borja *et al.*, 2000).

Simpson's Evenness (1-λ') (WFD criteria compliance **–** diversity, abundance)

Simpson's formula gives an indication of diversity based on how evenly spread the taxa are throughout a sample. The form of Simpsons chosen was 1-λ' which has a range between 0 (assemblage dominated by a single taxa) and 1 (abundance distributed evenly across all taxa).

Number of taxa (S) (WFD criteria compliance – diversity)

The number of taxa provides a representation of the structural diversity of a sample. Generally, a taxa number of 0 (azoic) is indicative of severely impacted conditions, with increasing numbers generally corresponding to improving ecological conditions.

2.4 Reference conditions

The use of appropriate reference conditions is essential for a meaningful WFD assessment of the benthic invertebrate community. The metrics within the IQI, used to describe the structure and function of macrobenthic assemblages, are influenced by a multitude of factors that are not directly associated with anthropogenic pressure. Factors which can bias the metrics include; i) true differences in the data (i.e. changes to the assemblages due to changing environmental conditions such as salinity) and ii) differences in how the assemblage data is collected (i.e. changes attributed to differences in sample collection and processing methods). Corresponding reference condition values are similarly influenced. The metric reference condition values for the IQI therefore had to be adapted to ensure that bias to the metrics resulting from changes in habitat (i.e. natural pressures) and measurement methods is not misinterpreted as an anthropogenic disturbance. For example, comparing a low salinity biological community (observed value) found in the upper regions of a transitional water body to a fully marine community (reference value) will return a false indication of a high level of disturbance (Figure 3).

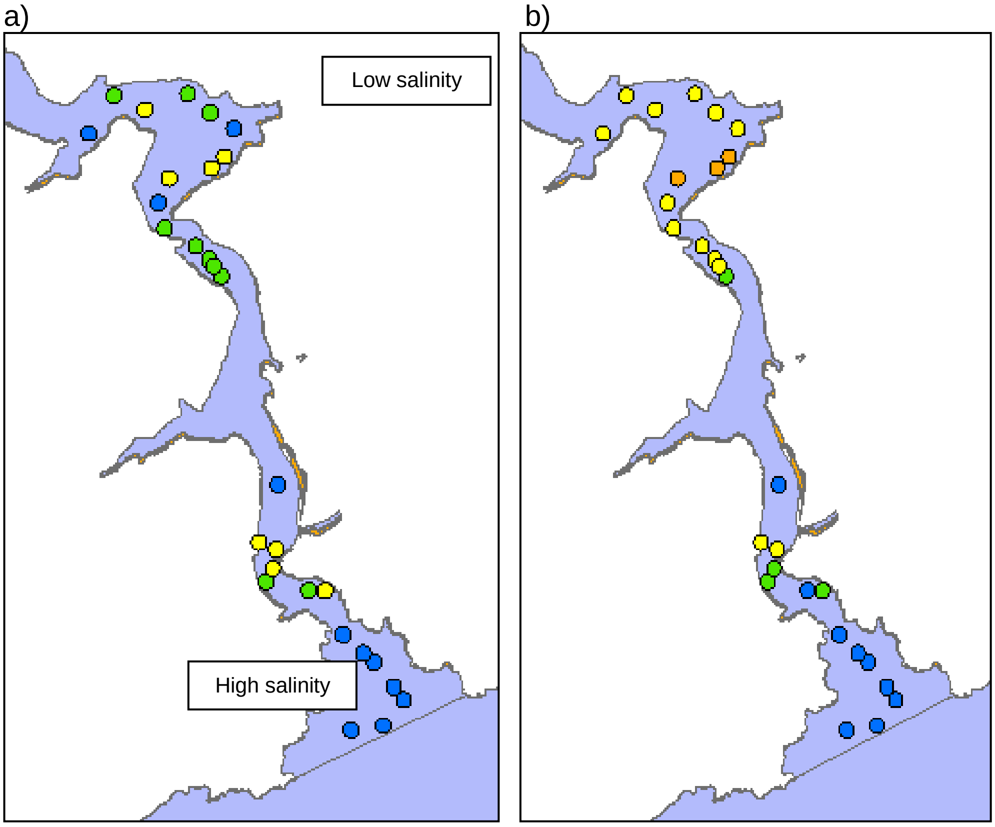

Figure 3: Illustration of the effect of natural salinity stress on ecological status of benthic invertebrate samples within a transitional water as derived by the IQI where (a) reference conditions take account of the salinity influence on the community and (b) the influence of salinity is not taken into account. (Blue = High status, Green = Good, Yellow = Moderate, Orange = Poor).

