Development of a Classification Scheme for the Marine Benthic Invertebrate Component, Water Framework Directive

Phase I & II - Transitional and Coastal Waters

R & D Interim Technical Report E1-116, E1-132

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This document provides guidance to Environment Agency staff, research contractors and external agencies on the development of a classification scheme to meet the requirements of the Water Framework Directive (WFD), European Council Directive 2000/60/EC.

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GLOSSARY OF ACRONYMS

AMBI	AZTI Marine Biological Index
AvTD	Average Taxonomic Distinctness
CEFAS	Centre for the Environment, Fisheries and Aquaculture Science
CIS	Common Implementation Strategy
CSV	Coastal Survey Vessel
CW	Coastal Water
EA	Environment Agency
EAV	Equivalent Assigned Value
EEA	European Environment Agency
EHS	Environment and Heritage Service
EMAP	Environment Monitoring Assessment Programme (US)
EN	English Nature
EQR	Ecological Quality Ratio
EUNIS	European Nature Identification System
EVA	Expert View Analysis
ICES	International Council for the Exploration of the Seas
IECS	Institute of Estuarine and Coastal Studies
ITI UK	Infaunal Trophic Index United Kingdom
JNCC	Joint Nature Conservancy Council
MAFF	Ministry of Agriculture, Fisheries and Food
MarLIN	The Marine Life Information Network for Britain and Ireland
MBITT	Marine Benthic Invertebrate Task Team
MDS	Multi-dimensional Scaling
MI ROI	Marine Institute of the Republic of Ireland
MTT	Marine Task Team
NMBAQC	National Marine Biological Analytical Quality Control
NMMP	National Marine Monitoring Programme
NMP	National Monitoring Programme
NRA	National Rivers Authority
OSPARCOM	Oslo & Paris Commissions
PCA	Principal Components Analysis
PML	Plymouth Marine Laboratory
PRIMER	Plymouth Routines in Multivariate Ecological Research
QA	Quality Assurance
R & D	Research and Development
RA	Rapid Assessment
RLA	Restricted Laboratory Analysis
ROI	Republic of Ireland
SEPA	Scottish Environmental Protection Agency
SNIFFER	Scottish and Northern Ireland Forum for Environmental Research
TSA	Timed Sorting Analysis
TW	Transitional Water
UK	United Kingdom
UK TAG	United Kingdom Technical Advisory Group
UWWTD	Urban Waste Water Treatment Directive
WFD	Water Framework Directive

EXECUTIVE SUMMARY

The Marine Benthic Invertebrate Task Team (MBITT) is currently testing benthic macroinvertebrate classification tools, in order to identify those suitable for assessing the ecological status of transitional and coastal waters for the Water Framework Directive (WFD). The project aims to identify WFD compliant classification tools for the marine invertebrate component by November 2004 (Phase III). Currently, MBITT is only considering soft sediment benthic invertebrate communities.

The first two phases of the Project have focused on sourcing and collating historic macrobenthic faunal abundance data into a biological database, UNICORN© (copyright© 1995-2004 Unicomarine Ltd). Without extensive, quality assured data, in an easily accessible format, adequate testing of the classification tools cannot be achieved. Modifications to the UNICORN© database have been developed to assist with testing of the WFD classification tools. Quality assurance (QA) of the electronic data and confirmation of those samples having undergone laboratory analysis has been carried out. The project database now holds over 400 benthic invertebrate surveys (13,000 samples) from UK coastal and transitional waters. The database therefore provides the resource for the project to help (i) establish reference conditions, (ii) set ecological class boundary criteria and (iii) test the suitability of proposed classification indices. Data truncation rules have been established to standardise datasets prior to statistical analysis (required due to discrepancies in the level of taxonomic identification in national datasets).

For benthic invertebrate assessment, 'habitat-specific' reference conditions will be required in order to establish the 'type-specific' reference conditions. Habitats will be defined by the European Nature Identification System (EUNIS) system and assessments carried out at EUNIS level 4. Suggested qualitative reference conditions relate to the EUNIS description for the dominant habitat/s in the water body type. Quantitative reference conditions will be set using expert opinion and existing spatial and temporal datasets to create 'virtual' reference conditions.

Classification tools relating to the benthic invertebrate community were reviewed in Phase I. The project does not aim to create new biological indices, rather it is assessing existing indices with respect to their use in WFD assessment. A 'multimetric' approach to ecological status classification will be adopted, as no single index is able to define the 'health' of the benthic community. The selection of metrics to be included in the multimetric will be established on a habitat basis through Principal Components Analysis (PCA) of the calculated metrics.

Many of the existing biological indices have previously been reviewed and as such the project is only evaluating their performance as part of the multimetric assessment. However, the individual performance of the two novel indices, Average Taxonomic Distinctness (AvTD) and AZTI Marine Biotic Index (AMBI), have been evaluated prior to considering their inclusion in the multimetric. Testing of these indices has been carried out on national datasets in order to assess their behaviour in the range of UK water body types. AMBI is being considered as a WFD compliant classification tool for UK coastal and transitional waters. Five hundred previously unassigned UK taxa have been identified and sent to the developers of the AMBI index, Borja *et al.*, for inclusion in the index taxon list (ensuring a 'master' European taxon list). The methods used by

Borja *et al.*, for establishing boundary criteria are also being followed by the project. Testing of AvTD identified the need for inclusion of a frequency distribution in the index before its potential for WFD assessment can be established. Phase III will continue to address this index when the modification has been completed.

A more rapid approach to the assessment of marine benthic invertebrate communities was considered (both field and laboratory assessment). Ecological assessment of the benthic community in the field could be of potential use for WFD surveillance monitoring. However, the assessment would be reliant on the inclusion of highly trained benthic invertebrate identifiers in the field teams. The cost-benefit of training taxonomic staff for field assessment relative to sending traditional samples to the laboratory is not known and will be further evaluated in Phase III.

A scheme for testing the classification tools has been established (habitat-specific, truncated data, comparative to normative definitions) and this will be followed in Phase III. The variability of the benthic invertebrate community and the risk of misclassification will be evaluated using macrofaunal samples collected specifically for WFD classification tool testing.

1. INTRODUCTION

The European Water Framework Directive ((WFD) Directive 2000/60/EC) substantially alters our approach to water management by establishing a framework for the protection of all waters (inland surface waters, transitional waters, coastal waters and groundwater). With regard to the marine environment, the main purposes of the WFD are to:

- prevent deterioration and protect and enhance the status of aquatic ecosystems and associated wetlands
- promote sustainable water use
- reduce pollution from priority substances
- protect territorial and marine waters

Central to the Directive is the concept of 'integration'. Not only does the Directive aim to integrate management and decision making but it also looks to integrate environmental assessments (disciplines, analyses and expertise). In order to determine the overall status of designated water bodies, the WFD incorporates an ecological status assessment in conjunction with hydromorphology and physico-chemical assessments. The determination of ecological status is itself an integrated process, combining the 'health' of several biological quality elements. For marine water bodies, i.e. transitional (estuarine) and coastal waters, the biological quality elements contributing to the ecological status assessment are phytoplankton, macroalgae, angiosperms and benthic invertebrates. In transitional waters, fish will also be assessed.

For each biological quality element, classification tools are required to give a statistically robust definition of the 'health' of the element in a designated water body. Under the Directive, the 'health' is measured against that described for reference (undisturbed) conditions. As such the classification tools and ecological status assessment are reliant on reference conditions being established which describe the optimum ecological status for a designated water body type. Further information on the establishment of water body types (typology), reference conditions and classification systems is detailed in the Guidance produced by the Common Implementation Strategy (CIS) COAST working group 2.4 (Vincent *et al.*, 2002).

The intention of the Directive is to restore all inland, transitional and coastal waters to good status by 2015 (Article 4(a)(ii)), ensuring that there is no deterioration of ecological status. The measurement of ecological status through suitable classification tools will determine whether the requirements of the WFD are being met. The current report addresses the use of the benthic invertebrate quality element in ecological status assessment of coastal and transitional waters. The plant and fish components are dealt with in separate technical reports.

1.1 Project Background

The Marine Benthic Invertebrate Task Team (MBITT) was established under the Environment Agency (EA) led Research and Development (R & D) Benthic Invertebrate Project for transitional and coastal waters. The project and project team have developed in stages, responding to the requirements of the EA WFD programme. The project phases are outlined below:

Phase I: (Project E1-116) Development of an estuarine classification scheme: benthic invertebrate component (April 2001 – Aug 2002).

This EA project was initiated in response to the national requirement to develop a classification system for estuaries. It was envisaged that the project would assist in the requirement to provide:

- 'headline indicators' for State of the Environment reporting in estuaries
- estuary classification for the Water Framework Directive
- some requirements of the Habitats Directive.

The key work areas in this phase were:

(i) an initial review of existing classification tools for benthic invertebrates
(ii) assessment of the status of benthic invertebrate records in the EA
(iii) sourcing and input of historic estuarine benthic invertebrate datasets into electronic format (biological database, UNICORN©)
(iv) consideration of a more 'rapid assessment' of the health of benthic invertebrate communities (field methodology).

Phase II: (Project E1 –132) Development of a classification scheme for transitional and coastal waters for the WFD: benthic invertebrate component (Aug 2002 – Nov 2003).

Phase II focused on the needs of the WFD. Following Phase I, emphasis was placed on the requirement to collate suitable historic data (both transitional and coastal waters) into electronic format for the testing of the classification schemes.

The key work areas in this phase were:

(i) input of benthic invertebrate datasets (coastal and transitional) into the biological R & D database (UNICORN©)
(ii) development of UNICORN© with respect to WFD classification tool testing
(iii) quality assurance of data held on the R & D database
(iv) rules for standardisation of datasets (i.e. data truncation) prior to calculation of classification indices
(v) establishment of 'habitat-specific' assessments (classification indices related to the habitat being assessed)
(vi) testing of novel classification schemes, Average Taxonomic Distinctness (AvTD) and the AZTI Marine Biotic Index (AMBI), on UK datasets

(vii) establishment of testing procedure for 'multimetric' assessment
(viii) continued development of 'rapid assessment' methodology for assessing ecological status based on benthic invertebrate communities.

Phase III in Progress: (Project E1 –139) Development of a classification scheme for transitional and coastal waters for the WFD: benthic invertebrate component (Nov 2003 – Nov 2004).

The Project is currently in Phase III, which is due for completion in November 2004. Funding for this phase has been supplied from both the EA (£60K) and the Scotland and Northern Ireland Forum for Environmental Research (SNIFFER, £10K), ensuring that classification tools are suitable for the full range of UK waters.

The key work areas in this phase are:

(i) continued input of suitable macrobenthic invertebrate abundance data to R & D database (UNICORN©)
(ii) expanded normative definitions linking the proposed classification tools to the normative definitions
(iii) linkage of proposed classification tools to normative definitions
(iv) establishment of type-specific reference conditions (habitat-specific)
(v) testing of proposed classification tools against known pressure gradients. This relies on access to gradient data (e.g. sediment chemistry) and matching chemical gradient data to biological abundance data
(vi) establishment of the behaviour of proposed classification tools in response to anthropogenic and natural pressures
(vii) testing the ability of the proposed classification tools to distinguish between ecological status classes
(viii) suitability of proposed classification tools in relation to specific habitats
(ix) quantification of the variability of ecological status assessment based on the natural variability of benthic communities (as shown through classification tools)
(x) quantification of the risk of misclassification of ecological assessment using the selected tools
(xi) comparison with benthic invertebrate classification tools being developed in other Member States (Intercalibration).
(xii) development of sampling and quality assurance protocols

The United Kingdom (UK) and Republic of Ireland (ROI) established the Marine Task Team (MTT) in order to ensure compliance with the Directive and implement an

integrated approach to meeting the requirements of the Directive across the UK and ROI. MBITT reports directly to, and takes guidance from, the MTT.
The MBITT project board (Appendix I) consists of representatives from the:

- Environment Agency (EA)
- Scottish Environment Protection Agency (SEPA)
- Environment and Heritage Service (EHS)
- Marine Institute, Republic of Ireland (MI ROI)
- Joint Nature Conservation Committee (JNCC)
- Centre for Environment Fisheries and Aquaculture Science (CEFAS)
- Institute of Estuarine and Coastal Studies (IECS)

In addition, links for external consultation and review have been established with marine benthic ecologists from academic, government and consultant institutions. These external contacts (Appendix I) took part in a workshop held in October 2003 by MBITT, which explored the aspect of "Expert Judgement", as described in the WFD.

This interim R & D project report outlines the work from Phase I and II carried out in the development of a methodology to classify the ecological status of benthic invertebrate assemblages. Some aspects of work from Phase III are also discussed. A final R & D report will be produced following Phase III of the project, suggesting the most suitable classification tools currently available for WFD assessment and the risk of misclassification when using the tools.

2. NORMATIVE DEFINITIONS

The criteria by which ecological status should be evaluated are detailed in the normative definitions in Annex (V(1.2)) of the WFD. Normative definitions describe the aspects of the benthic community that must be included in the ecological status assessment of a water body. It is therefore essential that any proposed classification scheme for WFD assessment includes indices (metrics) that address those parameters identified in the normative definitions for each of the five ecological status classes i.e. 'High,' 'Good,' 'Moderate,' 'Poor' and 'Bad'.

The normative definitions relating to benthic invertebrates are outlined below (Annex V (1.2.3 and 1.2.4) transitional and coastal waters, respectively). The main terms to be addressed by a benthic invertebrate classification scheme for WFD are underlined.

HIGH: The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions.

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

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

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

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

Taxa indicative of pollution are present.

Many of the sensitive taxa of the type-specific communities are absent.

POOR: Major alterations to the values of the biological quality elements for the surface water body type.

Relevant biological communities deviate substantially from those normally associated with the surface water body type under undisturbed conditions.

BAD: Severe alterations to the values of the biological quality elements for the surface water body type.

Large portions of the relevant biological communities normally associated with the surface water body type under undisturbed conditions are absent.

2.1 Expanded Normative Definitions

An example of how these normative definitions can be expanded with respect to benthic invertebrate classification tools is shown in Table 2.1 (Myles O'Reilly, SEPA). The classification tools discussed are those currently under consideration for use in WFD ecological assessment (see Section 5). As habitat-specific assessment will be required when considering the 'health' of the benthic invertebrate community, this example relates specifically to coastal, sublittoral soft sediments (EUNIS Habitat A4, Section 3.2).

Table 2.1 Expanded Normative Definitions: sublittoral soft sediments of Coastal Waters (EUNIS Habitat A4)
The indices used to assess each expanded interpretation are shown in brackets.

Quality Status	Normative Definition:	Expanded Interpretation
High	The level of diversity and abundance of invertebrate taxa is within the range normally associated with undisturbed conditions. All disturbance-sensitive taxa associated with undisturbed conditions are present.	<u>Invertebrate community shows no anthropogenic impact</u> • Species richness and diversity high (e.g. Number of species, Shannon, Fisher, Margalef, & Brillouin diversity indices). • Evenness high (Heip and Pielou indices). Abundance ratio (Abundance/Number of taxa) low. • Taxonomic range high (Taxonomic diversity, distinctness, and breadth indices). • Community Abundance (assessed by AMBI) – normal, unpolluted: Sensitive Taxa (Group I) of dominant abundance. Indifferent and Tolerant Taxa (Groups II & III) absent or of sub-dominant abundance. Opportunistic Taxa (Group IV) absent or of negligible abundance. Indicator Taxa (Group V) absent or of negligible abundance. • Trophic Structure (assessed by ITI UK) – normal: Dominated by water column and interface detritus feeders. • Abundance of important characterising, structural, or functional species unimpacted (e.g. seapens or burrowing decapods, large bivalves).

Quality Status	Normative Definition:	Expanded Interpretation
Good	The level of diversity and abundance of invertebrate taxa is slightly outside the range associated with the type-specific conditions. Most of the sensitive taxa of the type-specific conditions are present.	<u>Invertebrate community shows slight anthropogenic impact.</u> • Species richness and diversity slightly reduced (e.g. Shannon, Fisher, Margalef, & Brillouin diversity indices). • Evenness slightly reduced (Heip and Pielou indices). Abundance ratio slightly elevated. • Taxonomic range slightly reduced (Taxonomic diversity, distinctness, and breadth indices). • Community Abundance (assessed by AMBI) – slightly unbalanced, slightly polluted: Sensitive Taxa (Group I) abundance may range from high sub-dominant to absent. Indifferent Taxa (Group II) of low sub-dominant abundance. Tolerant Taxa (Group III) of dominant abundance. Opportunistic Taxa (Group IV) & Indicator Taxa (Group V) abundance may range from negligible or low to equi-abundance with Indifferent Taxa. • Trophic Structure (assessed by ITI UK) – normal or slightly changed: Dominated by detritus and deposit feeders. • Abundance of important characterising, structural, or functional species slightly reduced (e.g. seapens or burrowing decapods, large bivalves).

Quality Status	Normative Definition:	Expanded Interpretation
Moderate	The level of diversity and abundance of invertebrate taxa is moderately outside the range associated with the type-specific conditions. Taxa indicative of pollution are present. Many of the sensitive taxa of the type-specific communities are absent.	<u>Invertebrate community shows moderate anthropogenic impact.</u> • Species richness and diversity moderately reduced (e.g. Number of species, Shannon, Fisher, Margalef, & Brillouin diversity indices). • Evenness moderately reduced (Heip and Pielou indices). Abundance ratio moderately elevated. • Taxonomic range moderately reduced (Taxonomic diversity, distinctness, and breadth indices). • Community Abundance (assessed by AMBI) – Transitional unbalanced to moderately polluted: Sensitive Taxa (Group I) of negligible abundance or absent. Indifferent Taxa (Group II) of low sub-dominant abundance. Tolerant Taxa (Group III), Opportunistic Taxa (Group IV) & Indicator Taxa (Group V) co-dominate the abundance. • Trophic Structure (assessed by ITI) – shows moderate change: Dominated by interface deposit feeders. Abundance of important characterising, structural, or functional species moderately reduced. Some key species of negligible abundance or absent. (e.g. seapens or burrowing decapods, large bivalves).

Quality Status	Normative Definition:	Expanded Interpretation
Poor	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.	Invertebrate community shows major anthropogenic impact. • Species richness and diversity shows major reduction. (e.g. Number of species, Shannon, Fisher, Margalef, & Brillouin diversity indices). • Evenness shows major reduction (Heip and Pielou indices). Abundance ratio shows major elevation. • Taxonomic range shows major reduction (Taxonomic diversity, distinctness, and breadth indices). • Community Abundance (assessed by AMBI) – Transitional moderately to heavily polluted: Sensitive and Indifferent Taxa (Groups I & II) of negligible abundance or absent. Tolerant Taxa (Group III) of sub-dominant abundance. Opportunistic Taxa (Group IV) & Indicator Taxa (Group V) co-dominate the abundance. • Trophic Structure (assessed by ITI) – shows major change or degradation: Dominated by interface and sub-surface deposit feeders. • Many key species of negligible abundance or absent (e.g. seapens or burrowing decapods, large bivalves).

Quality Status	Normative Definition:	Expanded Interpretation
Bad	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 .	Invertebrate community shows severe anthropogenic impact. • Species richness and diversity shows severe reduction. (e.g. Number of species, Shannon, Fisher, Margalef, & Brillouin diversity indices). • Evenness shows severe reduction (Heip and Pielou indices). Abundance ratio shows severe elevation. • Taxonomic range severely reduced (Taxonomic diversity, distinctness, and breadth indices.) • Community Abundance (assessed by AMBI) – very heavily or extremely polluted: Azoic or if fauna present: Sensitive, Indifferent, & Tolerant Taxa (Group I, II, & II) absent. Opportunistic Taxa (Group IV) of sub-dominant abundance. Indicator Taxa (Group V) of dominant abundance. • Trophic Structure (assessed by ITI) – shows severe degradation: Dominated by sub-surface deposit feeders, or azoic. • All important characterising, structural, or functional species absent (e.g. seapens or burrowing decapods, large bivalves).

3. TYPOLOGY, REFERENCE CONDITIONS and BOUNDARY CRITERIA

3.1 Typology

The WFD requires that all coastal and transitional surface water bodies are characterised into 'types', with the assessment then relating to the defined water body type (i.e. a 'type-specific' assessment). The Directive sets out two methods (method A and B detailed in Annex II) by which characterisation of water bodies into types can be carried out. The end result is a physical characterisation of water bodies, which is also biologically relevant. The coastal and transitional waters of the UK and ROI have been assigned to six transitional water types (Table 3.1) and 11 coastal water types (Table 3.2) (see Typology guidance (UK TAG, 2003) for further details).

Table 3.1 UK and ROI Transitional Water (TW) Typology (UK TAG, 2003). All TW types occur in Ecoregion 1 (North Sea) and Ecoregion 4 (Atlantic) and are separated according to obligatory and optional factors (method B).

Type	Mixing Characteristics (optional)	Salinity (obligatory)	Mean Tidal Range (Obligatory)	Exposure (optional)	Depth (optional)	Substratum (optional)
TW1	Partly mixed/ Stratified	Mesohaline/ Polyhaline	Macrotidal	Sheltered	Intertidal/ Shallow subtidal	Sand and mud
TW2	Partly mixed/ Stratified	Mesohaline/ Polyhaline	Mesotidal	Sheltered	Intertidal/ Shallow subtidal	Sand and mud
TW3	Fully mixed	Polyhaline	Macrotidal	Sheltered	Extensive intertidal	Sand or mud
TW4	Fully mixed	Polyhaline	Mesotidal	Sheltered	Extensive intertidal	Sand or mud
TW5 Sea Lochs		Polyhaline	Mesotidal	Sheltered		
TW6 Lagoons	Partly mixed/ Stratified	Oligohaline/ Polyhaline	N/A	Sheltered	Shallow	Mud

Table 3.2 UK and ROI Coastal Water (CW) Typology (UK TAG, 2003). The Ecoregions in which each type occurs are shown. Types are separated according to obligatory and optional factors (method B).