Appropriate reference conditions need to be defined according to the specific habitat associated with each biological sample, rather than at a water body level. WFD competent authorities currently use a combination of methods (data from existing undisturbed sites or sites with minor disturbance, combined with expert judgement, and models to accommodate changes in habitat) to derive suitable reference conditions based on physiochemical conditions and sampling methodologies. For the first River Basin Management Plans, reference conditions were set specifically for fully marine, subtidal samples from a sand/mud habitat and processed using 1 mm mesh. This was done using expert judgement following the evaluation of existing UK and ROI data from a range of sites exposed to low levels of anthropogenic pressure. Developments since then have used the relationship between the IQI metrics and salinity, sediment measurements (particle size analysis) and sample methods to expand reference conditions to cover a wider range of habitats and sampling and processing methods. For further details on setting reference conditions please see Phillips *et al*. (2014).

IMPORTANT: Modelled reference (minimally disturbed) conditions are only as reliable as the data that they utilise. Where little quantifiable data exists for an expected community from a particular habitat, increased reliance on expert judgement will be required to interpret the derived ecological status.

2.5 Class boundaries

Class boundaries were initially developed through a UK process; defined using the changes in proportions of functional sensitivity groups of the benthic invertebrate communities over a quantifiable gradient of organic enrichment (Phillips *et al*., 2014). These were then modified to maximise the overall agreement to the status classes as derived using methods adopted by the Member States of the North East Atlantic Geographical Intercalibration Group (NEAGIG) (Phillips *et al*., 2014; Borja *et al*., 2007).

Deviation from reference condition for each WFD ecological status class was established by comparing the proportions of the taxa in the different ecological sensitivity groups, as defined by the AMBI, with the expected proportions of the groups found in each class as defined within the expanded normative definitions (see Table 1). During the boundary optimisation process, a common data set of benthic invertebrate data was collated using data from NE Atlantic Member States. Member State classification tools were used to assess the data and boundaries were subsequently optimised to maximise the overall agreement (assessed using Kappa analysis) as described by Borja *et al.* (2007). The ecological relevance of the original and optimised boundaries were considered in relation to the AMBI ecological groupings (Figure 4).

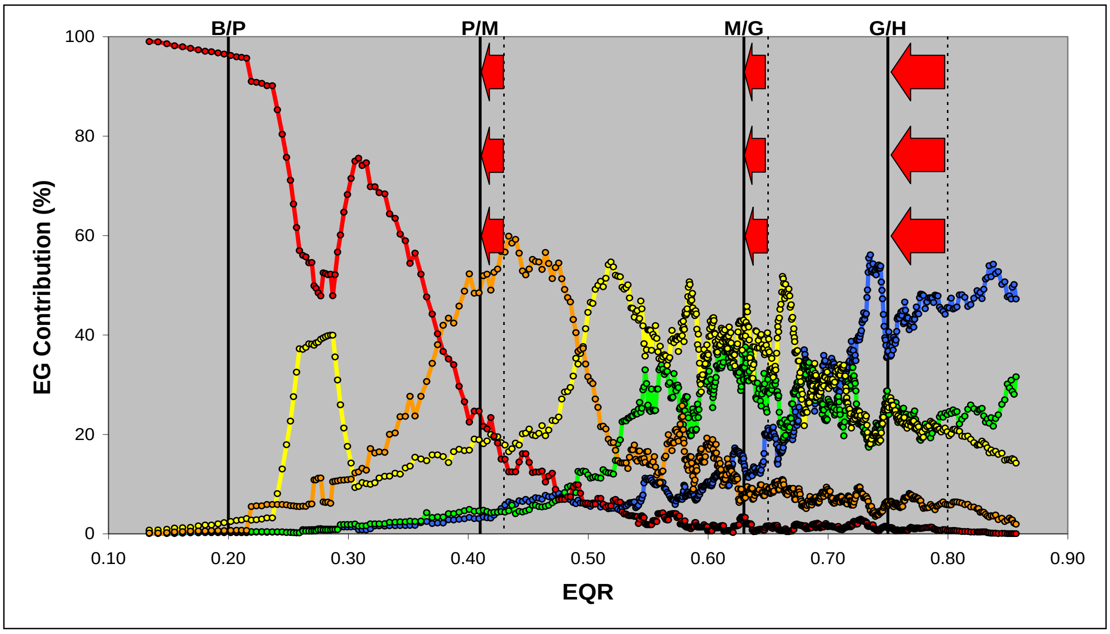

Figure 4: Proportion of AMBI Ecological Groups over the EQR scale within the North East Atlantic Geographical Intercalibration Group data used for boundary optimisation. The positions of nationally derived status boundaries (dashed lines) and status boundaries following optimisation (solid lines) are indicated (B/P = Bad/Poor boundary, P/M = Poor/Moderate, M/G = Moderate/Good, G/H = Good/High).

The resulting class boundaries (Table 2) are relevant to both transitional and coastal waters. The differences between the water categories are captured in the reference conditions. It is expected that the response to a specified pressure, in terms of the extent of departure from reference conditions, should correspond to the same state of ecological health in a benthic invertebrate community, regardless of water category.
These class boundaries remain unchanged since the 2009 first River Basin Management Plans.

Table 2: Ecological status boundaries for the $IQI_{v, IV}$

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

Note: EQR values exceeding one are possible where the observed values within a sample exceed the estimated reference condition values. Values exceeding one should be retained at the sample level, but water body average values should be limited to an upper value of one.

3. Undertaking an assessment

3.1 Summary Flow Chart

The process for undertaking a water body assessment for the IQI is summarised below (Figure 5).

Work Area	*Considerations*
Monitoring design ↓	• Is the IQI an appropriate assessment for the survey/investigation? (Suitable reference conditions? Consider level of natural variability and expected response to pressure) • Set appropriate sample numbers for habitat/aim of survey
Sample collection ↓	• Use of standardised methods (Sample type, sieve mesh, ISO 16665:2005) • Collect benthic infauna with supporting habitat information (salinity, depth, particle size)
Sample analysis ↓	• Use of standard laboratory methods • Full enumeration of biological samples identified at lowest taxonomic level • Use of standardised taxon list (WorMs) • Particle size analysis of sediment samples • Quality assurance procedures
Data treatment ↓	• Removal of non-invertebrate fauna • Removal of non-benthic invertebrates • Enumerate abundance as 1 for colonial and encrusting taxa.
IQI calculation ↓	• $IQI_{v.IV}$ (taxa number, (1-(AMBI/7)), Simpson's evenness) • Reference conditions derived from sample method and environmental data. • IQI (EQR) calculated at the sample level
Water body classification	• Derive WB average IQI (EQR) • Assign Class Status (use defined IQI boundaries) • Calculate IQI (EQR) Standard Error • Calculate Confidence of Class (CofC) and Risk of Misclassification (RoM).

Figure 5: Flow chart summarising the main stages involved in undertaking an assessment using the IQI.

3.2 Data requirements

Calculation of the IQI requires sample level, quantitative, benthic invertebrate abundance data, identified to lowest taxonomic level possible. Reference condition calculation requires a definition of (i) the habitat sampled (sediment parameters from PSA or qualitative description according to the Folk system (1954) or equivalent), (ii) the sampling methods (size of grab/core) and (iii) the processing methods (mesh size when sieving).

3.3 Sampling strategy

The number of samples required for an assessment is dependent on the survey aims and the variability of the EQRs in the habitat sampled. WFD water body assessments generally use single samples taken from stations spread across suitable habitats within a water body. The EQR is calculated at the sample level. (Multiple samples may be collected at a station, but the implications to the calculation of the standard error and representation of the area being assessed must be considered.) The water body status is then derived by calculating the mean EQR and relating it to the class status boundaries. Careful consideration must be given as to the aims of the survey before commencing sample selection. As for any investigation, enough samples must be taken to ensure the required degree of confidence in the final assessment is reached. In habitats with high inherent variability, the IQI may not be a suitable assessment method as the number of samples required would be disproportionately high.