Type	Name	Salinity (obligatory)	Mean Tidal Range (obligatory)	Exposure (optional)	Ecoregion (obligatory)
CW1		Euhaline	Macrotidal	Exposed	4 (Atlantic)
CW2		Euhaline	Mesotidal	Exposed	4 (Atlantic) and 1 (North Sea)
CW3		Euhaline	Microtidal	Exposed	4 (Atlantic)
CW4		Euhaline	Macrotidal	Moderately exposed	4 (Atlantic) and 1 (North Sea)
CW5		Euhaline	Mesotidal	Moderately exposed	4 (Atlantic) and 1 (North Sea)
CW6		Euhaline	Microtidal	Moderately exposed	4 (Atlantic)
CW7		Euhaline	Macrotidal	Sheltered	4 (Atlantic)
CW8		Euhaline	Mesotidal	Sheltered	4 (Atlantic) and 1 (North Sea)
CW10	Coastal Lagoons	Euhaline	N/A	Sheltered	
CW11	Sea Lochs (shallow)	Euhaline	Mesotidal	Sheltered	
CW12	Sea Lochs (Deep)	Euhaline	Mesotidal	Sheltered	

For ecological assessment to be carried out, each defined water body type requires biological reference conditions (high ecological status) to be established. These type-specific reference conditions form the anchor of the WFD ecological assessment (see Section 3.3).

In the case of benthic invertebrate communities however, type-specific reference conditions must take into account the substratum and the salinity zone (transitional waters) that the community inhabits. Benthic communities are, in the first instance, defined by these parameters. A range of habitat type-specific reference conditions is therefore necessary for the ecological assessment of such communities. These reference conditions must detail the ranges of abundance, diversity and sensitive taxa that would be expected at high status, as per the normative definitions. Initial tests of classification tools have confirmed that the variability shown in the benthic invertebrate communities between habitat types can mask the detection of anthropogenic impact on benthic communities. As such, a generic reference condition for the water body type alone would not be sufficient to derive the ecological status classes required by the Directive for benthic macrofauna. Instead a suite of habitat-specific reference conditions needs to be established, from which a selection can be used to form the relevant type-specific reference condition. For example, the Exe is a type 3 transitional water in south west England, but consists of a mosaic of ten habitat types (Figure 3.1). It may be suitable to monitor any one of the defined habitats, based on their spatial dominance or on their sensitivity to the pressures acting on the water body. For the chosen habitat/s, specific

reference conditions would be required in order to produce biologically relevant type-specific reference conditions.

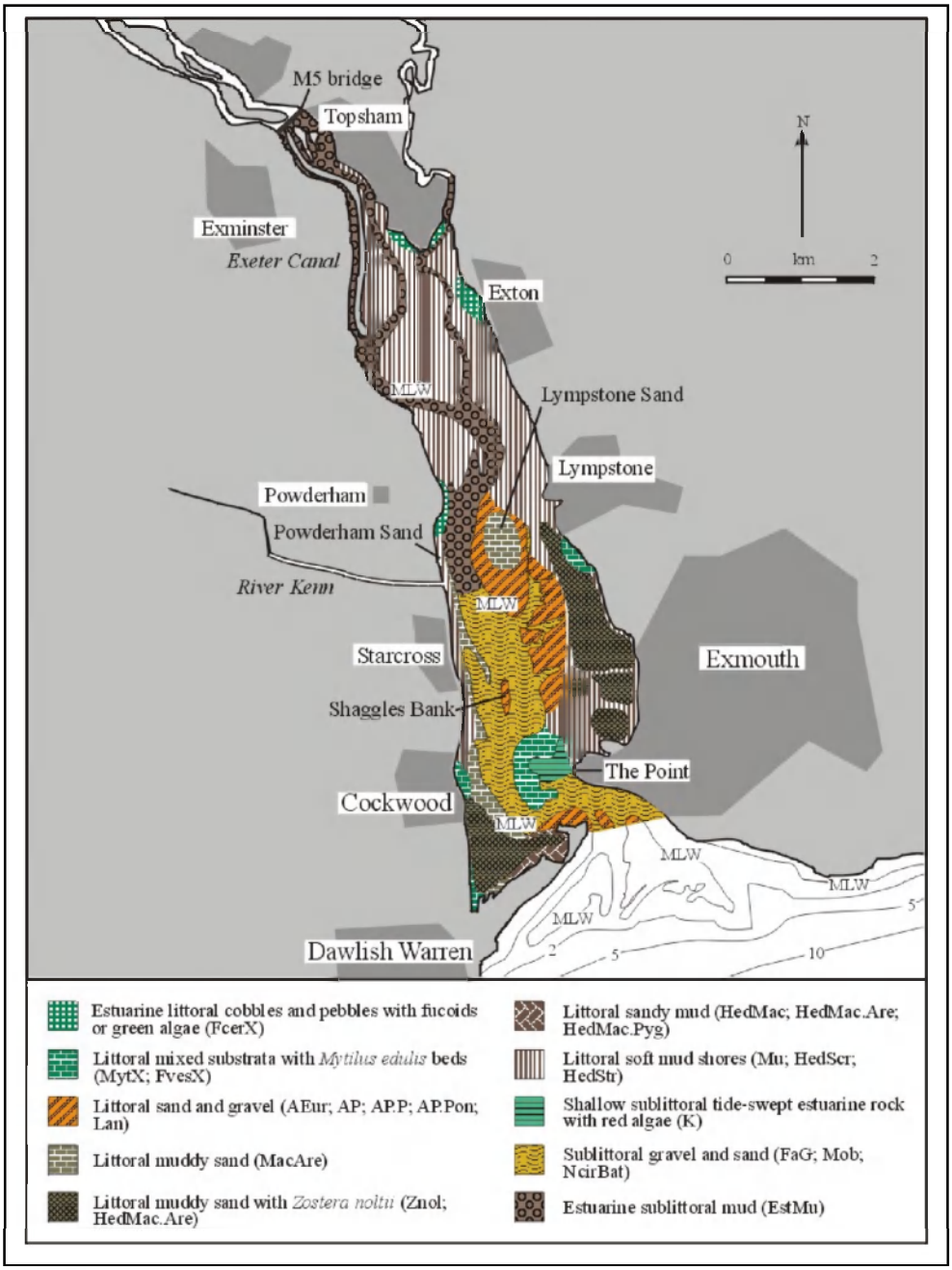

Figure 3.1 Indicative distribution of the main biotopes within the Exe, Southwest England. The Exe is one water body type (TW 3) but is comprised of ten different habitats (Moore *et al.*, 1999).

In producing guidance on establishing 'types', the WFD has outlined a procedure by which Member States can ensure that like is compared with like. By introducing the need for habitat-specific reference conditions, we also introduce the need for a system by which

to define habitats. This further level of definition is essential if monitoring, assessment and reporting on water bodies are to be carried out in a consistent and meaningful manner. As such, the European Nature Information System (EUNIS) of the European Environment Agency (EEA) has been adopted to ensure uniform nomenclature is used in naming habitats.

3.2 EUNIS Habitat Classification

EUNIS is a habitat classification established in 1996 to link all the major habitats, both terrestrial and aquatic, in Europe. With regards to marine habitats, it is cross-referenced with the Habitats Directive (92/43/EEC) and has been developed in collaboration with Oslo-Paris Commission (OSPARCOM) and International Council for the Exploration of the Sea (ICES). The full EUNIS habitat classification is available at http://mrw.wallonie.be/dgrne/sibw/EUNIS/home.html. EUNIS habitat classification provides a common currency between Member States and existing Directives to ensure consistency in comparing like with like habitats for ecological status assessment. It is therefore the ideal tool to incorporate in WFD assessment methodology.

EUNIS is based on a six level hierarchy (Table 3.3). Each level becomes increasingly defined, ending in a focused description (including characterising taxa) of a particular biotope.

Table 3.3 EUNIS level hierarchy based on an example of a marine intertidal habitat

Level	Description	Example	EUNIS Code
1	Environment	Marine	A
2	Broad Habitat	Littoral sediments	A2
3	Habitat complex	Littoral sands and muddy sands	A2.2
4	Biotope complex	Sand shores	A2.24
5	Biotope	Burrowing amphipods and polychaetes in clean sand shores	A2.244
6	Sub-biotope	Burrowing amphipods and polychaetes (often with *Arenicola marina*) in clean sand shores	A2.2441

The EUNIS level for which WFD habitat-specific reference conditions are required has been assessed by the project. The EUNIS level selected for the testing of the classification tools must allow the relevant reference conditions and associated deviations from these to detect change in ecological status due to anthropogenic impacts. It is beyond the scope of this project to establish the level for all possible marine habitat types found in UK transitional and coastal waters (out to 1 nm). Instead the project has focused on testing classification tools and identifying the necessary habitat definition, on a selection of five level 3 EUNIS habitat types:

- A2.2 Littoral sands and muddy sands
- A2.3 Littoral muds
- A4.2 Sublittoral sands and muddy sands
- A4.3 Sublittoral muds
- A4.4 Sublittoral combination sediments

This broad 'first cut' was selected on the basis that (i) these are some of the dominant habitats in UK water bodies, (ii) these habitats are routinely monitored by the Competent Authorities (e.g. National Marine Monitoring Programme) and (iii) the majority of benthic invertebrate data that the project holds relates to these habitats. Following initial investigation of the classification tools and discussion with benthic specialists, it was decided that EUNIS level 4 would be more appropriate for the development of reference conditions than level 3 (Table 3.3). The basis for this decision was that the EUNIS level used in ecological status assessment must enable the classification tools to detect the response of a benthic invertebrate community to perturbation. Levels of classification higher than 4 (levels 1-3) appear to obscure changes in ecological status resulting from anthropogenic stress, due to increased natural variability incorporated by the range of substrata at that habitat level. At a lower level of classification (levels 5-6), the detailed species information introduced can also obscure the detection of anthropogenic impact (i.e. 'fine-scale noise'). An analogy can be made with the level of taxonomic identification required (Section 6) where several authors (e.g. Warwick 1988, Olsgard *et al.*, 1997) have found that analyses at higher taxonomic levels are more likely to determine affects of anthropogenic impact. The biotope (level 5) and sub-biotope (level 6) have also been shown to change with time under natural conditions (Hiscock & Kimmance, 2003). The biotope complex (level 4) is based only on the physical description of the habitat and therefore provides a more stable description to compare separate sites with the same physical characteristics.

Classification tools are currently being tested on data separated at EUNIS level 4, with the aim of developing specific biotope complex reference conditions. Classification tools will be selected for their ability to detect anthropogenic impacts on the benthic invertebrate community of those biotope complexes for which reference conditions have been developed. At this stage, no decision has been made as to which habitats will be monitored for WFD surveillance monitoring. The selection of EUNIS level 4 pertains only to the current classification tool testing. During the course of testing, the suitability of the EUNIS level tested will continue to be evaluated.

3.3 Reference Conditions

The assessment of ecological status for WFD centres on the Ecological Quality Ratio (EQR):

> **Annex V 1.4.1. (ii)**
> *"the results of the (classification) systems"…"shall be expressed as ecological quality ratios for the purposes of classification of ecological status. These ratios shall represent the relationship between the values of the biological parameters observed for a given body of surface water and the values for these parameters in the reference conditions applicable to that body. The ratio shall be expressed as a numerical value between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero."*

A description of the benthic invertebrate reference conditions must therefore be established that permits the comparison of monitoring results with the reference conditions in order to derive the EQR. The values of the EQR then set for each ecological status class must ensure that the water body meets the normative definition for that status class given in Annex V (Tables 1.2, 1.2.3. or 1.2.4). As such the reference conditions form the anchor for the whole ecological assessment. Ecological status classes will be defined by their deviation from reference (Figure 3.2, Vincent *et al.*, 2002).

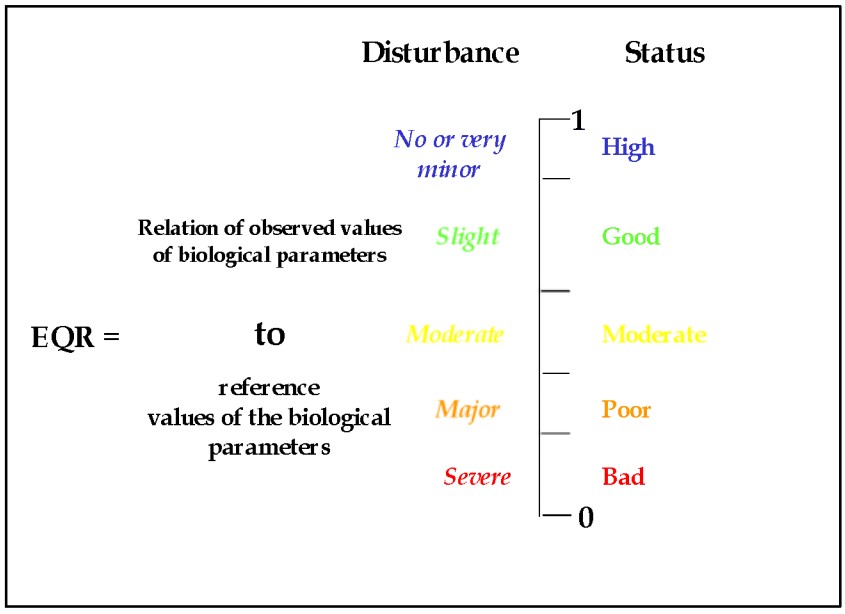

Figure 3.2 Suggested Ecological Quality Ratio according to Annex V, 1.4.1. The sizes of the bands differ because the boundaries between classes must align with the normative definitions, not a simple percentage. Note that all the deviations are measured from the reference condition (From COAST Guidance, Vincent *et al.* 2002).

For each water body type (see Section 3.1), type-specific reference conditions need to be established for the benthic invertebrate component at high ecological status i.e. the

benthic invertebrate community that exists, or would exist, if there were no, or very minor disturbances from human activities.

Type-specific reference conditions must summarise the range of possibilities and values for the biological quality elements over periods of time and across the geographical extent of the type (Vincent *et al.*, 2002). The Guidance appreciates that the natural variability of a quality element within a water body type may be as high as the natural variability between water body types. Creating habitat-specific reference conditions for EUNIS habitats (Section 3.2) helps to minimise this variability. The descriptive definitions within EUNIS have been used to suggest ***qualitative*** reference conditions for the coastal and transitional waters of UK and ROI. As several habitats will be present within a water body type, the qualitative reference conditions are described for the suggested predominant habitats within the water body type. For example, the qualitative reference conditions for transitional water type 3 describe EUNIS habitat A2.25, muddy sand shores:

> '*the drier sediment of the upper shore is characterised by the amphipods* Bathyporeia spp *and* Corophium spp *with a limited abundance of polychaetes and bivalves. Sediment of the mid and lower shore remains saturated throughout the tidal cycle and supports a lower abundance of amphipods but a wide range of polychaetes commonly occur, including* Nephtys hombergii, Scoloplos armiger *and* Pygospio elegans. *The bivalves* Cerastoderma edule *and* Macoma balthica *are also common.'*

The full list of suggested qualitative reference conditions is shown in Appendix II.

To complete the EQR (i.e. WFD assessment), ***quantitative*** habitat-specific reference conditions are required. Four methods of establishing quantitative water body type-specific reference conditions are set out in the WFD. In order of preference, these are the use of:

1. an existing undisturbed site or a site with only very minor disturbance
2. historical data and information
3. predictive statistical models
4. expert judgement.

These parameters will provide the basis for the classification tools. To establish such quantitative reference conditions using actual macrofaunal datasets (methods 1 & 2) in transitional waters, the salinity regime needs to be considered because habitats in lower salinity areas naturally support less diverse faunal assemblages compared to higher salinity areas. In addition, the dominant taxa groups in the targeted habitat will change with salinity. For instance in a low salinity area, a habitat may be dominated by oligochaetes and insects, whereas in higher salinity waters, the similar habitat would be dominated by polychaetes, bivalves and crustaceans (Hiscock & Kimmance, 2003).

In both transitional and coastal waters, the sampling methodology has to be taken into account when selecting and analysing suitable data. For testing purposes, data with standardised methodology (i.e. sample area, season etc) are used. Once reference conditions have been established on these matched datasets, the effect of variables, such as season, on the ecological status assessment can be evaluated. Without this specificity in

the reference conditions, it is unlikely the classification tools will be able to distinguish anthropogenic impacts from natural variation.

At present, there are no suitable predictive statistical models (method 3) for establishing marine benthic invertebrate reference conditions. A scoping study will hopefully be carried out to determine whether models such as those being pursued by the EA WFD freshwater teams (Walley *et al.* 2001) could be extended for use in the marine environment. The project is therefore using a combination of the methods 1, 2 and 4 set out in the WFD to establish reference conditions. Where possible, benthic data from undisturbed sites will be utilised. However, it is questionable as to whether undisturbed sites actually exist for all/any of the water body types within the UK. Method 1 cannot therefore be solely relied upon. Consequently, MBITT will depend heavily upon expert judgement (method 4), using historic data and enhancing it (with peer review) to reflect the benthic assemblage expected at high status. This approach has been used by Borja *et al.* (2003) who have considered 'virtual' reference locations based on the potential biological parameters and chemical concentrations of an area with no or minor disturbance.

3.4 Establishing Boundary Criteria

Once habitat-specific reference conditions are established for high ecological status, the departure from reference can be measured. The level of departure will be used to set boundaries for each of the ecological status classes. The boundaries between each of the status classes needs to be described and criteria established which reflect the normative definitions.

Borja *et al.* (2003) approached the setting of boundary criteria by first setting 'virtual' locations at reference status (Section 3.3). The potential parameters and concentrations that would constitute a severe alteration in the ecological status of the location are also established, in order to create 'virtual' locations at bad status. 'Virtual' locations are therefore created for the two ecological class extremes. These are plotted in a Principal Components Analysis (PCA) (Figure 3.3). The PCA is then used as a method to calculate EQR values, the high 'virtual' location being denoted as 1 and the bad 'virtual' location being denoted as 0. The position of real benthic data on this PCA is used to give an EQR between 1 and 0, as indicated by its distance from the 'virtual' high status. An iterative process is then required to determine the appropriate interval ranges for the ecological classes. Initially five equal intervals are set. The position of datasets and assigned ecological class status are then assessed against the normative definitions in the WFD. The intervals are then adjusted until the ranges reflect the derivations described in the normative definitions. Repeating this process for each of the soft sediment EUNIS habitats (level 4), would set the appropriate boundaries per habitat type.

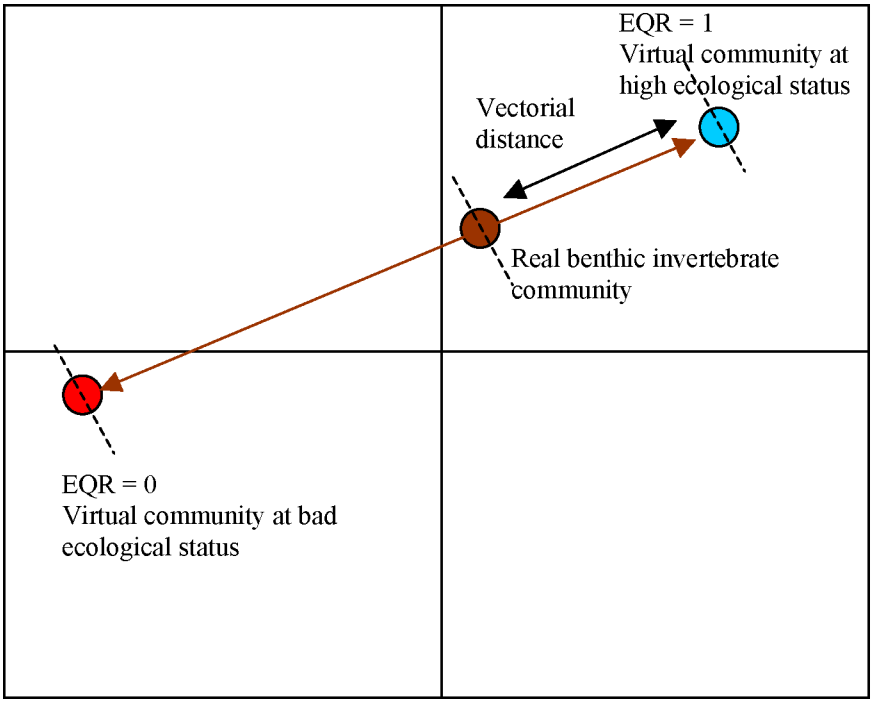

Figure 3.3 Principal Components Analysis showing virtual communities at high and bad ecological status. The position of real benthic invertebrate data on the axis can be used to give an EQR for that community (after Borja *et al.*, 2003).

Borja *et al.* (2003) have also described a method to derive an EQR through use of a multimetric. When combining indices into a multimetric, the varying scales of the metrics first need to be considered. For example, the Infaunal Trophic Index UK (ITI UK) ranges from 0 to 100, whilst the biotic coefficients for AZTI Marine Biotic Index (AMBI) Borja *et al.* (2000) (Section 5.2) range from 0 to 7. Borja *et al.* (2003) have used Equivalent Assigned Values (EAV) to normalise across indices. An EAV is simply a value between 0 and 1, which equates to a status class (5 equal parts). Assuming each individual metric has a range associated with the defined ecological class, it is possible to assign the EAV to the metric value. The EAVs are then combined within the 'multimetric'. The EQR is calculated by summing the EAVs and dividing by the number of metrics used (for example see Table 3.4).

Table 3.4 Ranges for selected indices to derive EQR through allocation of an Equivalent Assigned Value (EAV), according to the multimetric developed by Borja *et al.* (2003).

Ecological Status	H'(log2)	S	AMBI	EAV	EQR^{2003}
High	>4.8	>60	0-1.2	1	0.9-1
Good	3.6 - 4.8	45-60	1.2-3.3	0.75	0.7-0.9
Moderate	2.4 - 3.6	30-45	3.3-4.3	0.5	0.5-0.7
Poor	1.2 - 2.4	15-30	4.3-5.5	0.25	0.25-0.5
Bad	0 - 1.2	0-15	5.5-7	0	0 - 0.25

Phase III of the project will continue to investigate the appropriate ecological status class ranges for the EQR, ensuring that the benthic invertebrate community within each class range reflects the WFD normative definitions (Section 2).