It is recommended that samples are collected from a range of appropriate habitats representative of those present within a water body.

Power analysis is an approach used to estimate the probability in detecting differences in measurements. For the planning of WFD monitoring programmes, it is used to establish the number of samples which need to be collected in order to detect a difference in average EQR from a status boundary, for a given level of statistical certainty. The power (P) to detect change in the class status is influenced by a number of factors:

- number of samples
- the variability in the EQR (this reflects the level of inherent variability in the biological community)
- the amount of change in the EQR to be detected (a small difference will not be detected with the same degree of confidence as a large difference)
- the required probability at which significance is achieved (or Type I error, often set at 0.05).

EQR variability is in part dependent upon the habitat/s sampled. Early studies during WFD tool development indicate that in 60% of the water bodies sampled from well-characterised habitats (coastal, subtidal muds, sandy muds and muddy sands), a change of 0.1 (10%) in the average EQR of the water body could be detected with 90% confidence from 15 samples. In 50% of water bodies, a change of 0.05 (5%) in the EQR could be detected with 70% confidence from 15 samples (see Phillips *et al*., 2014).

3.4 Sampling methodology

The UK monitoring authorities follow the ISO/CEN standards for benthic invertebrate sampling and processing (ISO 16665:2005). EQRs are dependent upon sampling and processing methods, so these must be considered within the assessment (e.g. comparing a 0.1 m^2 Day grab sample sieved at 1 mm will give a false assessment of disturbance if compared to a reference value set for a hand core sieved at 0.5 mm).

The sample collection methods which are compatible with those currently used by the UK WFD competent authorities are $0.1m^2$ grabs (subtidal) and $0.01m^2$ core or 3 × $0.01m^2$ cores pooled (intertidal). Compatible sample processing methods are to 0.5 mm and 1.0 mm sieve mesh. Typically, 0.5 mm processing is undertaken for transitional water systems and 1.0 mm processing for coastal waters. However, this is not critical and discretion is recommended where the normal sieve mesh may insufficiently represent the benthic infaunal assemblage at the sampling location.
Established reference conditions have been modelled reflecting these sampling methods, although there is some flexibility to include alternative samples if it is feasible to adjust the data to ensure equivalency (Phillips *et al*., 2014).

Each biological sample should be accompanied by a sediment sample from the same location, which closely matches that of the biological sample, to undergo particle size analysis (PSA). Qualitative sediment descriptions in accordance with the Folk classification system (Folk, 1954) should be recorded if full PSA is not undertaken.

Salinity measurements should accompany each biological sample. Qualitative salinity descriptions in accordance with the Venice system (1959) should be recorded if salinity measurements are not available.

The pairing of sediment and salinity information with each biological sample is important to ensure that appropriate IQI reference conditions are applied.

3.5 Sample Analysis

Sample processing and laboratory assessment for WFD authorities follow the National Marine Biological Analytical Quality Control (NMBAQC) procedures (www.nmbaqcs.org). Benthic macroinvertebrate fauna should be identified to the lowest taxonomic level possible and enumerated per sample. Taxa need to be reported against a standardised taxonomic list; UK WFD authorities use the World Register of Marine Species (WoRMS).

Sub-sampling during the laboratory analysis stage should generally be avoided in order not to exclude rare taxa from the sample data. Sub-sampling of individual taxa in high abundance and multiplying back to the sample level may be undertaken if necessary, as the potential implications to the resulting IQI scores (EQRs) can be considered of low magnitude.

Standardised methodology and data treatment reduces the noise that factors such as differing enumeration protocols may introduce, and allows for greater certainty in detecting changes caused by anthropogenic pressures.

Sediment data should be analysed to derive the percent contribution (by weight) for nine size fractions (Table 3).

Table 3: Required PSA size fractions for fully quantitative derivation of IQI reference conditions.

Scale (µm)	Scale (Phi)
<63	>4
63 to 125	4 to 3
125 to 250	3 to 2
250 to 500	2 to 1
500 to 1000	1 to 0
1000 to 2000	0 to -1
2000 to 4000	-1 to -2
4000 to 8000	-2 to -3
>8000	<-3

Reference conditions should be derived according to the specific percent contribution of the different size fractions from PSA to attain the greatest accuracy in the reference condition values. However, typical size fractions for sediments described according to the Folk system (1954) have been established to allow reference conditions to be derived for the IQI where only qualitative descriptions are available. This increases the need for expert judgement in interpreting the resulting EQRs and water body classifications.

3.6 Data treatment

Data may require further processing prior to calculation of the IQI. This is to ensure that only appropriate benthic invertebrate fauna are used in the assessment and that the metrics derived for a dataset are compatible with those upon which reference conditions are based.

Assessment using the IQI requires taxa to be excluded if they are non-invertebrate, from an invertebrate family that are exclusively non-benthic or recorded as eggs. Taxa classified as either colonial or encrusting are enumerated as one (indicating presence only).

The IQI compatible taxon list with associated data truncation rules, are available within the IQI workbook (see section 3.7).

3.7 EQR calculation

As the IQI is a weighted ratio of the observed to reference condition values for each metric, the resulting IQI score is equivalent to the EQR.

The $IQI_{v.IV}$ is used to calculate EQRs at a sample level using the equation:

$$IQI_{v.IV} = \left(\left(0.38 \times \left(\frac{1-(AMBI/7)}{1-(AMBI_{\mathrm{Ref}}/7)} \right) \right) + \left(0.08 \times \left(\frac{1-\lambda'}{1-\lambda'_{\mathrm{Ref}}} \right) \right) + \left(0.54 \times \left(\frac{S}{S_{\mathrm{Ref}}} \right)^{0.1} \right) - 0.4 \right) \Big/ 0.6$$

where:

AMBI = {(0 x %GI) + (1.5 x %GII) + (3 x %GIII) + (4.5 x %GIV) + (6 x %GV)} / 100

The observed value of the parameter, Simpson's Evenness (1-λ'), of a sample should be calculated by the method of Simpson (1949) using the equation:

Observed value of Simpson's evenness = $$\frac{\sum_{p=1}^{S} n_p \times (n_p - 1)}{N \times (N - 1)}$$

where:

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

"S" is the number of taxa identified in the sample

"n_p" is the number of individual invertebrates of taxon "p" identified in the sample

"p" represents a taxon identified in the sample; "p" varies through values of 1 to "S"

An Excel workbook has been developed to aid calculation of the $IQI_{v.IV}$ (see www.wfduk.org.). Detailed instructions on the steps of how to use the workbook are provided within the workbook.

IMPORTANT: It is the responsibility of any organisation using this workbook to understand the classification methodology and the limits of the assessment for their data.

Metric reference condition values are specific to the environmental characteristics (sediment and salinity) at the sampling station and the sample collection and processing methods. The calculation of reference conditions, from the environmental parameters of samples available to the WFD competent authorities, is included in the IQI workbook. Comparability of IQI values may be reduced where reference conditions values are derived from different methods of setting reference conditions.

The IQI workbook requires the following information:

- Sample level quantitative benthic invertebrate abundance matrix (samples as columns, taxa as rows)
- Sample collection and processing methodology (limited selection of methods).
- Sediment data: either quantitative sediment data (% contributions from particle size analysis) or qualitative according to the Folk sediment classification system (Folk, 1954) or comparable
- Salinity data: either quantitative or qualitative according to the Venice system or general coastal/transitional description.

The IQI workbook automates the following processes:

- Data formatting (conversion of "present" abundance records to one, summing of abundance of duplicate taxa)
- Taxon check (cross references the input taxon list for compatibility to the AMBI and standardised taxon lists, highlights duplicate taxa)
- Assignment of AMBI ecological group scores
- Truncation: recalculates abundance for taxa that require removal or enumeration to one (presence only)
- Calculation of individual IQI metrics
- Calculation of reference conditions based on habitat and methodology

- Calculation of IQI for sample
- Calculation of mean IQI, standard error, Confidence of Class (CofC) and Risk of Misclassification (RoM) for the input data set.

Note: the AMBI taxon list (available through the AZTI website) must be updated when revised lists are issued so the version date of the workbook must always be considered.

3.8 Water body level classification

Water body classifications are based on the arithmetic mean EQR of all samples taken within a water body.