3.4.1 Testing the suitability of suggested ecological class boundaries

Once theoretical ecological class boundaries have been proposed it is necessary to test the suitability of the boundaries (with respect to the normative definitions and natural variability of the benthic invertebrate community). The class status assigned through the use of biotic indices has been compared to that assigned through 'expert judgement'. Expert judgement was based on (i) assessment of the general state of the water body by local (Area) Environment Agency staff and (ii) assessment of the faunal data matrix by benthic invertebrate ecologists from academia, consultant and government institutions. In the following exercise, AMBI (Section 5.2) is used to illustrate how the testing of boundary criteria has been approached.

(i) Assessment of the general state of the water body
Initially a general assessment of the water body, provided by staff local to the water body being considered, was used to ratify the biological metrics. Ecological assessment based on the biotic indices was compared to the status as indicated by the judgement of local staff based on their knowledge of pressures acting on the water bodies (e.g. discharges, physical disturbance).

It was hoped that a linear relationship between the calculated metrics and perceived ecological status could be established (e.g. Figure 3.4a). Following a series of exercises, however, no linear relationship could be established (Figure 3.4b).

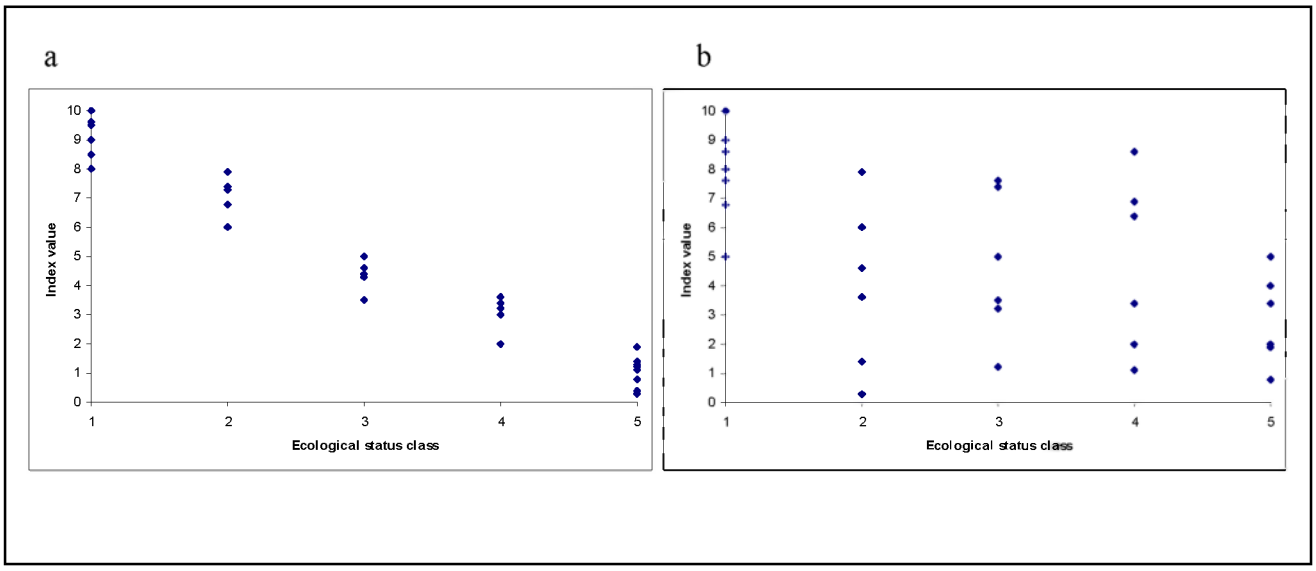

Figure 3.4 Comparison of ecological class status assigned through biotic indices and local assessment of pressures
a) expected outcome
b) example of results

Possible reasons for these disparities are:

1. the perceived pressures did not affect the benthic community
2. the severity of pressure on a water body was identified at a local (small geographical area) rather than at national level. This resulted in a large level of variability in assigning general status (based on pressures) of the water body. (This inconsistency has now been addressed as the EA pressures analysis has been carried out at a standardised, national level)
3. data at this time were only defined to EUNIS level 3 habitat (introduced variability due to natural habitat variation)
4. metrics considered were inappropriate for WFD assessment

It was considered that the variability introduced through points 1-3 meant that none of the metrics under consideration could be ruled out based on this exercise. Overall, there was no clear relationship between the metrics and the expert judgement of pressures on the water bodies.

(ii) Assessment of faunal data matrix
This compared the biological 'health' metric to the ecological status assigned by 'expert' interpretation of the taxa present and their abundance in the samples. The biotic metric used was AMBI (Borja *et al.* 2000, Section 5.2).

Three different datasets were considered, (i) NMMP (ii) Cardigan Bay and (iii) the Wash, during the workshop investigating the use of 'Expert Judgement' in October 2003. Details regarding the datasets are shown in Table 3.5.

Table 3.5 Datasets used in ecological status assessment based on marine benthic invertebrate taxa

	NMMP	Cardigan Bay	Wash
Year	1999-2002	2003	2002
Water body type	Various CW	CW 2 & CW 5	CW 4 & TW 3
Sediment type	Fine depositional sediment	Muddy sand (A 4.25)	Muddy sand, Mud
No. of stations	Numerous	12	66
No. of replicates at each station	5	4	3
Sample type	Day grab (0.1 m^2)	Day grab (0.1 m^2)	Day grab (0.1 m^2)
Mesh size (mm)	1.0	1.0	0.5

Although the *NMMP* targets fine, depositional sediments, the initial analysis of NMMP data indicated that samples were collected from a wide range of habitats. The variability this introduced was considered to be too large to allow status assessment based on the data. The NMMP dataset (1999-2002) has now been sent to James Allen (IECS) to assign stations to the correct EUNIS habitat type (methodology as for the establishment of the biotope classification of UK, Connor *et al.* 1997a,b).

Data from *Cardigan Bay* were collected by the project team as part of a 'rapid assessment' workshop (Section 6). The samples were collected using a standardised methodology (sample size, EUNIS habitat etc.). At the time of sampling, the overall impression of the sampled water bodies by the project team was that that they were at good to high status. This judgement was based on the lack of any visible pressures in the area and the macrofauna present in the sample (stable, diverse community). There was good agreement between the 'expert' judgement and the biotic index in this test (Table 3.6). Discrepancies were related to the ecological class weightings given to specific taxa in AMBI (Section 5.2).

Table 3.6 Comparison of the WFD ecological class assigned to samples from Cardigan Bay based on the metric, AMBI, and expert judgement based on the taxa present

EXPERT JUDGEMENT	AMBI					
	High	**Good**	**Moderate**	**Poor**	**Bad**	**TOTAL**
High	1	3				4
Good		8				8
Moderate						
Poor						
Bad						
TOTAL	1	11				12

The benthic invertebrate data from the *Wash* came from the Wash grid surveys (Section 6). The grid comprises of a total of 66 stations, approximately half of which were considered during the workshop exercise. The information considered by 'experts'

assigning ecological status was the ten most abundant taxa, the total number of taxa, the presence of any indicator species and details of the substratum.

A general idea of the ecological status of the water body was given by one of the workshop participants who regularly samples within the Wash:

> *'Although there is agricultural run-off and subsequent nutrient loads within the Wash, it is generally unpolluted. There is minimal light penetration due to high turbidity, sediment deposition and mixing from the estuaries. Consequently, there is little algal growth. Overall therefore, the Wash should be set at good to high status.'*

In general, the biotic metric indicated a higher ecological status than that assigned by the expert judgement (Table 3.7).

Table 3.7 Comparison of the WFD ecological class assigned to samples from Wash based on the metric, AMBI, and expert judgement based on the taxa present

EXPERT JUDGEMENT	AMBI					
	High	Good	Moderate	Poor	Bad	TOTAL
High	3	2				5
Good		18				18
Moderate	1	7	2			10
Poor		2				2
Bad						
TOTAL	4	29	2			35

These initial exercises emphasised the importance of taking into account habitat type and normalising sampling methodology (now incorporated in testing methodology in Phase III of the project). An assessment of the pressures acting on the water bodies in England and Wales has now been completed by the EA as part of the Risk Assessment exercise required by the WFD. This pressures matrix will be used in testing the proposed boundary criteria.

In addition, testing of boundary criteria will focus on benthic invertebrate data sampled along known impact gradients or data that have associated chemical measurements. As such the NMMP data will provide a valuable UK wide dataset, being comprised of matched biological and chemical parameters. In addition the project has highlighted a range of surveys carried out along known impact gradients (Appendix III). Kappa analysis (Fleiss, 1981) will be used to define the agreement between the biological 'health' metric and assessment of the water body based on known pressures or 'expert judgement' of the taxa present.

4. MACROBENTHIC INVERTEBRATE DATA

4.1 Historic Data

Comprehensive testing of classification tools relies on access to extensive, quality-assured benthic invertebrate data. Previous studies have highlighted the lack of access to such data. For example, Codling *et al.* (1995) stated that it had not been possible to demonstrate the feasibility of using the existing univariate measures as classification statistics because the existing datasets did not have the appropriate range and quality of information.

Historically benthic invertebrate data have been collected by the EA (previously NRA) for a wide variety of purposes. The majority of samples have been estuarine, with coastal water surveys being less common and tending to be focused around sewage discharges (Comprehensive Studies 1993-1995). The methods of collection of benthic invertebrate samples have varied over time (e.g. number of replicates, sampling gear and subsequent area sampled), as have the range of supporting natural environmental and pollution-related variables measured. This local, inconsistent monitoring, sometimes of unknown quality, has resulted in the comparison of sites being difficult on a national level.

Recognition of the need to co-ordinate marine monitoring in the UK led to the establishment of the National Monitoring Programme (NMP) in the late 1980's and the subsequent National Marine Monitoring Programme (NMMP, from 1999). The programme developed quality control procedures for chemical analyses and benthos identification (National Marine Biological Analytical Quality Control scheme, NMBAQC), which ensured that national consistent data of a high standard were obtained. All EA marine benthic invertebrate data, whether collected as part of the NMMP scheme or local initiative, are now subjected to the quality control procedures set out by the NMBAQC scheme.

The problem of inconsistent data sets has been compounded by the inaccessibility of the existing faunal data. Methods of storing data have varied from hard copies to electronically stored spreadsheets and quite often, this has not been stored in an easily retrievable format. Codling *et al.* (1995) recommended that a PC-based storage system be developed to store data related to macrobenthic surveys. The UNICORN© biological database system (Unicomarine Ltd.) has been used by the EA since 1992 and now forms a standard tool for holding macrobenthic data. The database has recently expanded to include marine fish and phytoplankton data. A significant resource of the MBITT has been spent in identifying and collating historic benthic invertebrate abundance data, including supporting parameters and then archiving the data onto the R & D database. In response to requests from the project, Unicomarine Ltd. has developed various modifications to UNICORN©, which have been necessary to test the classification tools. UNICORN4© is currently being rolled out within the EA.

The R & D database currently holds 413 separate benthic invertebrate surveys (13,095 samples, Figure 4.1). In addition to the EA R & D database, MBITT has access to the NI UNICORN© database that holds the macrobenthic data for EHS (NI) and the Marine Nature Conservation Review (MNCR) database. MBITT therefore now has access to macrobenthic invertebrate data from a substantial number of quantitative and qualitative surveys around the UK.

Checks on the quality of data held on the R & D database have been carried out to ensure that MBITT has confidence in the data and the subsequent testing of indices. Those surveys for which there are no supporting parameters or for which no quality assurance exists are still archived in the R & D database as the faunal data may provide useful background benthic macroinvertebrate data. Further data to be imported has been sourced, much of which has already been collated. Priority is allocated to datasets that match standard methodology, have good supporting data (e.g. particle size analysis, sediment chemistry) and are quality assured. In particular the project is keen to locate macrofaunal data that pertains to defined pressure gradients (see Appendix III).

The collation of the EA's historic macrofaunal data into a national database has provided a valuable resource for assessment of benthic macroinvertebrate communities. Data are linked through a GIS interface allowing MBITT to identify, for example, where relevant datasets for the water body type being assessed are located, and where data gaps exist. The benthic invertebrate data will be made available to the National Biodiversity Network (www.nbn.org.uk).

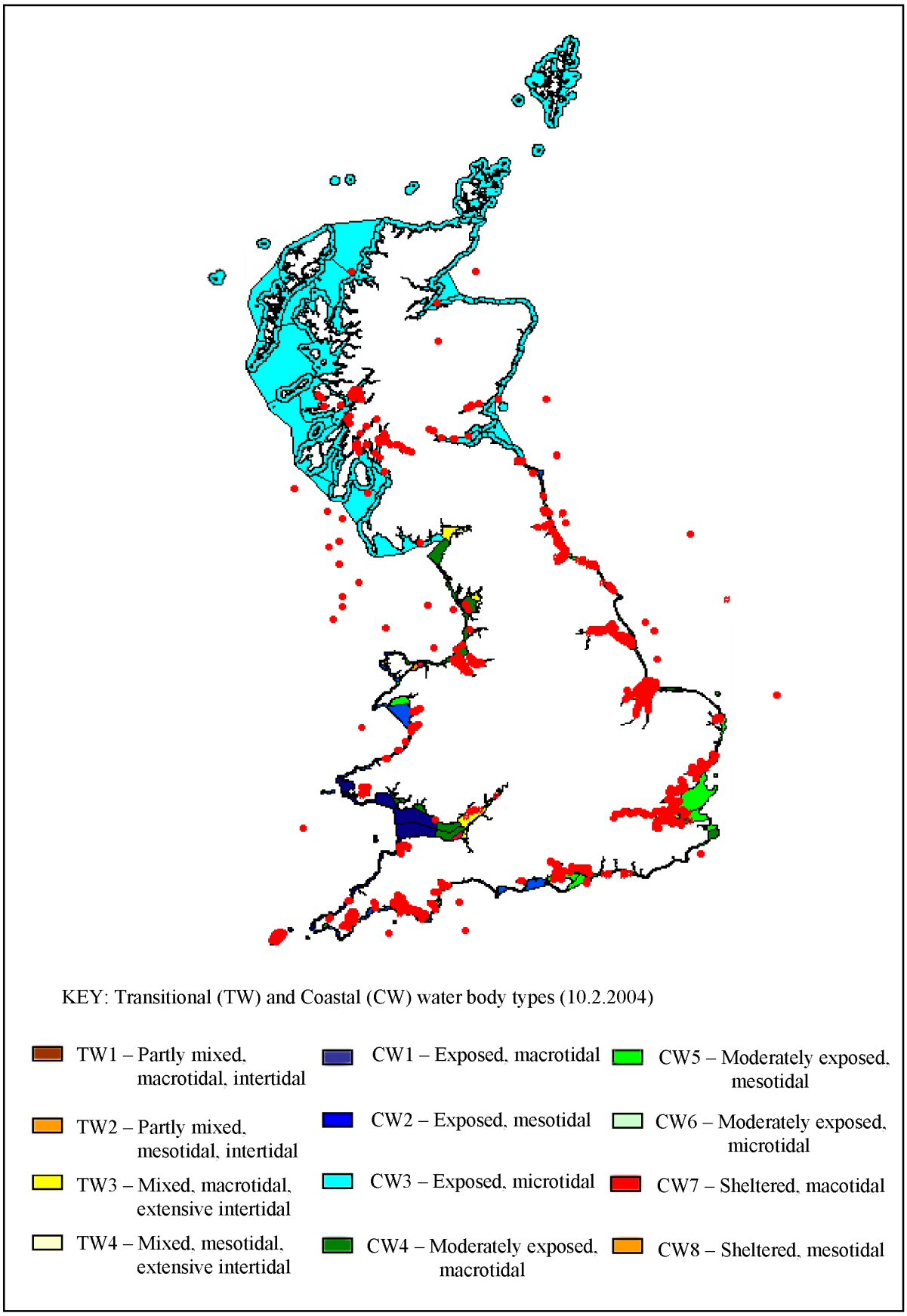

Figure 4.1 UK benthic sampling locations held on R & D database (UNICORN©) for which data are available (February 2004)

4.2 WFD sampling for classification tools 2004 (Phase III)

As well as analysing historic data, resources have been allocated within the EA WFD programme for the collection of dedicated macrofaunal data for the purpose of testing WFD classification tools and for the Intercalibration exercise. Intercalibration of classification tools and allocation of ecological status is a mandatory exercise which must be carried out by Member States to ensure consistency across Europe in assessing water bodies. For the majority of water body types in England and Wales (CW 1, 2, 4, 5, 7, 8 and TW 1, 2, 3, 4), samples are being collected from targeted EUNIS habitat types within a water body representative of each type. It is hoped that similar samples can be collected in Scotland, NI and ROI, in particular from water body types that are not found in England and Wales.

At this stage, sampling is all sublittoral, soft substratum and sampled between January and May (as defined in NMMP procedures). Within the targeted habitat type in the water body, three stations of five replicate macrofaunal samples are taken using a Day grab (standardised sample area). A sixth sample is taken for particle size analysis. Data are being quality assured according to the NMBAQC scheme. It is hoped that this targeted sampling will allow us to define the level of variability shown by the benthic invertebrate communities and how the variability is reflected in the classification tools. Data are being collected from areas that we perceive to have minimal levels of anthropogenic disturbance in order that these sites can be used in the establishment of reference conditions. Once these are established, macrofaunal samples collected from areas of known pressures will be used to help define the ecological status boundaries.

Sample collection to date, has highlighted problems with grab sampling in sandy sediments where at best only surficial sediments (often <5 cm) are sampled providing an unrepresentative sample for classification. Phase III will investigate more appropriate sampling methods for these types of sediments.

4.3 Data Truncation

Analysis has shown that to increase the sensitivity of the classification tools, sample data needs to be standardised for the habitat, salinity regime, sampling method, sample size and season. In addition, the identification of taxa and the level of discrimination of the taxa needs to be established. Benthic macrofaunal identification in the laboratory is now regulated through the NMBAQC scheme. In the majority of analyses (but not all), this will result in all taxa (including freshwater fauna and epifauna) being identified to the lowest possible taxonomic level. However, even with the strict quality assurance now in place, there is still some discrepancy between laboratories with respect to the lowest taxonomic level possible (related to the specialised taxonomic fields of the analysts). For example, Oligochaeta can be identified to just class level by some laboratories but to species level by others. Such disparities give the impression of greater richness in some data sets compared to others.

Historic data are often more variable compared to current analyses in terms of taxa that were identified (local expertise and pre-standardised quality assurance schemes). The habitat that was sampled can also be difficult to establish if records are not complete. As such, some caution needs to be used when interpreting historic data on a national scale.

On a local level the variability due to habitat and taxonomic identification may be a minor issue as data are more likely to be treated consistently. When data are compared on a wider geographical scale however, such variability in the sample taxonomic identification can render the classification tools impotent i.e. taxonomic variability creates sufficient background noise to obscure the detection of changes in ecological status due to anthropogenic impact. The robustness of the proposed classification tools towards taxonomic discrimination will continue to be considered by MBITT in Phase III.

Initial testing of the behaviour of classification tools for soft sediment benthic invertebrate communities highlighted the need to standardise data prior to analysis. MBITT has therefore worked to establish data truncation rules (at this stage only for soft sediment samples). The rules for truncation were discussed with external 'experts' at a WFD benthic invertebrate workshop (October 2003) using datasets supplied by MBITT. The following truncation rules were formed on the study of the datasets provided at the October 2003 workshop.

For all soft sediment data sets in sites with salinity greater than 10, the following groups were removed: algae, fish, insects, planktonic taxa, encrusting taxa and any freshwater taxa e.g. Cladocera. All juveniles were removed. Based on these rules, the taxa that were to be deleted are summarised in Table 4.1. Examples of the encrusting Cnidaria to be deleted are shown in Appendix IV. Taxa within the following taxonomic groups were combined to the level specified to negate the problem of inconsistent levels of identification: Oligochaeta, Nemertea, Platyhelminthes, Echiura, Chelicerata, Sipuncula, Phoronida, *Priapulus sp.,* and Types A, B, C etc to genus.

Any taxa only identified to phylum level were also removed (with the exception of fauna from the following Phyla; Nemertea, Platyhelminthes, Echiura, Chelicerata, Sipuncula, Phoronida).

Table 4.1 Taxonomic classification of taxa that should be deleted from soft-sediment data sets in salinities greater than 10. Taxa in bold are those that should be removed. Those not highlighted provide an aid for the taxonomic classification of those to be truncated

Phylum	Class	Order	Family	Genus & Species
Protozoa (all except *Astrorhiza sp.)*				
Porifera				
Ctenophora				
Nematoda				
Entoprocta				
Chaetognatha (all except *Spadella sp.*)				
Crustacea	**Branchiopoda**			
	Maxillopoda			
	Eumalacostraca	**Mysidacea**		
		Decapoda	**Crangonidae**	
		Euphausiacea		
Mollusca	Gastropoda	Anaspidea	Akeridae	***Akera bullata***
			Aplysiidae	***Aplysia depilans***
				Aplysia punctata
	Gastropoda	Notaspidea	**Pleurobranchidae**	
	Gastropoda	**Nudibranchia**		
Brachiopoda				
Bryozoa				
Tunicata				

These rules regarding the truncation of the soft sediment taxa are not absolute and all data sets must be analysed to identify any further encrusting or planktonic taxa, which have not yet been listed.

These truncation rules have been applied to all datasets on which indices have now been calculated. As an indication of the difference that truncation can make to a data set, 217 taxa (15%) were truncated from the NMMP data set held on the R & D database (February 2004) prior to index calculation. Once these truncation rules have been reviewed by benthic experts, it is hoped that they will be applied on a much wider scale for analytical quality control between WFD regulators and the scientific benthic community as a whole. Therefore, these rules will be put forward to the NMBAQC team and European AQC groups for review. These rules may require further development as further habitats, e.g. hard substrata, are included in testing for the establishment of classification tools.

5. CLASSIFICATION TOOLS

5.1 Metrics and the multimetric approach

The project was not established to develop new indices (metrics) for the assessment of benthic invertebrate communities, rather to assess the use of existing metrics for WFD ecological assessment. Statistics used in assessing benthic macrobenthic communities have been reviewed in many texts (e.g. Nixon *et al.* 1992a, Warwick and Clarke 1991, Miles and Price 2002). As such descriptions of the metrics being considered will not be included here, with the exception of those novel indices that have been tested specifically by the project. The metrics that have been considered are:

Univariate: Number of taxa (S)
Abundance (N)
A/T (ratio of dominance: Abundance / Number of taxa)

Diversity: Shannon Weiner (H')
Pielou (J')
Fisher's α
Brillouin (H)
Simpsons (1-λ')
Margalef (d)
Average Taxonomic Distinctness (AvTD) (Clarke & Warwick, 1998)

Functional: Infaunal Trophic Index (ITI) (Word, 1979), for the UK (Codling & Ashley, 1992)
AZTI Marine Biotic Index (AMBI) (Borja *et al.*, 2000).

The link between these indices and the normative definitions of the WFD is shown in Section 2.1.

In order to assess the use of the indices, testing needs be carried out on standardised datasets. The result of any index is a reflection of the contents of a sample (e.g. number of individuals, taxonomic relationships). Therefore, index values will vary in response to variables such as sampling method, sieve mesh size, number of replicates, inconsistencies in taxonomic identification, habitat type, as well as to changes in the benthic invertebrate community due to anthropogenic disturbance. In order to distinguish the latter, the 'noise' created by the former variables has to be minimised. Standard methodologies such as those laid out in the NMMP and the use of the National Marine Biological Analytical Quality Control (NMBAQC) scheme accredited laboratories, go some way towards minimising this variation. The project has further minimised 'noise' by interpreting metrics within the context of the habitat (as defined by EUNIS, Section 3.2), season, mesh sieve size and sample size.

Although all indices have particular benefits and drawbacks, a single metric used in isolation calculates only one measurable characteristic of a benthic community. Weisburg *et al.* (1997) found that a combination of metrics was able to distinguish between reference and degraded samples more effectively than one metric alone. They attributed this to the staged response of benthos to stress, in which different metrics display greater

response with different degrees of perturbation. As a result the project is following a 'multimetric' approach to the WFD assessment of benthic invertebrate communities.

The metrics included in the multimetric will be selected based on habitat type and salinity regime combinations. However, it is envisaged that the selected metrics may be relevant to a range of habitats. The individual metrics incorporated need to be able to detect change in a characteristic of the community. At the same time, the multimetric should avoid combining metrics that have strong linear correlation. For example, the Shannon-Weiner diversity index and percentage abundance of dominant taxa are highly correlated (Hamill & Ellis, 2003). These two metrics, therefore, explain the same variation in the data.

In order to apply the most appropriate indices within a multimetric, Principal Components Analysis (PCA) will be carried out on the full set of indices calculated on benthic invertebrate data from a particular habitat type and salinity regime. PCA provides a method by which to select those metrics that best describe the variation shown in the benthic invertebrate community. The distribution of indices within the PCA relates to their similarity. The indices that cluster together describe the same variation within the data. Indices that account for different parts of the variation will be selected for incorporation into the multimetric.

To illustrate the approach, a PCA was carried out on indices calculated for a subsection of NMMP benthic data (Figure 5.1) (This PCA has been included only for example purposes, as the data has now been further refined and the selection of metrics has not necessarily remained the same). Abundance (N) and the Infaunal Trophic Index (ITI UK) are quite separate from all the other univariate, diversity and functional indices (Figure 5.1a). If N and ITI UK are then excluded from the PCA analysis, species richness (S) and AZTI Marine Biotic Index (AMBI) emerge to explain further the variation within the dataset (Figure 5.1b). N, ITI, S and AMBI would therefore be considered for inclusion within the multimetric.

Before indices can be included in the multimetric, the way in which they respond to pressures (e.g. organic enrichment, hazardous substances, and physical disturbance) needs to be taken into account. The predicted response of the benthic invertebrate community to pressures and the potential ability of the proposed metric to measure such a response therefore needs to be defined. The response of many of the statistical indices has been previously considered (e.g. Nixon *et al.* 1992b, Warwick and Clarke 1991) but less is known about the application of the two novel indices, AMBI and AvTD. As such the project used the UK benthic invertebrate datasets to examine the behaviour and limitations of these metrics, prior to their possible inclusion in a multimetric (sections 5.2 and 5.3).

The suitability of indices included within the multimetric for each habitat type and salinity regime combination will be considered on an individual basis, according to knowledge of the way in which they respond to particular pressures.

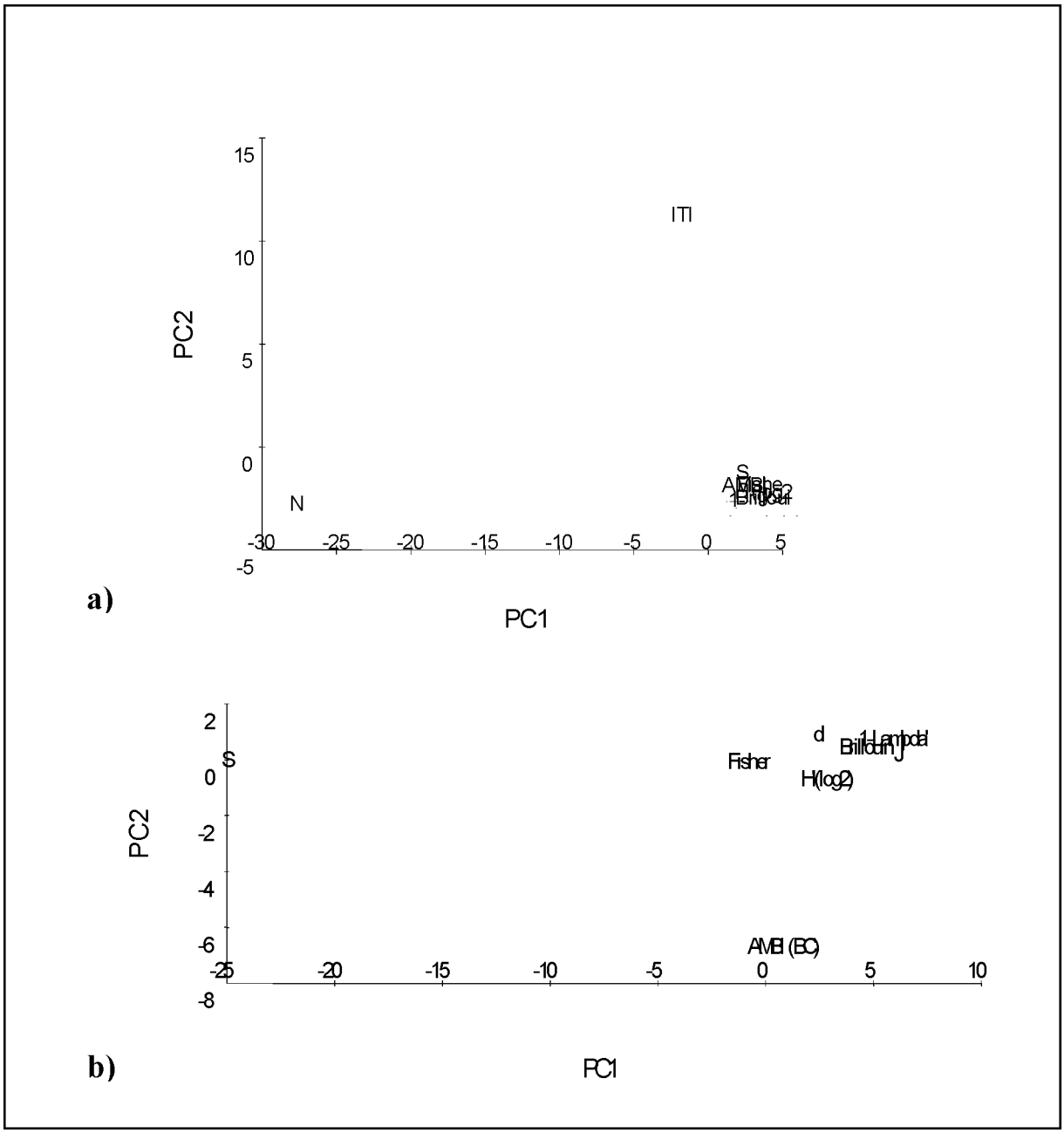

Figure 5.1 **a) PCA of univariate, diversity and functional indices calculated for 95 NMMP Day grab (0.1 m^2) samples (>0.5 mm mesh) (83.9% of variation is explained in PC1).**
b) Expansion of Figure 5.1a to show indices in cluster, with N and ITI removed from the analysis (92.2% of variation is explained in PC1).

5.2 AZTI Marine Biotic Index (AMBI, Borja *et al.*, 2000)

The AMBI index Borja *et al.* (2000) is an expansion of the ideas of Grall and Glémarec (1997). AMBI was devised to establish the ecological quality of European coasts, using the response of soft-bottom communities to natural and man-induced changes in water quality. The index is derived from the proportions of individual abundance in five ecological groups (Table 5.1), which are related to the degree of sensitivity/tolerance to an environmental stress (organic) gradient (Figure 5.2) (Pearson & Rosenberg, 1978).

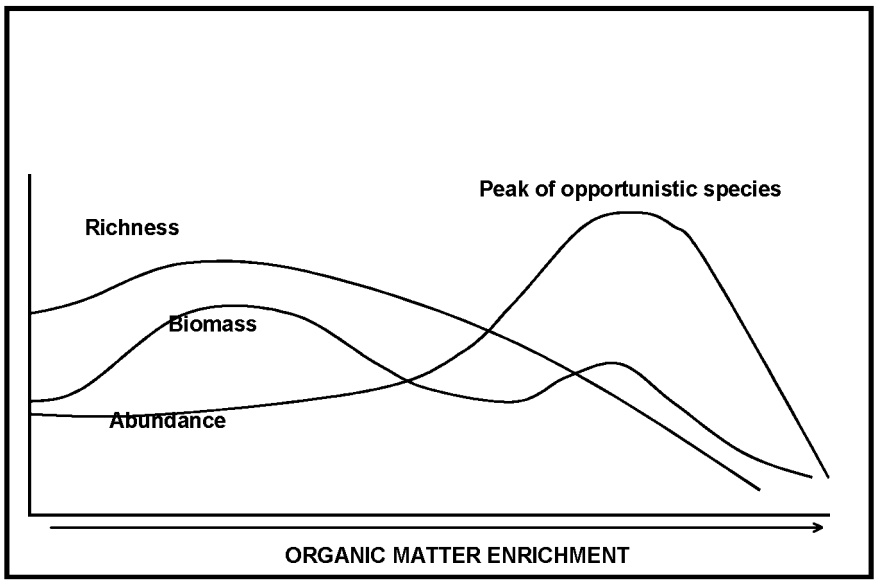

Figure 5.2 Model developed by Pearson and Rosenberg (1978) to show changes in species richness, biomass and abundance in response to an environmental stress (organic) gradient.

The relative proportion of each ecological group gives an indication of the level of disturbance at the site (Figure 5.3). Predominance of the sensitive (GI) taxa indicates undisturbed conditions. During slight unbalanced situations, GI taxa decline whilst species indifferent and those tolerant of enrichment (GII and GIII, respectively), rise in abundance. These are eventually superseded by second order opportunistics (GIV) and first order opportunistics (GV) as the gradient increases towards pronounced unbalanced conditions. Finally, when the gradient is so severe and azoic conditions occur, all groups are removed (Figure 5.3). The proportions of taxa at each stage of the organic gradient can be related to the normative definitions of all or most sensitive taxa present with respect to high and good status, respectively, and many sensitive taxa absent with regard to moderate status.

Table 5.1 Description of the ecological groups (GI to GV), including examples of possible indicator taxa, used in the calculation of AMBI (Borja *et al.*, 2000).

Groups	Description	Indicator Species
GI	Species very sensitive to organic enrichment and present in unpolluted conditions (initial state). These include most of the specialist carnivores and some deposit feeding tubiculous polychaetes.	Sensitive species different from one community to another.
GII	Species indifferent to enrichment, always present in low densities with non- significant variations with time (from initial state, to slight unbalance). These include most of the suspension feeders, less selective carnivores and scavengers.	*Nephtys hombergii*, *Marphysa belli*, *Glycera spp.*, *Nereis caudata*, *Platynereis dumeri*
GIII	Species tolerant to excess organic matter enrichment. These species may occur under normal conditions, but their populations are stimulated by organic enrichment (slightly unbalanced situations). They are mainly surface deposit-feeding species.	*Spio martinensis*, *Notomastus latericeus*, *Phyllodoce mucosa*, *Pygospio elegans*, *Nereis diversicolor*, *Prionospio malmgreni*, *Abra alba*
GIV	Second order opportunistic species (slight to pronounced unbalanced situations). Mainly small sized polychaetes, e.g. subsurface deposit feeders, such as cirratulids.	*Capitomastus minimus*, *Polydora spp.*, *Cirratulus cirratus*, *Cirriformia tentaculata*, *Chaetozone setosa*, *Heterocirrus sp.*, *Staurocephalus rudolphi*
GV	First order opportunistic species (pronounced unbalanced situations). These are mainly deposit feeders, which proliferate in reduced sediments.	*Scololepsis fuliginosa*, Oligochaeta, *Capitella capitata*, *Capitellides giardi*

To calculate AMBI, taxa are allocated to one of the ecological groups (Table 5.1). The following formula is then used to calculate the biotic coefficient (BC) for a sample:

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

The biotic coefficient is based upon the proportion (percentage of abundance) of each ecological group within a sample. The formula provides a value between zero and seven (zero indicates that all GI taxa are present and seven indicates that the sample is azoic).

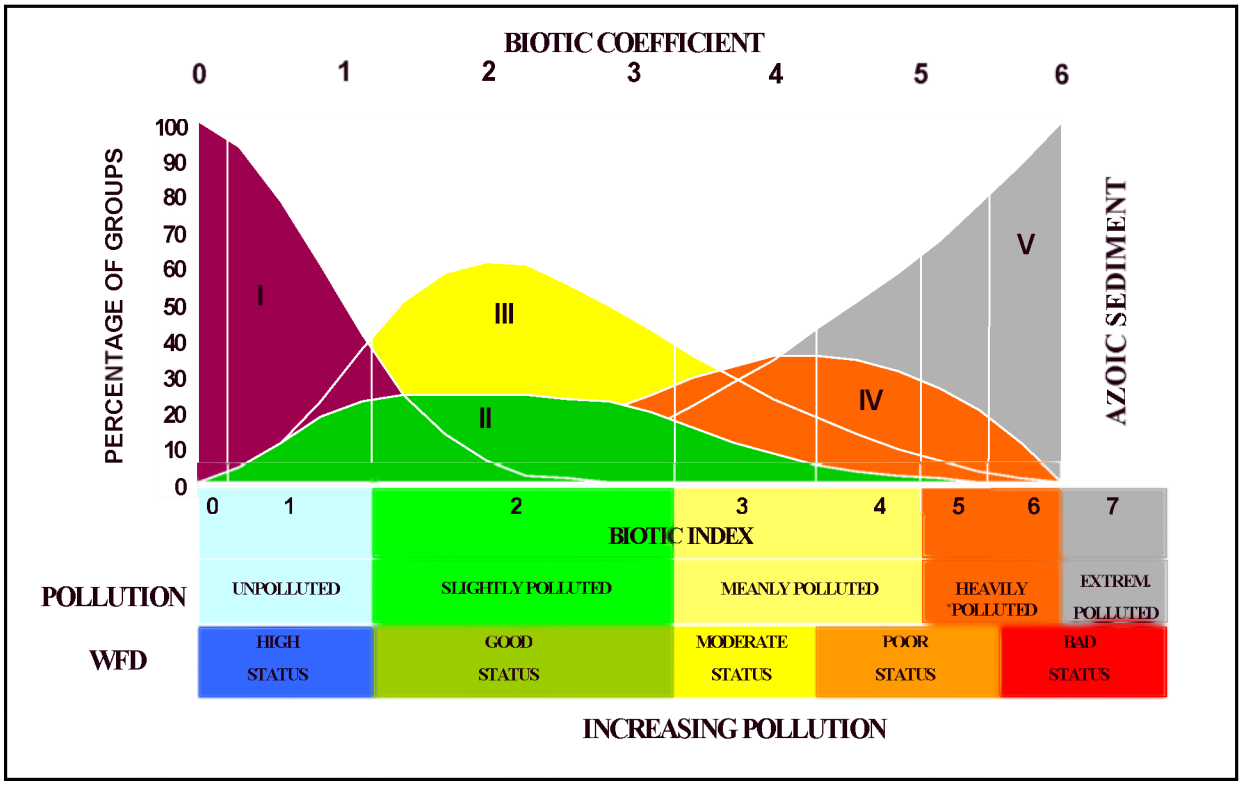

Figure 5.3 **Diagram illustrating how the AMBI biotic coefficient (BC), calculated from the ecological groups present in a sample, can be used for benthic invertebrate assessment. Pollution classification boundaries (Borja *et al*, 2000) and suggested class boundaries for WFD (Borja *et al.*; 2003) are displayed.**

Borja *et al*. (2000) used the AMBI BC values to assign pollution classification boundaries (Table 5.2). These demarcations have, in turn, been used to suggest possible class boundaries for the Water Framework Directive (Borja *et al*., 2003).

Table 5.2 **Range of AMBI biotic coefficient values, associated pollution classification and suggested WFD ecological classes, as per ranges described in Borja *et al*., 2000.**

Biotic Coefficient	Pollution Classification	Suggested WFD Ecological Status
0.0< BC ≤1.2	Unpolluted	High
1.2< BC ≤3.3	Slightly polluted	Good
3.3< BC ≤5	Meanly polluted	Moderate
5< BC ≤6	Highly polluted	Poor
>6	Extremely polluted	Bad

The project is currently testing the AMBI biotic coefficient values to determine whether it is necessary to modify these preliminary boundaries set by Borja *et al*. (2003) in order to

use AMBI for WFD assessment in the UK and ROI. Further work will establish the robustness of the classification tool with respect to salinity regime and habitat (see worked example of the Colne transitional water, Appendix V).

The project has been in close contact with the key developer of the AMBI index, Angel Borja. To calculate the index, Borja has created a Microsoft© Excel look-up table (used by the project) and a downloadable program. These are freely available from aborja@pas.azti.es and at www.azti.es. Following testing of AMBI in relation to a large range of pressures, Borja *et al.* (2000) have concluded that AMBI is sensitive to a range of different impact sources but is insensitive to seasonal variability. As such, Borja *et al.* suggest that the index could be used in the determination of ecological status in the WFD. The pressures investigated include engineering works (Borja *et al.* 2000, 2003), heavy metal inputs (Borja *et al.* 2003), organic enrichment (Borja *et al.* 2000, 2003), oil platforms and hydrocarbons (Borja *et al.* in prep.)).

Whilst testing AMBI using UK datasets, 500 taxa have been identified by the project that do not have ecological groups assigned to them. Within the index, if 10% or more of taxa within a sample are unassigned (i.e. no ecological group is assigned to the taxa) there is a higher risk of misclassification. As a temporary solution, the project allocated ecological groups to some taxa by assigning the group associated with related taxa. For instance, if all other species within the same genus already had an ecological group assigned, the group was extrapolated to the unassigned taxon. This list was sent to Borja and colleagues to assess and include in the AMBI index species list. The inclusion of these additional taxa by Borja (as custodian of the list) ensures uniformity across the users of AMBI throughout Europe.

The AMBI list has been sent (February 2004) to UK benthic experts for further review on the allocation of taxa into particular ecological groups. Feedback has also been requested on taxa for which little or no ecological information has been compiled. Once gaps in ecological information have been highlighted it may provide a focus for any future research to expand on the ability of presence/absence of taxa to indicate impacts. In addition to this gap analysis, it is hoped that feedback will include further sources of literature that might aid in the allocation of ecological groups. This will prove useful to both MBITT and Angel Borja.

The project team continues to test the performance of AMBI in relation to WFD assessment. Under certain circumstances, caution needs to be exercised with AMBI scores e.g. where the number of individuals and the number of taxa within the sample are low. For instance, in upstream low salinity environments or estuarine intertidal sites, the fauna may have a naturally low diversity. If the taxa present have high ecological group e.g. Oligochaeta, this would give an overall high biotic coefficient, indicating an impacted environment where this might not actually be the case.

Despite such precautions, the AMBI index is proving to be very useful as its response to a variety of pressures has been tested (Borja *et al.*, 2000, 2003). The ranges of AMBI values have also been used to formulate pollution classification boundaries, which correspond to WFD ecological status classes. Results of tests carried out using AMBI have been discussed with Angel Borja and it is likely that AMBI will prove invaluable within the multimetric approach.

5.3 Average Taxonomic Distinctness (AvTD, Warwick and Clarke, 1998)

5.3.1 Introduction

Average Taxonomic Distinctness (AvTD, Δ^+) is a measure of biodiversity based on taxonomic distance between species. AvTD measures the relatedness of taxa by considering the phylogenetic structure of a benthic invertebrate assemblage. An assemblage comprised of a group of closely related species is considered less diverse than one with the same species richness, but with more distantly related species (Clarke and Warwick, 2001a). Therefore, AvTD can be thought of as measuring the taxonomic breadth of a sample. This is done by measuring the average distance apart of all pairs of species in a sample, traced through a taxonomic tree (Clarke and Warwick, 2001b). Traditional diversity indices are based on abundance data, whereas AvTD is calculated using presence/absence data.

The taxonomic tree used for the investigation of AvTD is based on the species directory for the British Isles developed by Howson and Picton (1997). This has been further developed within the functionality of the UNICORN© database to produce a six level phylogenetic hierarchy (no sub- or super- levels):

- Species
- Genus
- Family
- Order
- Class
- Phyla

An advantage of this index is that it incorporates an expected mean AvTD value. In terms of WFD assessment, this could be considered to represent reference conditions. The expected mean AvTD is based upon a master list of taxa, which is reflective of the species pool of a region/biogeographic area. The null hypothesis of AvTD is based on the expectation that the species present at any one place or time behave like a random selection from the species pool. Benthic invertebrate samples taken from undisturbed sites within the region should likewise meet the expected AvTD values of the master list.