3.9 Understanding the certainty of the assessment

Providing an estimate of the statistical uncertainty of water body assessments is a statutory requirement of the WFD (Annex V, 1.3). Water body assessments based on estimates of ecological quality from sample data may be subject to elements of spatial bias (e.g. changes in salinity), temporal bias (e.g. seasonal changes), random sampling/measurement and random error from ecological processes. When assigning discrete ecological status classes, variability means that, depending on the proximity of the water body assessment result to a class boundary, there is a likelihood that the "true" status (i.e. that status if the EQR for the total population was known with zero error) is different to that assigned. This is termed the 'risk of misclassification' (RoM). Conversely, the statistical confidence that the status assigned from the sample population falls into each of the five ecological status classes is referred to as the 'confidence of class' (CofC).

The approach developed to define and report the CofC and RoM for WFD transitional and coastal benthic invertebrates is described by Ellis and Adriaenssens (2006) (in terms of its' general application to the WFD ecological quality elements). This approach to CofC and RoM requires the following information for a given assessment:

- Mean EQR
- Ecological class status boundaries
- Standard error (SE) of the assessment data.

It is not possible to describe the full background to this method in this document (see Phillips *et al*. 2014). It is important, however, to understand the RoM/CofC when considering; (i) the implications of sample numbers when planning a survey and (ii) when interpreting the IQI (class status) results.

For a given degree of variability, increasing sample numbers reduces the RoM and increases CofC. In the example below (Figure 6), increasing effort from 3 to 15 samples reduces the RoM to close to 0 near the centre of 'poor' and 'moderate' ecological status, and from ~37% to ~5% near the centre of 'good' status. *Note:* in all cases the increase in sample effort will not reduce the RoM at the status boundaries to below 50%.

The CofC for an assessment tends to be near its maximum where the average EQR is at its furthest from a status boundary. It is possible for the CofC to drop below 50% where there is a high enough probability that the true status for an assessment could be one of three or more different status classes represented by the mean EQR. Increasing sample effort from 3 to 15 samples increases the CofC as illustrated below (Figure 6).

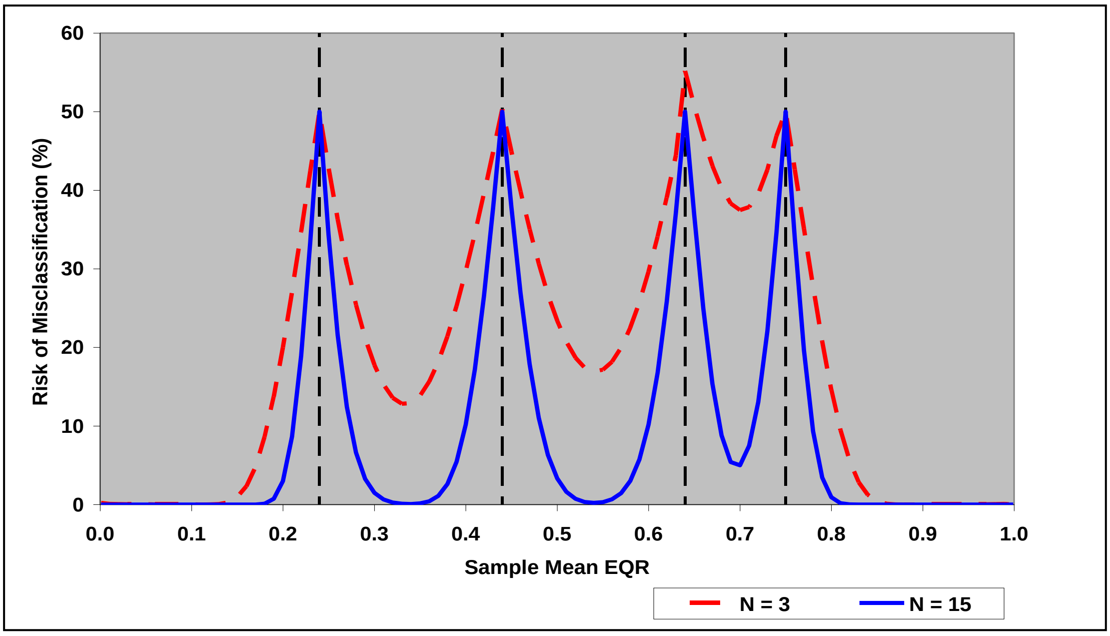

Figure 6: The effect of sample numbers on the Risk of Misclassification.