A second advantage of this index is that it incorporates a statistical framework from which to measure departure from expected (reference condition). Clarke and Warwick (1998) devised a randomisation test to compare the observed value of AvTD against an expected value derived from the master list of species. The AvTD randomisation test is carried out in the Plymouth Routines in Multivariate Ecological Research (PRIMER© (Copyright© 2001 PRIMER-E Ltd, www.primer-e.com)) statistical package, using the TAXDTEST routine. This calculates whether the species in a sample are representative of the biodiversity expressed in the full master list of species (Clarke and Warwick, 2001a). Random sub-samples (typically 1000) of a fixed number of species are drawn from the master list to calculate the distribution of AvTD values. This distribution is plotted as a frequency histogram showing the range of expected AvTD values. The values falling outside of the 95% probability interval are considered to have a lower than expected taxonomic spread (Clarke and Warwick, 1998). This establishes the statistical framework that allows detection of departure from expectation (Somerfield *et al.* 2003). Repeating this procedure for different numbers of species, the upper and lower limits of the 95%

probability intervals for each sublist size can be plotted. Connecting the 95% probability interval points creates a funnel plot. Likewise connection of the mean AvTD value for each sublist size displays a mean line through the funnel (Figure 5.4). The AvTD values of real sample data can then be plotted onto the funnel plot to give a visual display of their proximity to the 95% probability limits and the expected mean AvTD (Figure 5.4). The project has investigated AvTD for use as a WFD compliant classification tool with the developers, K.R. Clarke, R.M. Warwick and P.J. Somerfield at Plymouth Marine Laboratory (PML). The work developed in three stages:

- DEFRA indicator study on NMMP data
- WFD initial trials (regional data)
- WFD final trials (EUNIS habitat type and salinity)

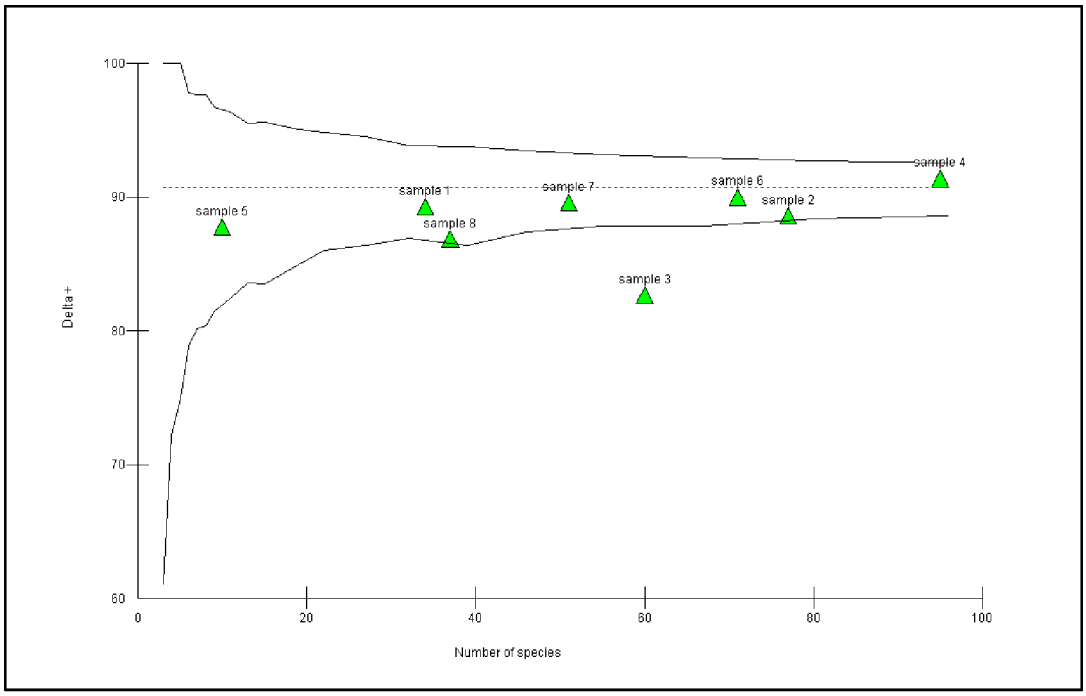

Figure 5.4 **Probability funnel to illustrate AvTD. Points represent the AvTD scores for eight samples. The horizontal line represents the simulated mean AvTD derived from random sub-samples (typically 1000) of a fixed number of species, this is repeated for each species sublist size. The converging lines above and below the mean are 95% probability limits.**

5.3.2 DEFRA indicator study - NMMP data

In January 2003, MBITT participated in an exercise to investigate the NMMP dataset (1999-2001). The overall aim was to assess the utility of AvTD as a measure for detection and monitoring of environmental change as laid out for DEFRA indicators. Three particular aspects were explored:

- the temporal and spatial patterns of the NMMP dataset

- variation in environmental variables
- comparison of AvTD with commonly used marine benthic indices

In order to address inconsistencies in identification of taxa, the NMMP data was truncated according to the rules set out by the NMBAQC committee (16th October meeting). Truncation reduced the master taxon list (species pool) from 1137 to 862 taxa. This proved to be a time consuming exercise and raised the importance of rules for data truncation. The replicates at each station were pooled to give a station-year combination. Factors were then used to discriminate between the sampling occasions on the basis of year, site, region, number of replicates, sampling method and whether samples were from transitional or coastal water bodies. The study found that the more traditional measures of diversity (S, N, d, J', Brillouin, Fisher's α, H' and 1-λ') varied significantly between sampling occasions, which varied according to location, sample size, year and sampling method (Somerfield *et al.*, 2003). In contrast, AvTD did not vary significantly with these factors. This lack of response to temporal, spatial and sampling aspects of the dataset, combined with an expected mean AvTD which is independent of the number of species, led to the suggestion that a common reference condition may be a possibility for this index (Somerfield *et al.*, 2003).

The response of the indices according to sediment metal concentrations (Pb, Ni, Cu, Zn and Cd) and total organic carbon (TOC) was also explored. The AvTD values for the coastal samples had a significant positive correlation with TOC (Table 5.2). Transitional water samples had a significant negative correlation with TOC and metal concentrations (Table 5.3). The only other biological index to show a significant correlation with the environmental variables was number of taxa. This index displayed a significant positive correlation with metal concentrations. Based on the NMMP study, AvTD was considered the more sensitive measure of biodiversity and environmental health (Somerfield *et al.*, 2003).

Table 5.3 Coastal water NMMP (1999 – 2001) samples: Correlations between a range of indices, TOC and metals for all NMMP data (1999-2001). Values of the correlation coefficient above 0.21 are significant at the 95 % probability level (*) (Somerfield *et al.*, 2003).

Measure	TOC	Metals
S	0.114	0.098
J'	-0.204	0.038
H'	0.023	0.026
1-λ'	-0.044	0.072
Δ^+	0.285*	-0.157
Metals	0.089	

Table 5.4 **Transitional water NMMP (1999 – 2001) samples: Correlations between a range of indices, TOC and metals for all NMMP (1999-2001) transitional water samples where data were available. Values of the correlation coefficient above 0.26 are significant at the 95 % probability level (*) (Somerfield *et al.*, 2003).**

Measure	TOC	Metals
S	0.118	0.243*
J'	-0.042	-0.063
H'	0.084	0.037
1-λ'	0.085	0.094
Δ^+	-0.310*	-0.330*
Metals	0.514*	

Within the NMMP study, the question of how AvTD might be used to classify samples according to their ecological status was addressed. Figure 5.5 illustrates a possible scheme. This scheme only considers a decline in AvTD as being detrimental because an anthropogenic impact is unlikely to increase the taxonomic diversity of a sample (Somerfield *et al.*, 2003). Samples which have AvTD values lying outside the 95% probability limit were defined as 'moderate or worse'; samples with values lying between the 90 and 95% probability contours were defined as 'good', and samples with values above the 90% contour were defined as 'high' (Somerfield *et al.*, 2003). It is hoped that the classification of samples into ecological status bands will make the interpretation of the index much easier. Further testing of the index will be required in order to set appropriate probability level bands for each ecological class.

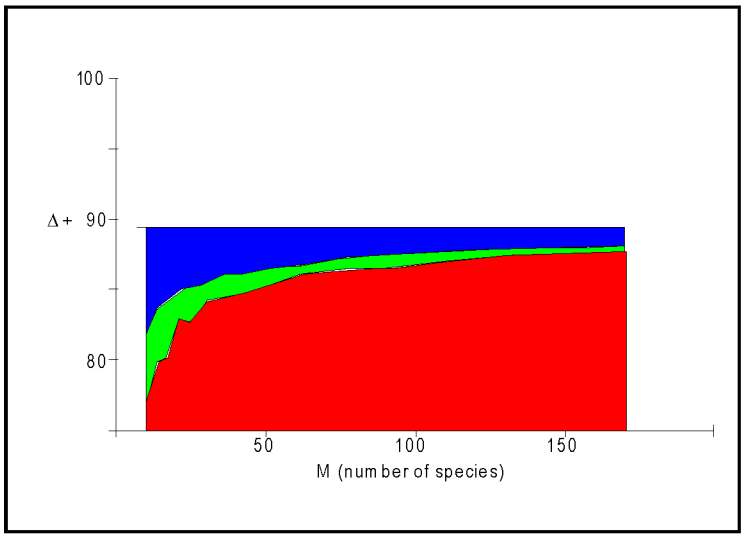

Figure 5.5 **Illustrating how a scheme may be constructed defining status on the basis of AvTD and probability contours. Samples falling within the blue area of the graph are classified as 'high', samples in the green area as 'good' and samples in the red area are 'moderate or worse' status (Somerfield *et al.*, 2003).**

Following these investigations, the NMMP report recommended:

- Refinement of the appropriate species lists for different regions or sets of conditions
- Determine further the relationships of taxonomically-based indices with environmental variables
- Define how AvTD can be used in the construction of metrics for management and monitoring purposes
- Determine further the relationships of taxonomically-based indices with ecosystem function

There are many advantages to AvTD, not least its independence from numbers of species and the fact that it has in-built reference conditions (Somerfield *et al.*, 2003). Both of these benefits and the recommendations were further examined by MBITT. The results of these investigations were discussed with the AvTD developers during two periods in April and October 2003.

5.3.3 Regional Data (April 2003)

The data held within the R & D UNICORN© database was divided into Environment Agency Regions for trial of wider data. Rather than having biological relevance, this division provided management units for data trials. The taxa from the data for each of these Regions were used as the species pool (master list). The replicates at each station were pooled and the AvTD values for each station-year combination were plotted against the appropriate Regional funnel. For example, Figure 5.6 shows the AvTD values for the Wash grid survey (1991, 1993 and 1999) dataset. The master list funnel was created from all the benthic surveys within Anglian Region transitional and coastal waters (Humber, Wash, Ore, Alde, Stour, Roach, Crouch, Orwell, Deben, Colne and Blackwater) and was used to create the reference funnel plot and mean AvTD (Figure 5.6). It was found that a substantial number of sample points fell outside of the funnel, indicating they showed a significant departure from expected, i.e. mean AvTD. This indicated that the diversity of the samples was less than expected.

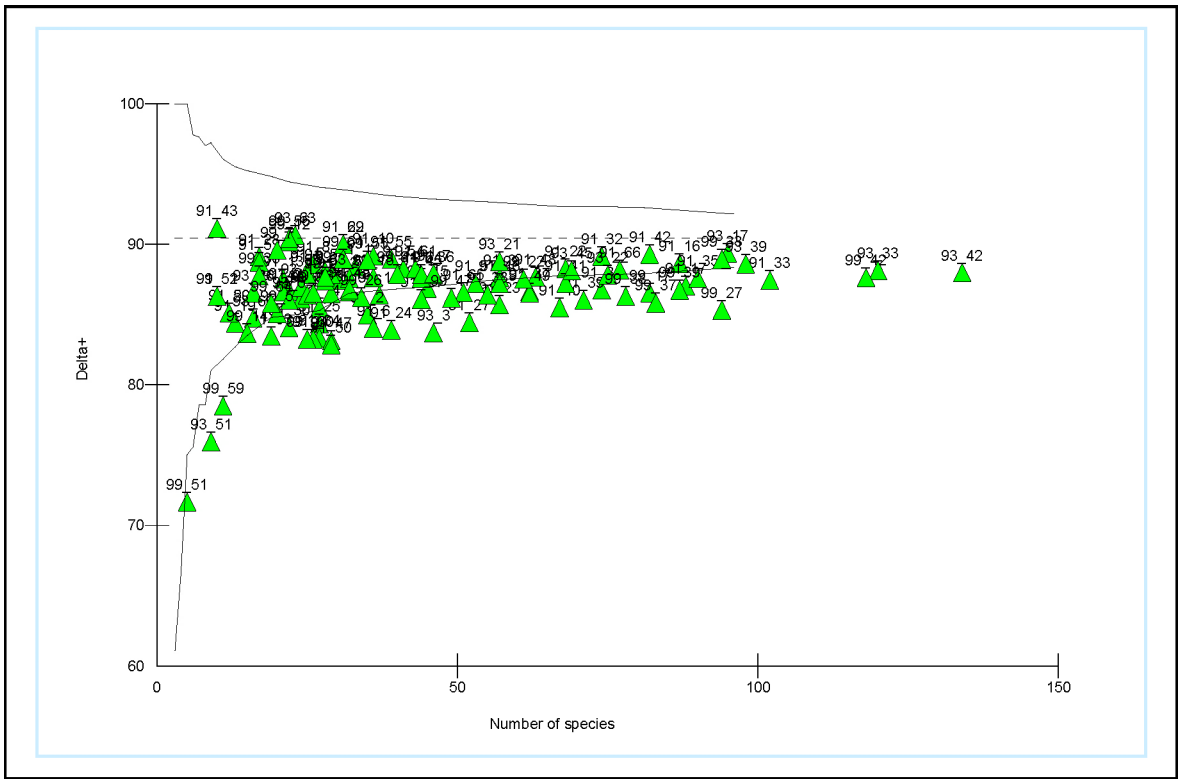

Figure 5.6 **AvTD values of Wash grid survey stations (combined replicates) sampled over three years (1991, 1993 and 1999), plotted against the 95% probability limits funnel based on all marine benthic data from Anglian Region (the expected mean AvTD is indicated by the dotted line).**

As shown in Figure 5.6, the sample points appeared to form the shape of a funnel that was below the expected funnel created by the master list. This result was seen for the majority of data plotted against respective Regional funnels. This consistent pattern over the large amount of data interrogated led to the further investigation of the master taxon list and how its formation affects the resultant AvTD values. Initially, it was thought that the taxon list could be quite broad and based upon biogeographic area (Clarke and Warwick, 2001a). The results, which were consistently falling below expected AvTD, suggested that the master list needed to be further defined.

It was considered that the master list should be specific for salinity. Anglian data were therefore pooled to one of four salinity ranges; coastal (30+), outer (20-29), mid (10-19) and upper (0-9) and plotted against the master taxon list (Figure 5.7). Data from the upper and outer salinity ranges departed significantly from expectation whilst the mid and coastal salinity range data fell on the 95% probability limit. As none of the salinity ranges fell within the expected AvTD, it was suggested that the master list needed to be more specific with respect to salinity.

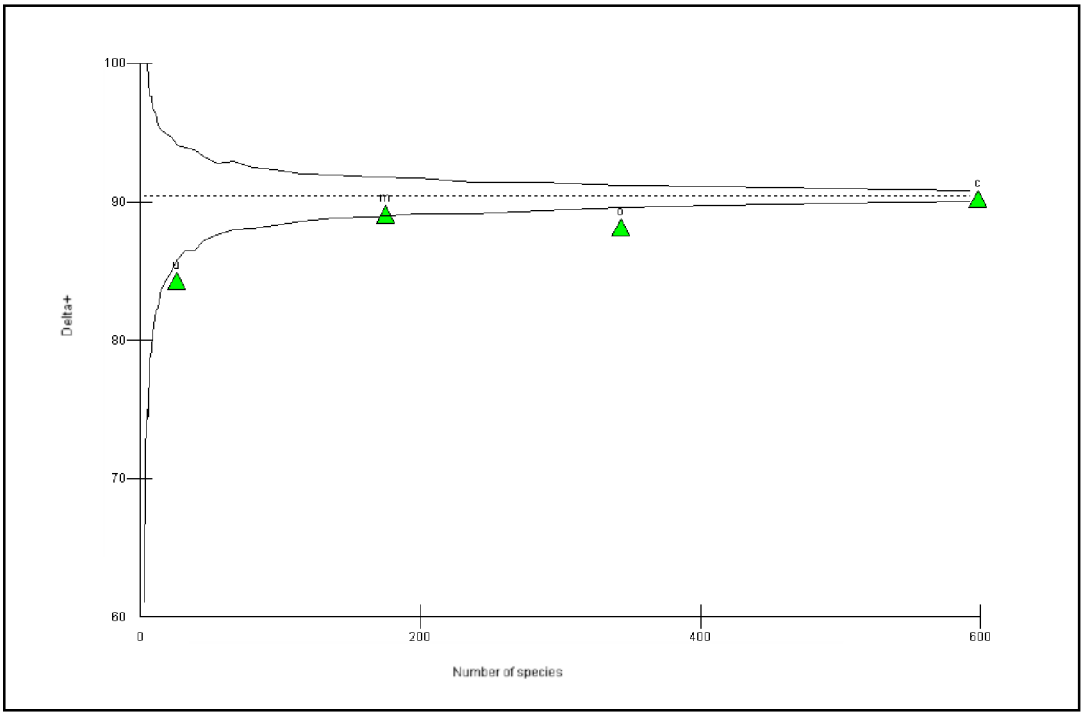

Figure 5.7 **Benthic data for Anglian Region pooled according to salinity range, c–coastal (30+), o–outer (20-29), m–mid (10-19), u–upper (0-9). Plotted against funnel from master list for Anglian Region.**

The Regional analysis indicated that the overall AvTD investigation needed to be more specific as none of the sample points fell within their expected ranges. As well as being more defined with respect to salinity, it was decided that the master list might also need to be habitat specific rather than Region specific. Furthermore, the master list is subject to the inconsistencies that might exist in the identification of taxa between laboratories over time. As well as investigating AvTD at the habitat and salinity level, truncation of the master list was therefore explored at three levels:

- Taxa such as Nematoda and encrusting taxa that are inconsistently recorded were removed
- Inconsistencies of the level to which taxa are identified were addressed by looking at the results of AvTD when it is calculated at genus and family level
- Looking at only surrogates e.g. the four main phyla (Annelida, Crustacea, Mollusca and Echinodermata) explored the effect that excluding rarer taxa has on the mean AvTD

These investigations were addressed during October 2003.

5.3.4 Data split for Habitat and Salinity (October 2003)

For the final section, data subdivided for salinity and EUNIS habitat type (level 3) was used to assign a habitat type to samples. The allocation of EUNIS habitat was based upon available sediment descriptions and/or PSA results for each sample. Data were then further subdivided into the salinity ranges described above. The WIMS (Environment Agency environmental variables database) database was used to allocate a salinity range for each sample by using the minimum salinity recorded nearest to the sample location. A

funnel plot was created for the coastal, outer, mid and upper salinity ranges within EUNIS habitat type A4.2 (sublittoral sand and muddy sand) (Figure 5.8). Although data were specific to habitat type (EUNIS level 3) and salinity, results were similar to those shown in the Regional data, whereby the points formed a funnel below that created from the master taxon list. Investigations therefore continued into whether the truncation of the master list and samples was a factor in causing this anomalous result.

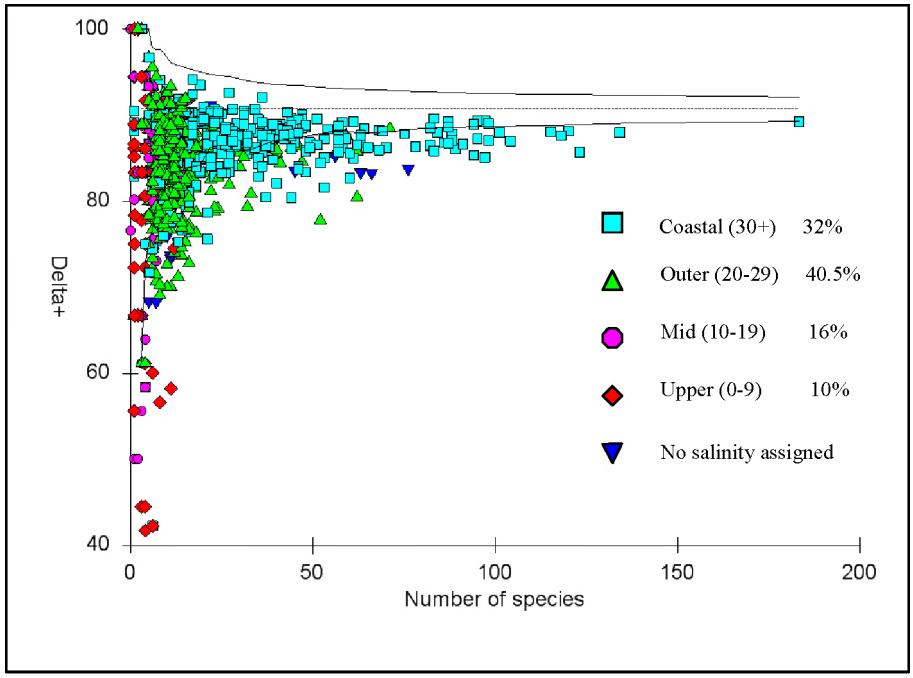

Figure 5.8 **AvTD values of all EUNIS habitat A4.2 (sublittoral sands and muddy sands) samples, plotted against the 95% probability limits funnel based on the master taxon list created from all EUNIS A4.2 habitat samples. The percentage contribution of each salinity band is also shown. The expected mean AvTD is indicated by the dotted line.**

5.3.5 Data truncation

This exercise was undertaken prior to the data truncation rules (section 4.3) being established. As such the master taxon list initially included very rare and also non-soft sediment taxa. By including such taxa in the master taxon list, it was thought that a bias was created in the expected AvTD because all taxa were being weighted equally. To establish how much of an effect these rarer taxa and non-soft sediment taxa were having, they were removed from the dataset. Protozoa, Insecta, Porifera, Bryozoa and Cnidaria (except *Nematostella* and Edwardsidae) were deleted. However, even with this truncation, the pattern shown in the data still formed a funnel below that created by the master list. Therefore, a stronger truncation was explored by analysing AvTD at the genus and family level.

Species, Genus, Family level

In addition to the truncation of the master taxon list, it was thought that inconsistencies in taxonomic identification might have an affect on the results. Taxonomic distinctness is based on a taxonomic aggregation file, which describes the six level phylogenetic hierarchy. In the species column of the aggregation file, a taxon may be identified to any taxonomic level, e.g. phylum, class, order, family, genus or species. This means that many of these entries could be describing the same species, therefore giving the impression of a greater number of taxa in the master list.

Tests were carried out to determine whether AvTD varies significantly between a six-level taxonomic hierarchy (species to phylum), a five-level hierarchy (genus to phylum) and a four-level hierarchy (family to phylum). Coastal stations within EUNIS habitat A4.2 (350 station-year combinations) were used. Although actual AvTD values altered, it was of interest to see if the behaviour of the indices remained consistent when considered at the relevant taxonomic level. In order to ensure that the results at each of these levels were consistent, the station-year combinations were ranked on their AvTD scores for each hierarchy and tested using Spearman's rank correlation. There was no significant correlation between the stations for each hierarchy (Table 5.4), i.e. taxonomic integrity was not maintained between these levels. At this stage it would therefore imply that the analysis should be carried out at species level. However, although the rank order between station-year combinations change significantly, the ecological status class may not, i.e. station-year combinations may rearrange in rank order at the different taxonomic levels, but they remain within a status class. This would need further investigation.

Table 5.5 Spearman's rank correlation comparing rank order of AvTD for coastal EUNIS A4.2 stations at three levels of phylogenetic hierarchy. R_s indicates the level of association between the two levels. 1 would indicate a perfect positive correlation. The probability values show the significance of the correlation at the 95% probability level.

	R_s value	p-value
Species - Genus	0.143	0.007
Genus - Family	0.014	0.794
Species - Family	0.109	0.041

Surrogates

To determine why the data was continually falling below its expected range, further truncation of the data was explored. By analysing only the dominant taxa (Annelida, Mollusca, Crustacea and Echinodermata), the effect of rarer taxa was removed. However even with this truncation, the data continued to form a funnel below that created by the master list. Figure 5.9 shows the funnel plot for coastal A4.2 station-year combinations.

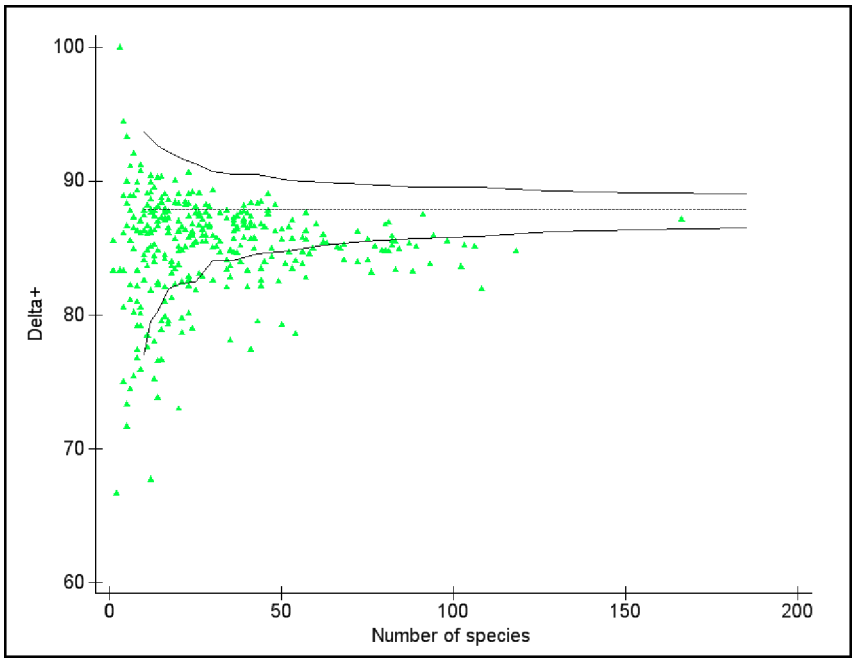

Figure 5.9 **AvTD values for coastal A4.2 station-year combinations considering only Annelida, Crustacea, Mollusca and Echinodermata (sample points and master taxon list).**

Aggregation of Samples

Because various divisions and truncations of the data had not resulted in any of the data sets falling within their expected range, it was decided to aggregate the data to test AvTD at a higher level than at the station-year combination. The data was aggregated according to water body, so that an AvTD score would be created for each water body rather than for each station-year combination. However, even when aggregated to this gross level, the data points still fell within a funnel outside of that created by the master taxon list (Figure 5.10).

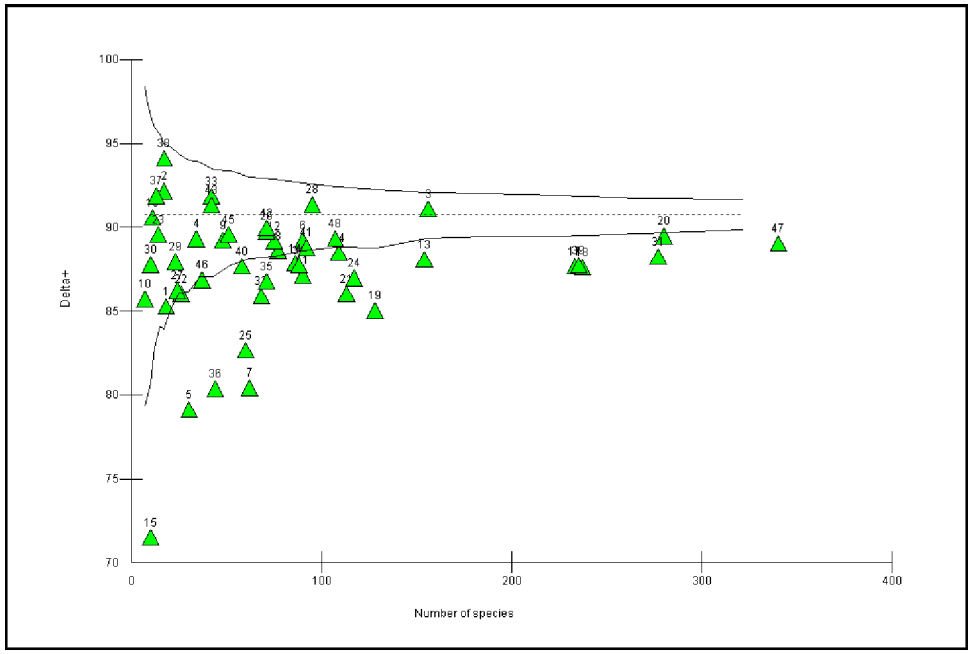

Figure 5.10 AvTD values for water bodies in England, Wales and Scotland. All samples within each water body were combined to give one AvTD value. The funnel was created from a master list of EUNIS A4.2 habitats. The key for the water body codes is shown in Appendix VII.

5.3.6 Frequency weighting of taxa

Investigations have shown that even by applying various divisions (regional, habitat and salinity) and truncations to the data, the recurrence of the data points falling outside the expected range created by the master taxon list remained unexplained. The null hypothesis of AvTD was therefore tested, i.e. that all the taxa are equally weighted and consequently, every taxa in the master list is as likely as any other to occur. The developers of PRIMER© tested this by altering the algorithm that runs the TAXDTEST routine in PRIMER to take account of the frequency distribution of taxa. This test was carried out using data from the outer Blackwater stations designated at high ecological status. Status was assigned through local knowledge of the pressures impacting on the area. Having applied this frequency distribution code, a funnel was created and the sample points were found to lie within their expected taxonomic breadth. Previously, these same sample points had departed from the funnel created from the master taxon list without an incorporated frequency distribution.

This finding means that taxonomic distinctness is sensitive to the frequency of occurrence of taxa across the samples. This is contrary to the null hypothesis on which AvTD is currently based and takes account of the natural spatial variation caused by reproductive strategies within benthic communities. Our investigations have highlighted that account of frequency distribution is necessary for AvTD to be used as a WFD compliant classification tool. It is imperative that the frequency distribution is developed for high status, in order to set a strong reference from which to measure departures.

PRIMER-E© are currently developing a revision of the algorithm that runs the TAXDTEST routine, which will be included in the next version of PRIMER© (PRIMER© 6). This algorithm will take account of frequency distribution for selecting species differentially at random (Clarke, K.R. pers. comm.).

Conclusions

The overall aim of the testing carried out was to determine whether AvTD can detect anthropogenic impact and to test whether the behaviour of the index relates to WFD. An advantage of AvTD is that the master list can be used as a biodiversity reference. However before the index can be considered for use in a multimetric, the results from the modified TAXDTEST routine incorporating the weighted frequency distribution will have to be related to anthropogenic pressures to ensure that they can distinguish impacted from non-impacted benthic assemblages. If this index is found to be appropriate for WFD purposes, the funnel plots can be modified to include probability level bands that relate to the boundaries between ecological status classes e.g. Figure 5.5. These boundaries will be set according to the habitat (section 3.2).

Analyses of the way in which AMBI and AvTD function has involved thorough testing by MBITT, which has highlighted the necessity for the further development of AvTD. Subsequent testing will be carried out to determine whether these indices can detect changes within benthic assemblages due to anthropogenic impact. If so, AMBI and AvTD will be used within the multimetric approach carried out for the assessment of benthic invertebrate communities. Furthermore, because these indices are not purely information statistics as traditional univariate and diversity indices are, they provide more information about variation within the benthic assemblage; by taking account of the taxa's sensitivities to impacts (AMBI, organic enrichment) and change in taxonomic structure as a result of impacts (AvTD).

5.4 Indicator Taxa

The normative definitions of the WFD refer to 'sensitive taxa', 'disturbance-sensitive taxa' and 'taxa indicative of pollution'. For an index to be able to detect anthropogenic impact over natural environmental variation, it is imperative that the life cycles of benthic invertebrate taxa, their roles within an ecosystem and the way in which they respond to different pressures are understood. The way in which a benthic community responds to pressure forms a model, for example that of Pearson & Rosenberg, 1978, on which biotic indices, such as AMBI, are based. Biologically based indices incorporate knowledge of life histories and functions and how these change along an impact gradient.. For instance, 'r' strategists are characterised as small in size with a short life cycle and a high reproductive capacity (Gray, 1979). Given a rapidly changing environment, the 'r' strategists can flourish and as such are often linked to communities that have been subjected to anthropogenic impact. Hence, 'r' strategists are often known as 'opportunists' or 'taxa indicative of pollution'. However, it should be appreciated that under extreme natural conditions e.g. under salinity stress within a transitional water, 'r' strategists may also dominate, despite the absence of an anthropogenic stress. It is, therefore, not possible to use the presence of r-strategists as indicator taxa without further consideration of the environment sampled. Their overall abundance needs to be considered to determine whether or not their relative presence indicates a habitat with degraded ecological status.

The decrease of sensitive species within an area is considered as an initial 'signal' of the negative influence of a pressure. Sensitive species are usually k-strategists, having a long life-cycle (> 1 year), slow growth, large size and late sexual maturity. K-strategists e.g. large burrowing crustaceans are considered sensitive because they are not equipped with the capacity to adjust to environmental perturbation (Pearson & Rosenberg, 1978). They are usually indicative of stable environments where they can out-compete many other taxa. When a pressure acts on a community, K-strategists will be affected first and will remain absent for as long as the pressure remains, or for the time required for the recovery of the species. Sensitive species can act as key structural species, with their loss creating cascading affects on the community.

The subject of sensitive taxa was initially discussed at the "Expert Judgement" in October 2003. It is important to clarify the definition of sensitivity when attempting to identify sensitive taxa. For example, ICES/OSPAR working groups consider that a sensitive species is one that is easily depleted by human activity and, when affected, is expected to recover over a long period or not at all. As such the term 'sensitivity' takes into account both the tolerance to a pressure and the time needed for recovery from the pressure. 'Sensitive' taxa are often referred to as 'intolerant'. However, the term 'intolerant' takes no account of the time required to recover from a pressure. As such, all taxa may be intolerant to the pressure, dependent on the scale, severity and duration of the particular pressure.

The Marine Life Information Network (MarLIN, www.marlin.ac.uk) uses the following definitions, which have been currently adopted by MBITT:

'*Intolerance*' is the susceptibility of a habitat, community or species (i.e. the components of a biotope) to damage, or death, from an external factor. Intolerance must be assessed relative to change in a specific factor.

'*Recoverability*' is the ability of a habitat, community, or species (i.e. the components of a biotope) to return to a state close to that which existed before the activity or event caused change.

'*Sensitivity*' is dependent on the intolerance of a species or habitat to damage from an external factor and the time taken for its subsequent recovery. For example, a very sensitive species or habitat is one that is greatly affected by an external factor arising from human activities or natural events and which has low recoverability (i.e. >10 or up to 25 years). Intolerance and hence sensitivity must be assessed relative to change in a specific factor.

Sensitive taxa that are also rare may not be detected by typical sampling procedures and will therefore not be easily incorporated within a classification tool. For this reason, the frequency of occurrence should be taken into account by weighting the taxa. The weighting allocated to a taxon will be proportional to its significance within an ecosystem. Sensitive keystone species crucial for the functioning of an ecosystem, such as *Sabellaria spinulosa* (Polychaeta) will have higher weighting than other less significant species.

A species can only be an indicator if it exists naturally under undisturbed conditions. Indicator species are likely to be habitat-specific e.g. amphipods such as *Bathyporeia* sp. may be appropriate in a sandy habitat but would not be suitable disturbance-indicators in a muddy habitat. .

The sensitivity of taxa to certain pressures was discussed during the October workshop. In subtidal sediments, the absence of bivalves such as *Mya sp.*, *Macoma sp.* and *Arcica islandica* could be indicative of an impact, particularly fishing or dredging. Identifying disturbance sensitive taxa in the intertidal area is difficult because species are inherently robust in order to inhabit this naturally stressed environment. There are a variety of natural and anthropogenic activities that could disturb intertidal taxa (Table 5.6).

Table 5.6 Summary of notes on sensitive taxa made during the October workshop. Examples of taxa sensitive to particular pressures and reasons for their sensitivity are shown.

Pressure	Sensitive Taxa	Reasons for impact/sensitivity
Sediment inputs to water column, e.g. from dredging.	*Nephtys cirrosa* *Paraonais* sp. *Scolopolos* sp. *Scolelepis* sp. *Spiophanes bombyx*	Sensitive to inputs of fine sediment
Water abstraction	*Streblospio shrubsollii* *Heterochaeta costata*	Abstraction can cause incursion of high salinity water from downstream, affecting taxa typical of low salinity habitats upstream.
Increase in water temperature e.g. at discharge sites for power station cooling waters	*Corophium volutator* *Tubificoides benedii*	Sensitive to high temperatures
Chemical input e.g. industrial and sewage treatment by-products, anti-fouling paints used in shipping	*Scrobicularia plana*	Sensitive to TBT
	Corophium arenarium	Sensitive to synthetic contaminants
Organic enrichment	*Pontocrates arenarius*	Sensitive to pollution

For the development of classification tools, it is essential that the presence and abundance of sensitive taxa, such as those in Table 5.6, be linked to the normative definitions. The distribution of sensitive taxa for different habitat types could be plotted against the concentrations of known contaminants. Predictions of the changes in abundance of taxa in relation to particular pressures could then be made and incorporated into a classification tool.

The identification of marine indicator species from time-series and other studies is now being carried out by Hiscock *et al.* at MarLIN. The work, funded through JNCC and EA (WFD), relates indicator taxa to the pressures identified in the risk assessment of coastal and transitional waters. This work will be reported in the Phase III R & D report.

6. Trialing of a more Rapid Ecological Assessment

Managers assessing the ecological status of the transitional and coastal waters have long been concerned that the benthic community monitoring approach is highly labour intensive and therefore expensive. Indeed, sample analysis to species level is frequently time consuming and requires a high degree of taxonomic expertise and standardisation (Warwick 1993). Although both poor sorting and mis-identification of samples can lead to false 'trends' or 'classifications,' the high degree of taxonomic expertise required is generally an undervalued skill.

It is necessary to identify organisms to a taxonomic level sufficient to meet the objectives of any study (Ellis 1985). In this instance, the objective is the ecological status assessment of the water body as defined under the WFD. There has been, and still remains to some degree, a general belief that the ability to detect an effect at a site is enhanced by identification of taxa to the lowest possible taxonomic level, namely species. However, several authors (e.g. Drake *et al.* 1999 and references therein) have suggested that a higher level of taxonomic resolution can be used without a significant loss of information. In fact, an increasing number of studies show that analyses at higher taxonomic levels are more likely to reflect a contamination gradient than analyses based on species (Warwick 1988, Olsgard *et al.*, 1997). When added to the fact that several studies (e.g. Elliott & Pomfret 1994) have shown a significant (50%) time saving in identifying to family level instead of species, the question as to the level of taxonomic identification required for robust ecological status assessment gains importance. In guidance as part of the US Environmental Monitoring and Assessment Program (EMAP), Gibson *et al.* (2000) state that "… the cost and effort to sort, count and identify benthic invertebrate samples can be significant, requiring trade-offs between expenses and the desired level of confidence in decisions based on collected data." Many workers (e.g. Warwick 1993) recommend that priority be given to examining a large number of stations/replicates at the level of higher taxa in preference to a small number of stations at the species level.

WFD assessment may well require a combination of taxonomic levels, with potentially higher levels of identification being used to assess community structure (abundance and diversity) but species level identification being used for specific sensitive and disturbance indicator taxa. The following exercises were initiated to consider the benefits and disadvantages of a more 'rapid' assessment of the benthic invertebrate community.

6.1 Approaches to Rapid Assessment

Benthic invertebrate communities are sampled by taking a number of replicate samples at defined stations. The position of the stations (sampling design) is defined by the primary objective of the survey. Traditionally, sediment samples for faunal analysis are sieved in the field, the residue preserved in formalin and the sample sent to laboratory for the picking and identification of the macrofauna. The analysis of macrobenthic samples can therefore become a time-consuming and expensive process.

Historic data are being assessed by MBITT to evaluate the behaviour of the metrics (i.e. resulting assessment status) at different taxonomic levels. At the same time, the possibility of assessing ecological status by evaluating field samples or considering changes to the laboratory analysis were considered.

It is not realistic to suggest that all sampling can be reduced to a field assessment. Even when incorporating a field evaluation into the WFD assessment of a water body, the full laboratory analysis of samples at targeted stations would be required for quality assurance of the assessment. These approaches were therefore examined with the view of increasing the number of stations that could be assessed within a water body, the field assessment 're-enforcing' the assessment from the targeted sampling. There are several stages where a more rapid approach could possibly be introduced into the assessment procedure. These fall into the following categories:

(i) *Field assessment* of samples on a broad scale, so larger number of stations can be assessed for anthropogenic pressure, carried out in conjunction with targeted sampling for full laboratory assessment to quality assure field assessment. This allows more samples to be assessed with the resources available.
(ii) *Laboratory assessment* of samples to a higher taxonomic level.

Without question, the use of such assessments would result in some loss of information regarding the benthic invertebrate community. What is not clear is whether this loss of information would result in a different 'ecological status' being assigned to the water body as compared to that assigned following full taxonomic identification of the sample. The risk of misclassification due to a less thorough assessment (more rapid approach) would require clear quantification before such methods could be considered for inclusion in a monitoring programme.

Under Phase I, MBITT began to consider possible methodologies for a more rapid assessment (RA) of the benthic invertebrate community. The objective was to determine whether an accurate assessment of the ecological status of a water body could be determined with a less rigid sampling procedure. The WFD calls for the ecological status of all our waters to be evaluated. Currently 121 transitional water bodies and 70 coastal water bodies have been identified in England and Wales alone (the final number of water bodies has yet to be confirmed). If a method of RA of the benthic invertebrate community could be incorporated for surveillance monitoring, resource implications could be substantial. The use of RA methods for 'Operational' and 'Investigative' monitoring has not been considered.

Approaches for RA methods have been carried out in three phases:

(i) Wash (transitional embayment type 4 and transitional water type 3)
(ii) Fal system (coastal water type 5 and transitional water type 4)
(iii) Cardigan Bay (coastal water type 5 and type 2)

6.1.1 Wash (August 2002)

(Transitional embayment type 4 and transitional water type 3)

This survey followed initial thoughts regarding the possibility of introducing a 'field assessment' into WFD surveillance monitoring. The feasibility of such an approach was unknown so initial approaches were considered while carrying out an established benthic survey. The Wash grid survey planned for August 2002 provided a suitable basis in terms of resources for the work.

Background

The National Rivers Authority (NRA) carried out the first large subtidal benthic grab survey in 1991 (NRA, 1994). A total of 66 stations (Figure 6.1) were sampled with three replicate sediment samples taken at each. A fourth sediment sample was taken for measurement of particle size, organic carbon, heavy metals and certain pesticides. Further partial surveys were carried out in 1993 (14 stations) and 1999 (31 stations) and are detailed in Table 6.1.