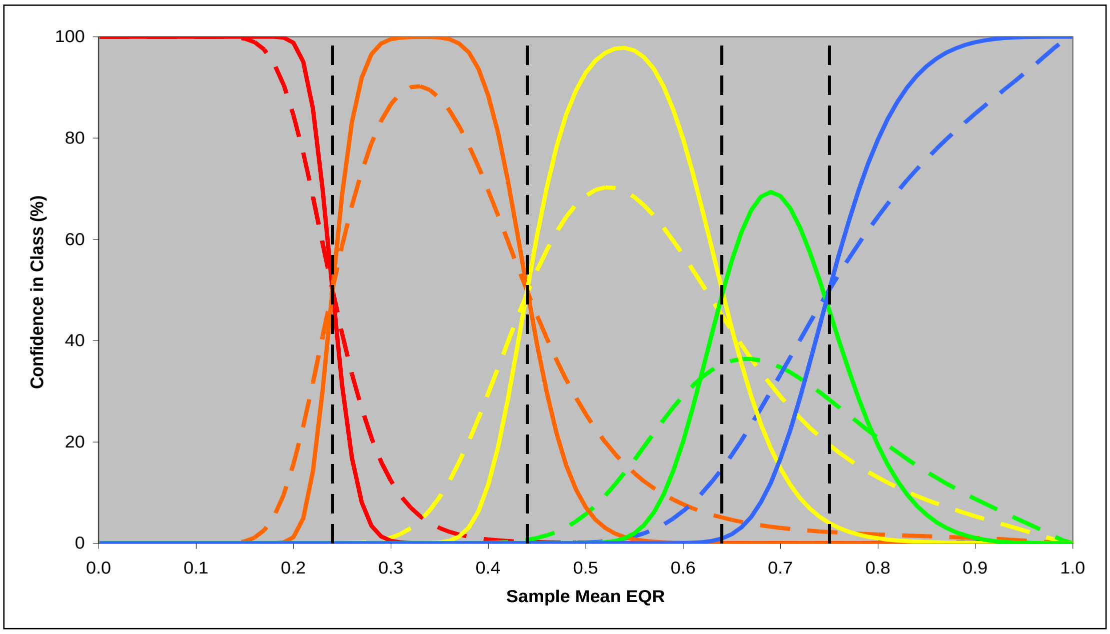

Figure 7: Example of the Confidence of Class (CofC) over the EQR scale for values based on 3 (dashed lines) and 15 samples (solid lines). Blue = CofC at High status, green = CofC at Good status, yellow = CofC at Moderate status, orange = CofC at Poor status, red = CofC at Bad status.

When interpreting an IQI assessment both the mean EQR and associated class value as well as the CofC and RoM values must be considered. The EQR provides the ecological class into which the site falls and the RoM and CofC values provide the confidence of that classification being true for the water body. When looking at the outputs it is important to consider that an EQR with a low RoM/high CofC value means that a high degree of statistical certainty exists that the assigned status corresponds to the true status of the water body. A classification with a high RoM/low CofC should be used with extreme caution.

4. Worked Example (Sample level)

A sample was processed with the following results:

Number of taxa (S)

The number of taxa (*S*) found in the sample was 30 (post truncation).

AZTI Marine Biotic Index (AMBI)

Individuals from each taxa were attributed to ecological groups (as described by Borja *et al*. (2000) and according to the AMBI taxon list). The number of individuals in each group are shown in Table 4.

Table 4: Contributions of taxa for the AMBI ecological groups for the worked example.

AMBI Ecological Group	Abundance	% Contribution
EGI	400	8
EGII	500	10
EGIII	900	18
EGIV	1200	24
EGV	2000	40
	Total abundance = 5000	

AMBI = {(0 x %EGI) + (1.5 x %EGII) + (3 x %EGIII) + (4.5 x %EGIV) + (6 x %EGV)} / 100

Therefore the observed AMBI value
AMBI = {(0 x 8) + (1.5 x 10) + (3 x 18) + (4.5 x 24) + (6 x 40)} / 100 = 4.17

Simpson's Evenness

The total abundance (N) was 5000 for S = 30.