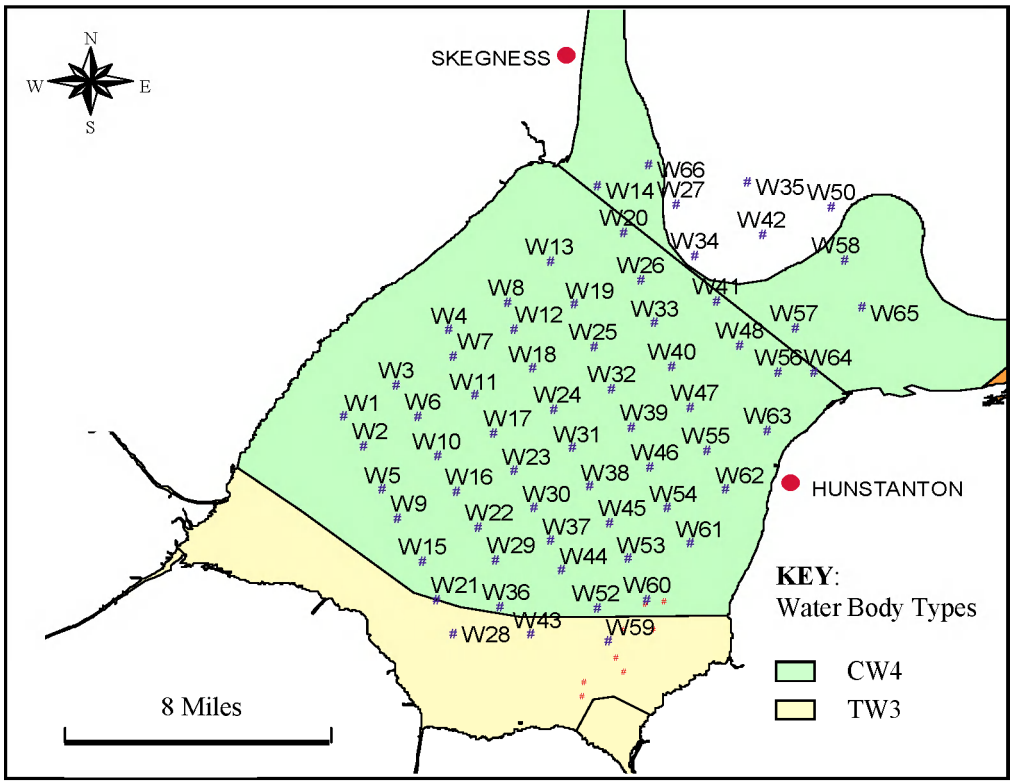

Figure 6.1 Wash benthic invertebrate grid survey stations. Water body types are indicated.

Table 6.1 **Wash Grid Survey stations (Stn) and the years in which the benthic invertebrate communities were sampled (blue blocks), stations where Rapid Assessment was trialed in 2002 (yellow blocks). (W-Wash, WW-Welland & Witham, E-Great Ouse).**

Stn	91	93	99	02	02	Stn	91	93	99	02	02	Stn	91	93	99	02	02	Stn	91	93	99	02	02
W1						W18						W35						W52					
W2						W19						W36						W53					
W3						W20						W37						W54					
W4						W21						W38						W55					
W5						W22						W39						W56					
W6						W23						W40						W57					
W7						W24						W41						W58					
W8						W25						W42						W59					
W9						W26						W43						W60					
W10						W27						W44						W61					
W11						W28						W45						W62					
W12						W29						W46						W63					
W13						W30						W47						W64					
W14						W31						W48						W65					
W15						W32						W49						W66					
W16						W33						W50						E10					
W17						W34						W51						WW19					

In August 2002, English Nature (EN) commissioned a full benthic grid survey of the Wash for Habitats Assessment, co-funded by the EA. For the purposes of the EA, the survey addressed several issues:

1. The operational requirement of a ten yearly assessment of the health of the Wash.
2. Provision of supporting data with regard to countering the Reasoned Opinion from Europe that the Wash should have been designated as a Sensitive Area (UWWTD).
3. Provision of information to a local initiative, the Wash forum, trying to discover why the shell fisheries have not been able to recover from their decline.

This comprehensive survey of the Wash provided an ideal opportunity for MBITT to (i) collect data for WFD assessment of the Wash and provide a dataset for classification tool testing and (ii) to assess the feasibility of using a field assessment of the benthic macroinvertebrate community for ecological status assessment. Members from MBITT project joined the survey, providing additional staff resource for the primary goals, while the full taxonomic assessment of the samples from the survey provided quality assurance for comparison to the field assessment. As such, the resources (costs) for testing the feasibility of a RA were kept to a minimum, while ensuring that maximum utilisation was made of the survey data.

Methodology

The benthic grid survey was managed by the consultancy Eco-maris, and took place on the EA Coastal Survey Vessel, Water Guardian in August 2002. The grid consisted of the 66 stations (Fig 6.1), with three replicate Day grab samples taken for benthic infauna and a fourth replicate used for particle size analysis at each station. Samples were sieved on board (0.5 mm) and immediately preserved in buffered formalin (4%). Attempts to assign a field ecological assessment of the samples (Fig 6.2) were fitted in around the primary objectives of the field survey.

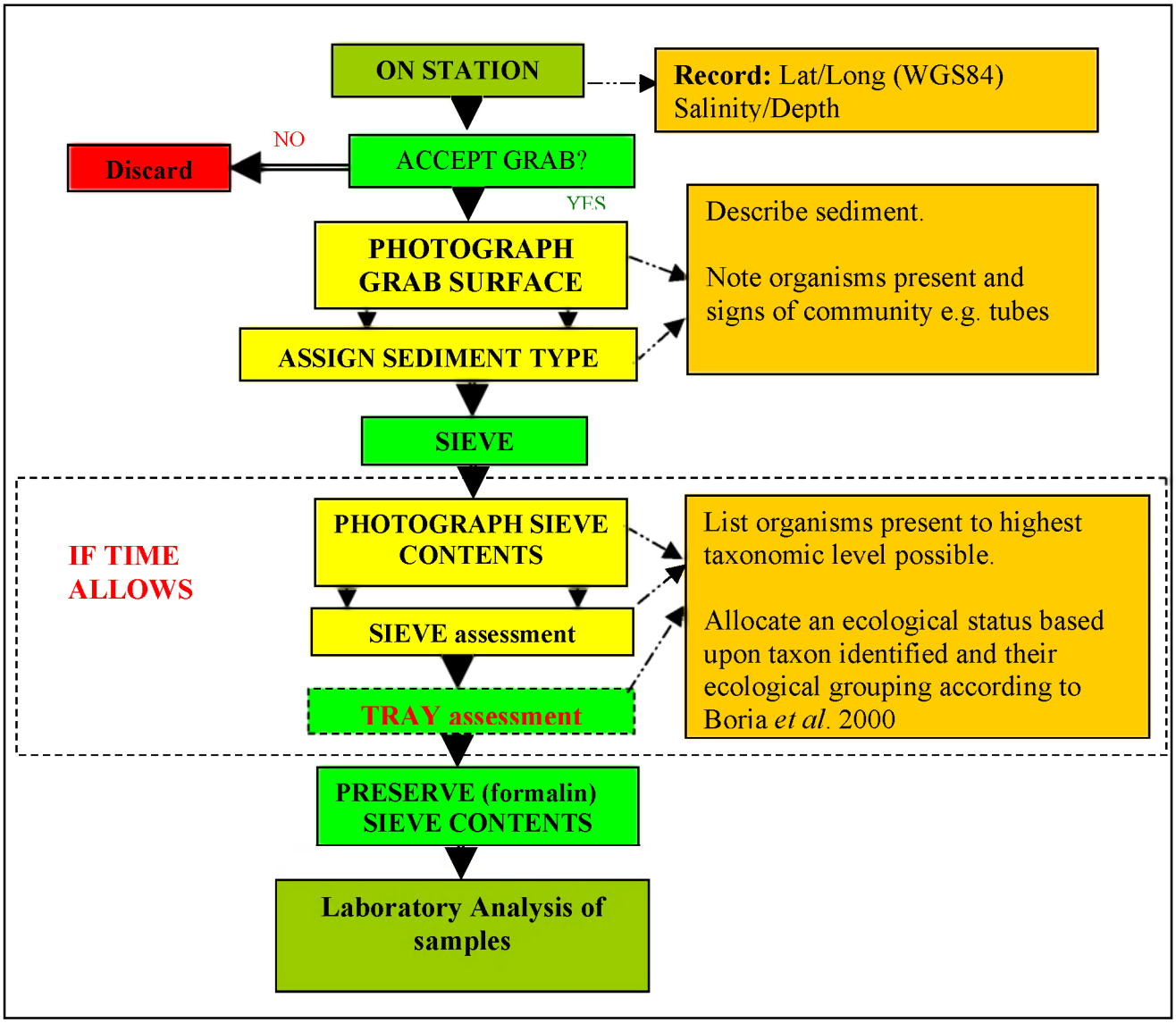

Figure 6.2 Process diagram detailing stages in Rapid Assessment during the Wash grid survey 2002.

Where time allowed, assessment of the 'visible' benthic invertebrate community took place in three stages:

1. The sediment surface in the grab - sediment description, oxic depth, visible infauna, tubes, burrow holes recorded (as for NMMP assessment)
2. Sieve contents (after sample was sieved through a 0.5 mm mesh) - taxon presence and estimate of abundance recorded
3. Sieve contents into sorting tray (limited sort time, picking of infauna) - taxon presence and estimate of abundances recorded

Photographic records of the grab surface and sieve contents were also made to provide a record of the sample. In order to compare ecological status based on field and laboratory assessment, the faunal abundance results were used to calculate a biotic index. For this exercise, the biotic index, AMBI, was used (AMBI is described in Section 5.2).

Discussion

Due to exceptionally calm conditions, the survey was completed in four days instead of the planned ten days. Unfortunately this had the adverse affect of reducing the time available to the staff for carrying out the field assessment. As the RA was not the main aim of the survey, time could not be increased for field trials alone. Although this minimised the extent of the RA trials, the survey provided a valuable insight into the requirements for further field trials. In particular:

(i) the need for 'expert' trained technical staff if field assessment, at any level, is to be considered.
(ii) the use of digital photography was recognised in defining and recording PSA/habitat. This would provide records that could be quality assured against particle size samples
(iii) the potential of a rapid assessment in defining the habitat areas and recognising 'good ecological status' in coastal waters
(iv) the potential in using field assessment to target sampling for WFD assessment.

The laboratory analyses of the macrofaunal samples have only been completed recently (March 2004). Comparisons between field and laboratory data have therefore not yet been carried out. Data will be compared and the results presented in the Phase III report. The preliminary AMBI scores calculated for the Wash grid surveys using the full laboratory analysis are however shown in Figure 6.3. According to AMBI, the majority of stations had good ecological status, with only one station at poor and seventeen at high status.

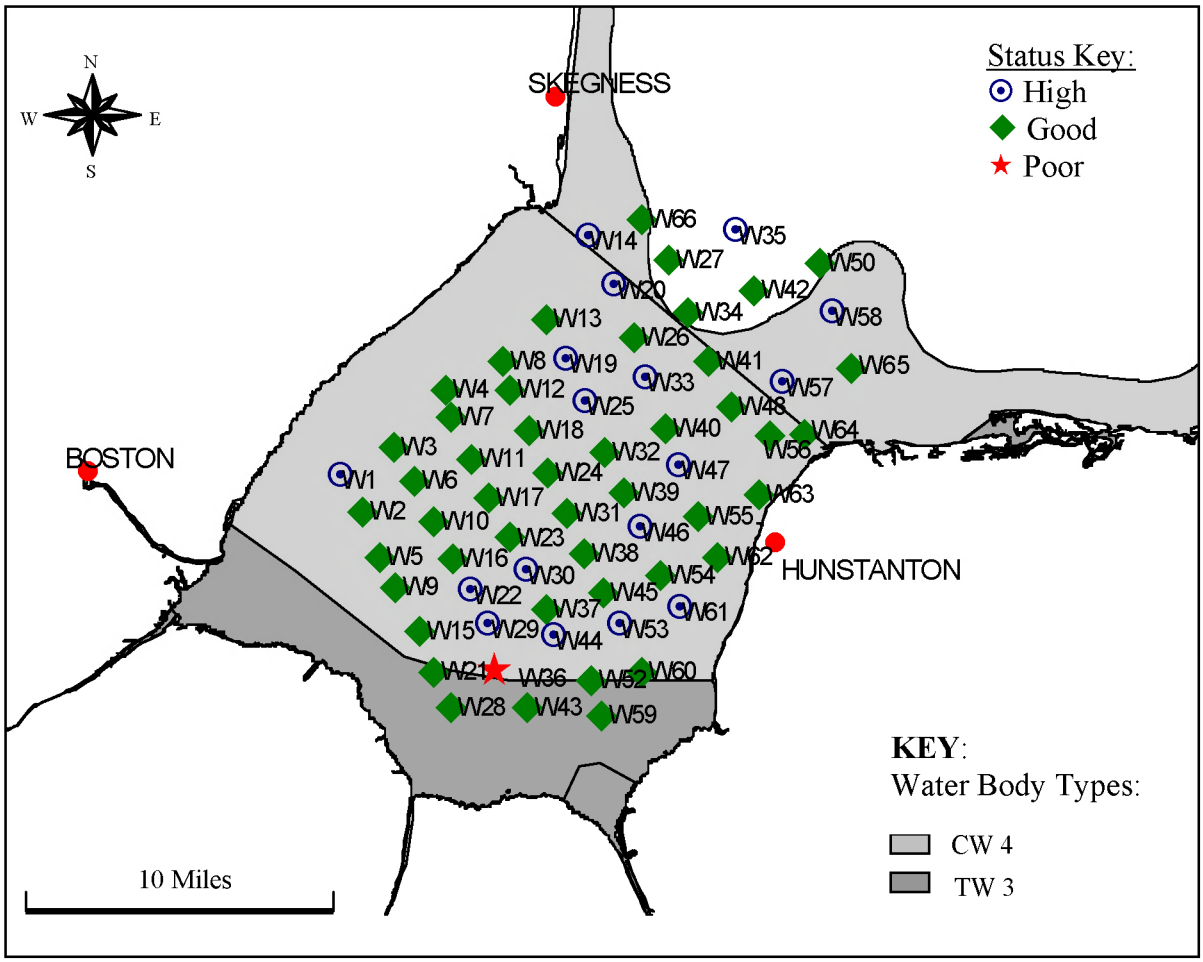

Figure 6.3 **Ecological status of stations within the Wash according to AMBI. Data based on 3 x 0.1 m^2 Day grab replicates at each station in August 2002 and analysed to species level within the laboratory. Ecological status demarcations based on Borja *et al.*, 2003. Water body types are indicated.**

6.1.2 Fal system (September 2002)

(coastal water type 5 and transitional water type 4)

The second phase was carried out in the Fal system in SW England. This represented the first dedicated sampling for determining a more rapid benthic invertebrate assessment and built on the experiences of the Wash survey. Organised by the EA project team, the workshop was run in conjunction with staff from EN, JNCC and EHS (NI). Field assessment and a more rapid laboratory assessment were trialed.

The objectives of the workshop were:

- to assess how an 'expert view' of a sieved sample assessed in the field without any magnification relates to a full sample analysis in the laboratory (EVA - Expert View Analysis)
- to assess whether a subsample extracted during a pre-determined time period could be representative of the whole sample. This was addressed by Timed Sorting Analysis (TSA)
- to evaluate whether a sample analysed by an expert in the laboratory with basic magnification (1.5x magnifying lens) equates to the more usual microscope aided identification. This was tested using a protocol termed Restricted Laboratory Analysis (RLA)
- to validate these objectives with complete full analysis following NMBAQC protocols.

Funding for this field workshop was provided by MBITT. JNCC provided funding for the laboratory analysis and testing of modified laboratory protocols.

Background

The NRA carried out a spatial survey in 1990 of the Fal estuary system (29 intertidal and 19 subtidal stations) (Baker, 1994). Its purpose was to fulfil the objectives of the proposed National Classification Scheme, and provided a baseline survey of quantitative data. Parts of the Fal estuary complex, notably Restronguet Creek, are affected by historic mine waste. The elevated concentrations of metals found in the sediments and the resulting stress of the environment has been shown to impact the benthic invertebrate communities (Bryan *et al.*, 1987) of the area. As such the Fal system presented an opportunity to investigate whether RA could detect any disparity between a known impact in one area (e.g. Restronguet Creek) and a relatively unimpacted area of the same substratum and salinity range (e.g. Ruan Creek).

Methodology

The workshop was based on the CSV Vigilance during September 2002. Both intertidal and subtidal sampling was carried out (Figure 6.4). Where water depth allowed, Day grab samples (0.1 m^2) were taken (Malpas, Greatwood and Messack). However in the transitional waters, intertidal cores (0.01 m^2) (Tresillian, Ruan Creek, Restronguet Creek) or Van Veen grab (0.05m^2) (Ruan Creek, Restronguet Creek) were used, depending on access. Field processing of the samples was carried out on the CSV.

Figure 6.5 shows the outline of the methodology followed, which was similar to that used in the Wash. At the time of the workshop, there were no typed water bodies defined for

UK waters. As a result the staff defined indicative water bodies based on salinity and geomorphology of the area.

In order to assess the habitat type in a defined area (defined to represent a water body) a grid pattern was used, consisting of points at 200m intervals. Initially, this was carried out on the north east quadrant of Carrick Roads (Messack). A single Day grab sample from each of 14 stations was sieved (1.0 mm mesh). Sediment type (habitat) and an initial analysis of the diversity of the infauna were used to assess each sample. Following this initial screening, three more replicate samples were taken at a representative station of the area in terms of substratum. Further replicate sampling was also carried out at the station that had the highest diversity of infauna. As such this provided a 'best case' assessment of the benthic community of an area. The north west quadrant of Carrick Roads, Greatwood, was also sampled in a scattered pattern (single 0.1 m^2 Day grab). The Creeks Ruan and Restronguet were sampled along their length to take account of the salinity gradient. Samples taken during the workshop therefore covered a range of habitat types and salinity bands.

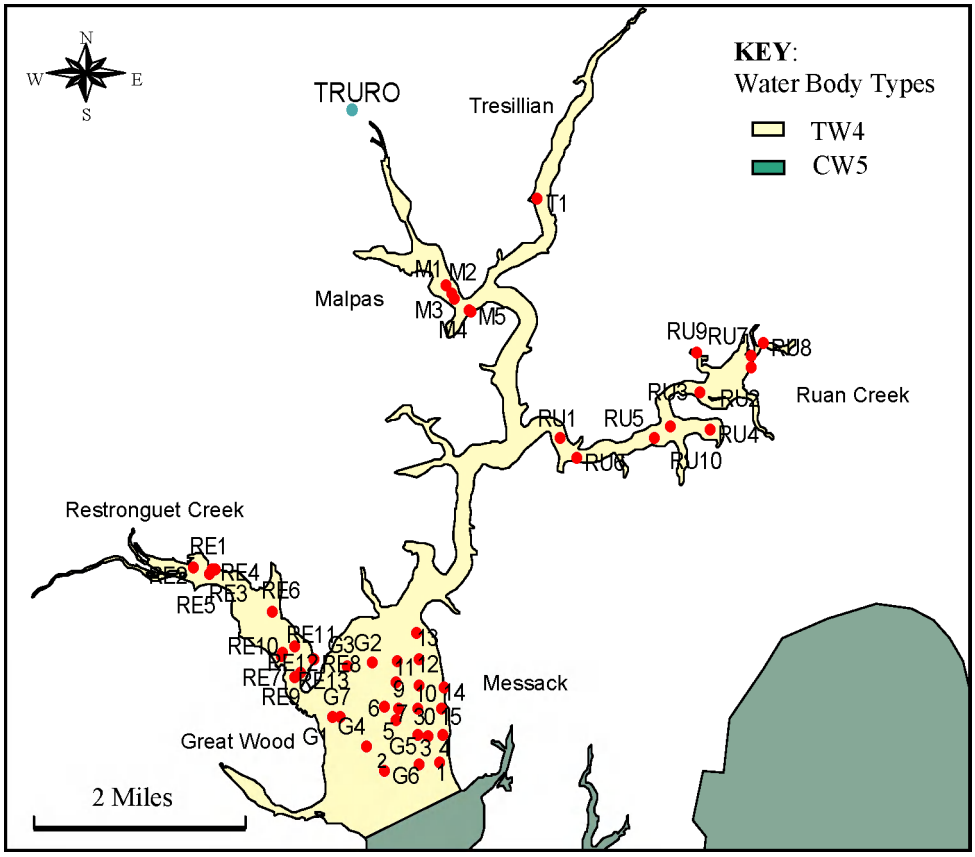

Figure 6.4 **Location of stations for the Rapid Assessment exercise in the Fal estuary complex (September 2002). Water body types and names of different parts of the complex are indicated.**

Two approaches were taken in the identification of the obvious taxa in the samples (field assessment):

(i) designated experienced benthic identifiers identified and listed taxa during the sieving process
(ii) sieved samples were picked (15 and 30 minute periods) and identified by staff with three different levels of experience (expert, intermediate, beginner).