The number of organisms found for each taxon and Simpson's evenness was calculated, results are shown in Table 5.

Table 5: Components of Simpson's evenness calculation for the worked example.

Taxon P	n_p	$n_p (n_p - 1) \div (N (N\text{-}1))$
1	773	0.023875015
2	654	0.017085897
3	554	0.012256931
4	469	0.008781436
5	397	0.006289738
6	336	0.004503301
7	284	0.003215523
8	241	0.002314063

Taxon P	n_p	$n_p (n_p - 1) \div (N (N-1))$
9	204	0.001656811
10	172	0.001176715
11	146	0.000846969
12	123	0.000600360
13	105	0.000436887
14	88	0.000306301
15	75	0.000222044
16	63	0.000156271
17	54	0.000114503
18	45	0.000079216
19	38	0.000056251
20	33	0.000042248
21	28	0.000030246
22	23	0.000020244
23	20	0.000015203
24	17	0.000010882
25	14	0.000007281
26	12	0.000005281
27	10	0.000003601
28	9	0.000002881
29	7	0.000001680
30	6	0.000001200
Totals	**5,000**	**0.084115 (= λ')**

IQI calculation
Reference conditions for this sample (considering habitat, sampling and processing methodology):
$[1 - AMBI_{Ref} \div 7] = 0.75$
$[1 - \lambda'_{Ref}] = 0.96$
$S_{Ref} = 35$

$EQR_{IQI} = [0.38 \times (1 - AMBI \div 7) \div (1 - AMBI_{Ref} \div 7) + 0.08 \times (1-\lambda') \div (1-\lambda'_{Ref}) + 0.54 \times (S \div S_{Ref})^{0.1} - 0.4] \div 0.6$

$= [0.38 \times (1 - 4.17 \div 7) \div 0.75 + 0.08 \times (1 - 0.08) \div 0.96 + 0.54 \times (30 \div 35)^{0.1} - 0.4] \div 0.6$ **= 0.69**

= **Good** status

5. References

Borja, A., Franco, J., & Pérez, V. (2000). "A Marine Biotic Index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments". Marine Pollution Bulletin, 40 (12), 1100-1114.

Borja, A., Josefson, A. B., Miles, A., Muxika, I., Olsgard, F., Phillips, G., Rodríguez, J. G., & Rygg, B. (2007). An approach to the intercalibration of benthic ecological status assessment in the North Atlantic ecoregion, according to the European Water Framework Directive. Marine Pollution Bulletin, 55, 42–52.

Ellis, J. & Adriaenssens, V. (2006). Uncertainty estimation for monitoring results by the WFD biological classification tools. Bristol: Environment Agency

Folk, R. L. (1954). The distinction between grain size and mineral composition in sedimentary rock nomenclature. Journal of Geology, 62 (4), 344-359.

ISO 16665:2005 (2005). Water quality - Guidelines for quantitative sampling and sample processing of marine soft-bottom macrofauna, International Organization for Standardization, Geneva, Switzerland.

Phillips, G. R., Miles, A. C., Prior, A., Martina, L. J., Brooks, L., & Anwar, A. (2014). Infaunal Quality Index: WFD classification scheme for marine benthic invertebrates. R&D Technical Report. Bristol: Environment Agency.

Prior, A., Miles, A. C., Sparrow, A. J., & Price, N. (2004). Development of a classification scheme for the marine benthic invertebrate component, Water Framework Directive. Phases I & II – Transitional and coastal waters. Bristol: Environment Agency, R&D Interim Technical Report, E1-116, E1-132: 103 pp (+appendix).

Simpson, E. H. (1949). Measurement of diversity. Nature, 163, 688.

Venice system (1959). The final resolution of the symposium on the classification of brackish waters. Archo Oceanography Limnology, 11 (suppl), 243–248.

Water Framework Directive 2000/60/EC of the European Parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. Official Journal of the European Communities L 327: 1–72

WFD CIS Guidance Document No. 5 (March 2003). Rivers and Lakes – Typology, Reference Conditions and Classification Systems. Published by the Directorate General Environment of the European Commission, Brussels, ISBN No. 92-894-5614-0, ISSN No. 1725-1087.