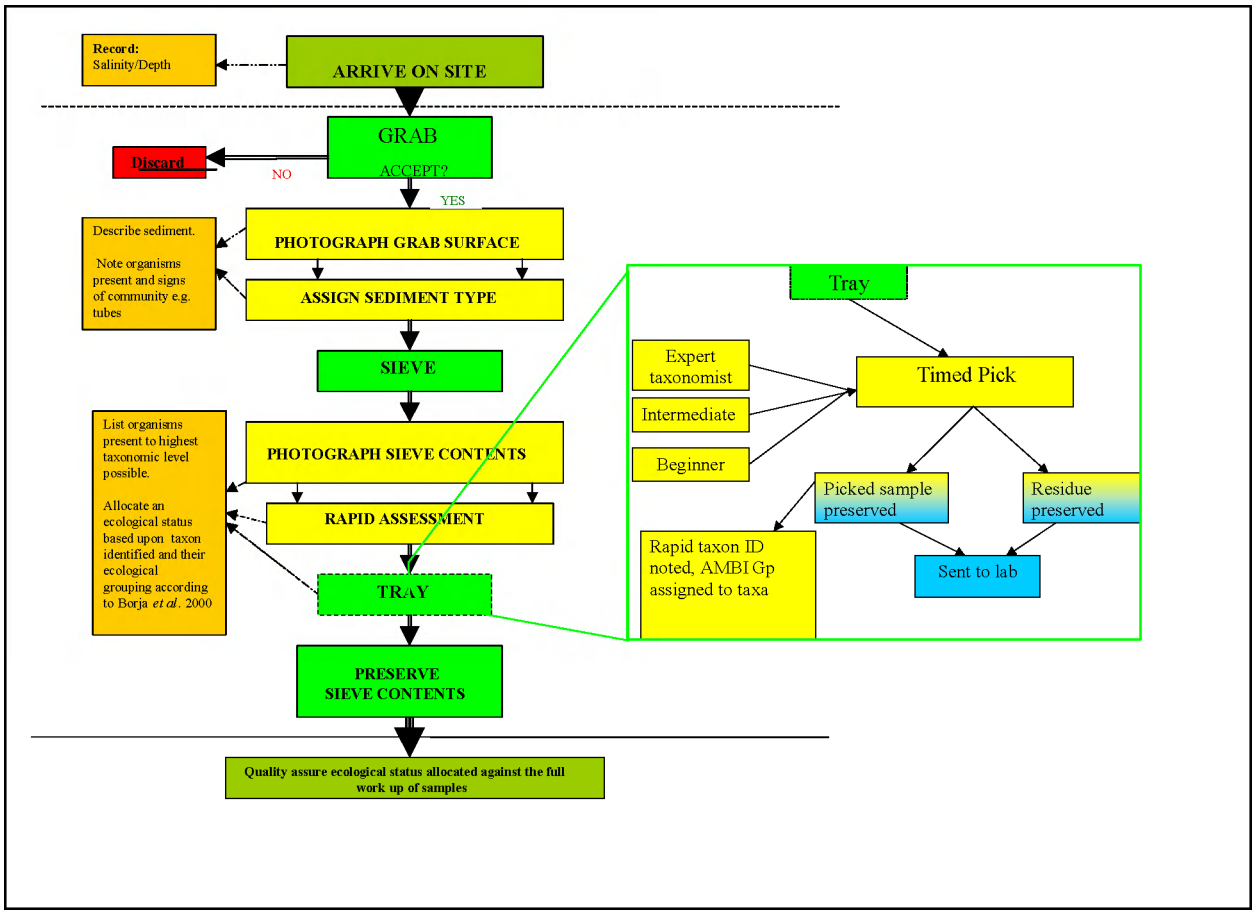

Figure 6.5 Flow diagram of procedure followed during Fal Rapid Assessment exercise

The sample residues, including those picked and identified in the field, were sent to an external contractor (Institute for Estuarine and Coastal Studies, IECS) for full taxonomic identification (samples NMBAQC assured).

Samples therefore consisted of (i) sieved but not processed (traditional method) (ii) sieved, taxa identified in the field process but individuals not picked and (iii) picked individuals (identified in the field) and residues (varying level of expertise of the staff). The laboratory work focused on two extreme habitat-types; a fully marine sublittoral gravel (high diversity, species rich) and a low salinity mud (low diversity, species poor).

Ecological status assessment was compared between the field and laboratory results (EVA). Analysis of the residues also allowed assessment of the taxa missed by carrying

out a field assessment. If these taxa are identified as disturbance sensitive taxa or pollution indicators, the consequences in terms of WFD assessment could be significant. During the laboratory analysis, IECS was also asked to consider and trial aspects of the sample analysis that could be adapted to more rapidly assess samples (RLA and TSA). For the TSA, 15 and 30 minute sorting periods were considered (as used in the field assessment). For RLA treatment, the sorted animals were identified and counted as far as possible using a 1.5x desk magnifier. These samples were then checked "blind" (i.e. without reference to these data) by a second member of staff, using standard microscope techniques (Jarvis *et al.*, 2004).

Results and Discussion

The final report on the findings of the laboratory analysis and rapid assessment trials by IECS was received in March 2004 (Jarvis *et al.,* 2004). Only a preliminary summary of the results is therefore included here. The full report is included in Appendix VIII.

Expert View Analysis (EVA): Expert level identification achieved a mean of 83% of species richness compared to that determined in the laboratory. Appendix VIII details the taxa that were missed and misidentified in the field. Intermediate level identification achieved a mean of 60% species richness when compared to laboratory analysis. Where numerical data were available, AMBI (Borja *et al.*, 2000) scores revealed no difference in the resulting ecological status designation between the expert assessment in the field and the full laboratory analysis.

Timed Sorting Analysis (TSA): AMBI (Borja *et al.,* 2000) was used to compare the various treatments in terms of implications for the ecological assessment. For the TSA of the 'gravel' habitat samples (high natural diversity and abundance), a 15 minute sorting period of the 1mm sieve mesh fraction constituted 3.4% of the total sorting time i.e. about 97% reduction in sorting effort. During the 15 minute period it was possible to extract a mean of 8.8% of the fauna and 38.2% of the sample species richness. However, the AMBI scores revealed very little difference in the ecological status assessment assigned to the samples sorted within 15 minutes and those sorted in the full workup. The assignment of status from the 0.5mm fraction was much more variable than that from the 1mm fraction.

Restricted Laboratory Analysis (RLA): In many cases the use of a 1.5x magnifying lens prevented identification to species level and was therefore generally similar to identification at genus or family level. However, when analysing the whole sample, the method of identification did not affect the assignment of pollution classification as assigned through AMBI (Borja *et al.,* 2000).

The Fal workshop highlighted potential considerations for the RA of benthic macrofaunal samples. Due to the range of issues considered at this workshop (e.g. sample type, salinity, habitat variability, range of taxonomic expertise, field assessment, laboratory assessment), the number of replicate tests for any single variable was considerably lower than required for robust statistical validation of the method. Further testing with higher statistical power would be required before any of the considered methodologies could be accepted. As such the workshop highlighted possibilities for testing rather than providing definite solutions for sampling methodology. Further discussion of these test methodologies will be included in the Phase III report, once MBITT has had time to analyse the results fully and consider the implications of the laboratory analysis versus the field analysis.

6.1.3 Cardigan Bay (June 2003)

(Coastal water type 2 and 5)

The second dedicated RA exercise was carried out in the coastal waters of Cardigan Bay (Wales), building on the experiences of the previous field trials. The Cardigan Bay trial was therefore focused on a single habitat, with standardised sampling to ensure a high statistical power in testing the RA in the field against the full work up of samples in the laboratory. Following the Fal RA exercise, it was clear that field assessment required staff to be have a high level of skill in identifying taxa. To this end two experienced benthic identifiers were invited to take part in the field trials: Myles O'Reilly (SEPA) and Nigel Proctor (IECS).

Based on initial analysis of pressures by MBITT, Cardigan Bay was estimated to be at high/good ecological status. In addition to RA, the samples may therefore also provide data for (i) establishing reference conditions for the coastal water habitat types sampled (ii) the Intercalibration exercise (Section 4.2) and (iii) testing of classification tools.

Methodology

Sampling was carried out in Cardigan Bay aboard the CSV Vigilance from the 2nd-6th June 2003, using a Day grab (0.1 m^2).

The null hypotheses for this study were:

H_o - for a given habitat type the benthic invertebrate community sampled at stations within that habitat (~1/4 nm apart) were not different.

H_o - the ecological status derived from RA was not different to that derived from the full work-up of samples.

The same substratum (muddy sand) was sampled at sites within Cardigan Bay, in order to evaluate within-habitat variation. Initially sites were all located within a single water body type, however a further typology split (defined after the workshop) resulted in sites spanning two water body types (CW 2 and 5). Three sites, each with four stations, were sampled. Four replicate benthic invertebrate samples were taken at each station (Fig 6.6). Samples were sieved (1 mm), picked and fully analysed on board. Sediment samples for particle size analysis were taken at each station to ensure that our allocation of sediment type was accurate. The picked taxa were returned to the respective residues and following the workshop, the samples were sent to Unicomarine for full laboratory workup (NMBAQC assured). Where possible, fauna were identified to species level.

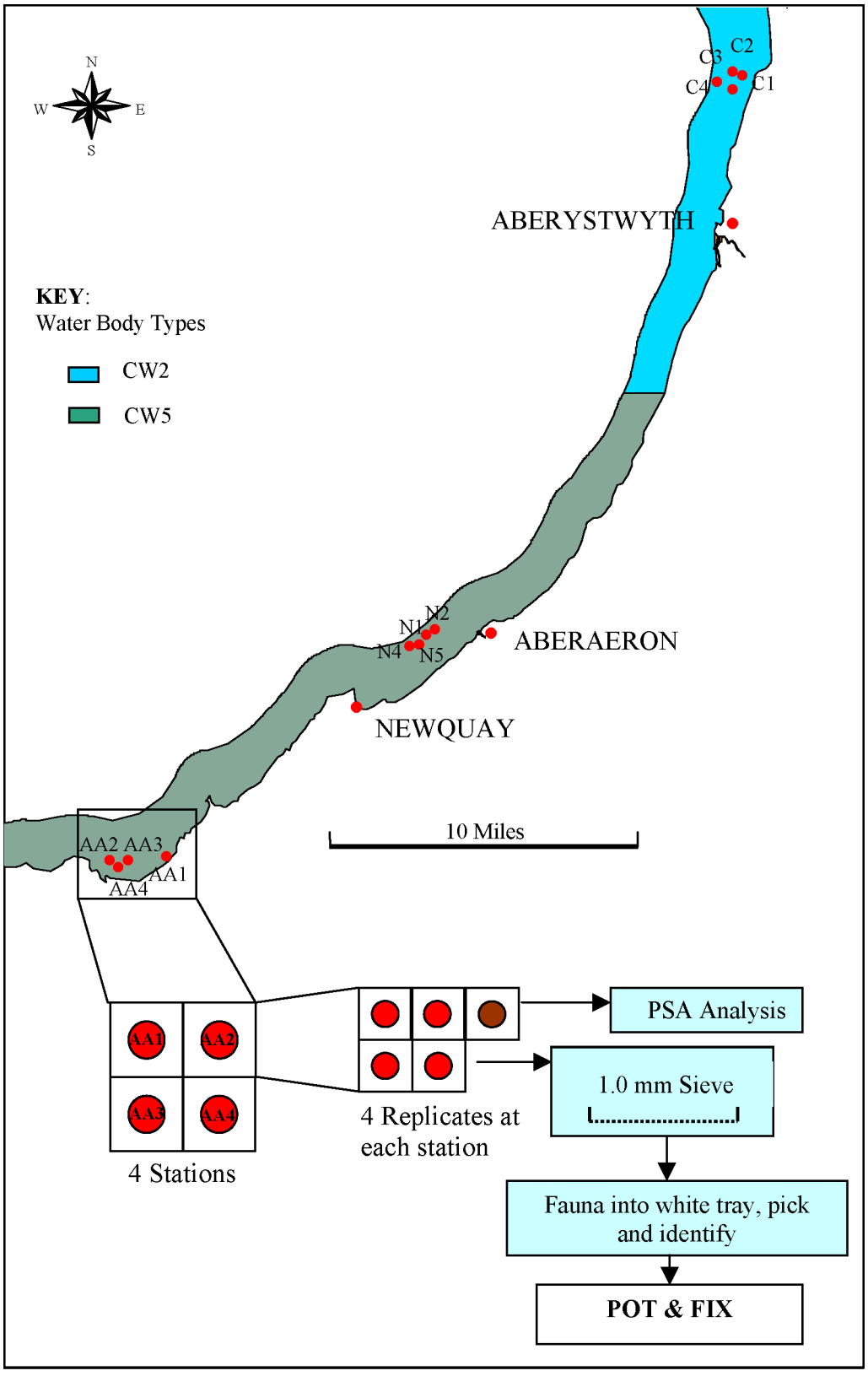

Figure 6.6 **Location of stations for the Rapid Assessment exercise in Cardigan Bay (June 2003). Water body types are indicated. A flow diagram of how the samples at each site were processed is shown.**

Results

Due to the muddy sand habitat sampled in this exercise, sieving was a relatively quick process and there was no backlog of samples during field analysis (as experienced when processing the gravel habitats of the Fal). Little sediment residue was retained on the 1 mm mesh, so faunal samples were relatively 'clean.' As such these samples represent the 'best case' scenario for field assessment.

Table 6.2 shows the number of taxa (at a variety of taxonomic levels) identified in the samples through field and laboratory analysis. Data have been truncated following the rules in Section 4.3.

Table 6.2 Number of taxonomic groups identified at each taxonomic level during the field assessment and laboratory assessment of the Cardigan Bay samples

	Field	Laboratory
Phylum	10	8
Class	15	15
Order	39	33
Family	80	84
Genus	95	109
Species	107	128

The two extra phyla identified in the field compared to the laboratory were *Platyhelminthes* and *Ctenophora*. At most other taxonomic levels, an increased number of taxa were identified in the laboratory as compared to the field. Approximately double the number of individuals was found in the laboratory analysis compared to the field assessments (Table 6.3). This was however, very taxa dependent.

Table 6.3 The number of individuals found within each phylum for Cardigan Bay samples analysed in the field and laboratory

Phylum	Field	Laboratory
Cnidaria	8	13
Ctenophora	5	0
Platyhelminthes	2	0
Nemertea	23	33
Sipuncula	30	23
Annelida	640	880
Crustacea	74	92
Mollusca	862	1932
Phoronida	4	23
Echinodermata	30	28
Totals	***1678***	***3024***

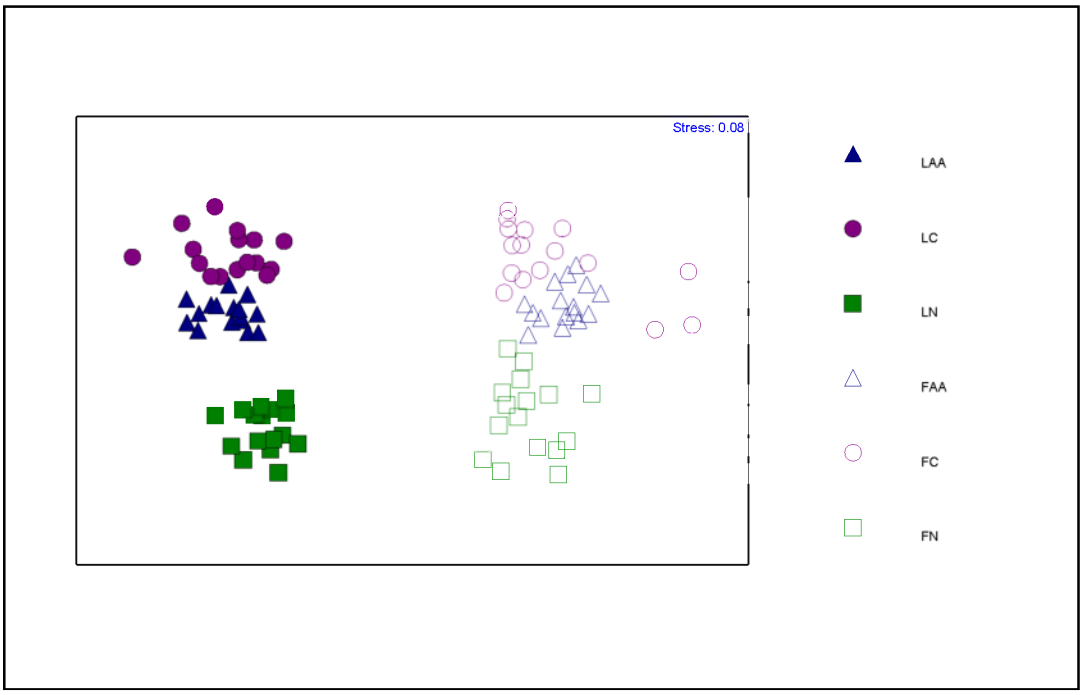

Figure 6.7 **Multi-dimensional scaling plot based on a Bray-Curtis similarity matrix (square root transformed) of taxa abundance data sampled in Cardigan Bay in June 2003. Data is based on 4 x 0.1 m^2 Day grab replicates taken at each station. Factors indicate field (F) and laboratory (L) analyses of the data. AA, C & N are site codes.**

The field and laboratory data were compared using an MDS plot in PRIMER© (Figure 6.7). Although the field and laboratory data are separate, primarily due to the differences in taxa abundance recorded between the two analyses, the site ordination within each data set indicates that the majority of inter-site variation was explained by both types of assessment. There was less intra-site variability shown in the laboratory samples than within the field samples, shown by the tighter clustering of the samples in the MDS.

Both the field and laboratory data were then used to calculate AMBI scores (Section 5.2) for an example comparison of the ecological assessment of the water body using RA (Figure 6.8). The ecological status assigned by AMBI to each station was the same for the majority of samples, whether they were analysed in the field or in the laboratory. For stations N1 and N4 however, there was a difference in the status assigned by AMBI between the field and laboratory analyses. For both stations, the field analysis indicated high status while the laboratory analysis indicated good status. The reason for the discrepancy between the two methods of analysis is due to the higher count of *Corbula gibba* recorded in the laboratory. The numbers of *C. gibba* recorded within the laboratory for stations N1 and N4 were 20 and 30 times greater, respectively, than the numbers that were recorded in the field. *C. gibba* is a Group IV taxa and the higher abundance found in the laboratory analysis resulted in an increased AMBI BC at both N1 and N4 (1.8 and 2.8, respectively). This higher AMBI BC caused the ecological status to be reduced from high to good. The fact that *C. gibba* was not identified during the field assessment indicates the individuals must have been particularly small and could possibly have been ephemeral

juveniles. This highlights a need for clear criteria on the identification of juveniles as opposed to small adults, this issue has been raised with the NMBAQC.

The RA exercise within the field generally provided a good representation of ecological status when stations were classified according to AMBI. Where discrepancies occurred, the field assessment provided a higher ecological status than the assessment based on the laboratory analysis.

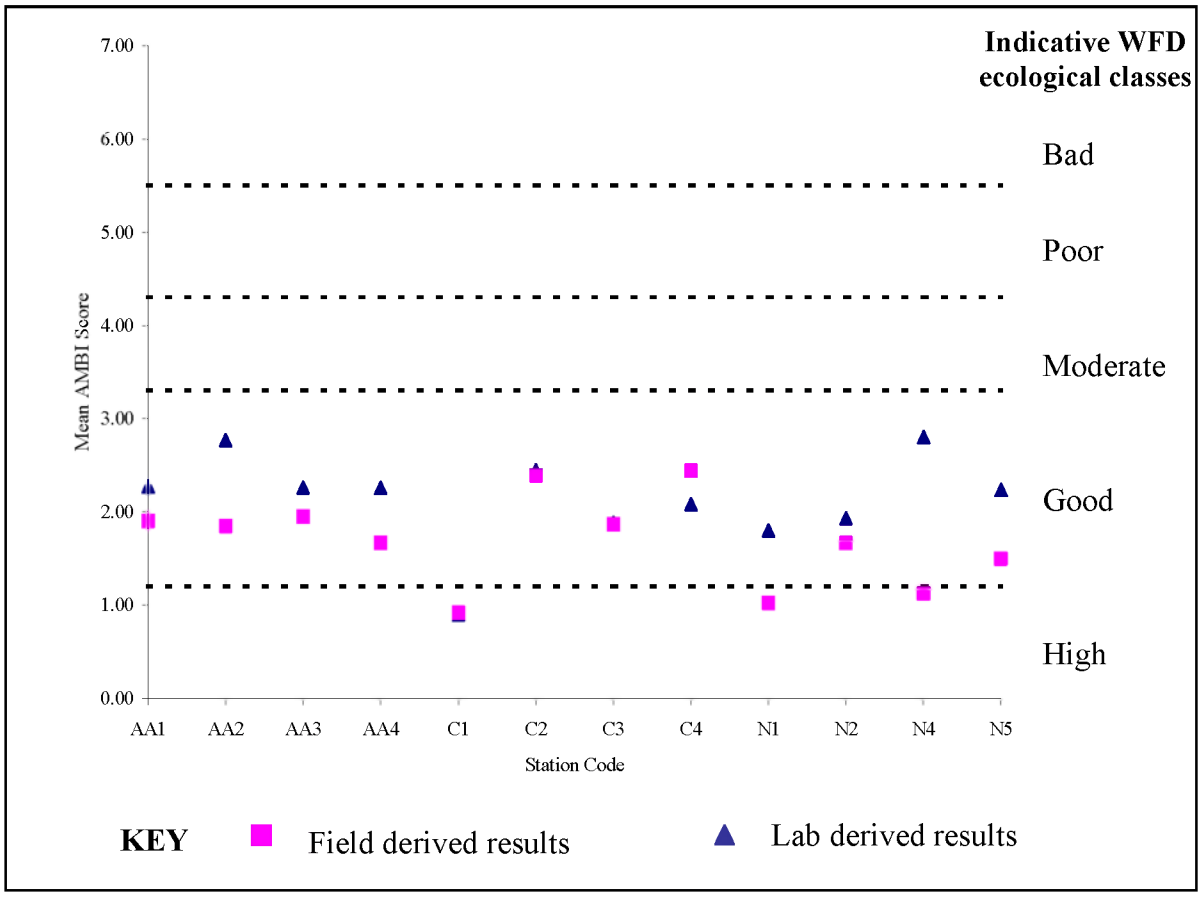

Figure 6.8 AMBI scores of stations surveyed in the Cardigan Bay Rapid Assessment exercise. The results from both field and laboratory analyses are shown. Data based on 4 x 0.1 m^2 Day grab replicates taken at each station in June 2003. Indicative WFD ecological status demarcations according to Borja *et al.*, 2003 are shown.

6.2. Rapid Assessment – Preliminary Conclusions

As results for the Wash and Fal have only recently been received it is not yet possible to reach definite conclusions regarding the use of RA in ecological assessment for WFD. The cost benefits of carrying out a modified assessment and the implications for

surveillance monitoring (risk of misclassification) have not yet been assessed. These will be included in the Project Phase III report (November 2004).

Initial results however, do indicate the potential of such methodologies if biotic metrics such as AMBI (Borja *et al.* 2003) will be used for assigning ecological status. The success of the modified methodology is dependent on habitat sampled and primarily, the expertise of specialised staff (a high level of taxonomic expertise is required). Without staff with the appropriate taxonomic skills, it will not be possible to contemplate field assessment.

No further testing or modification of a field methodology has been planned in Phase III of the project as MBITT resources are now fully committed with identifying WFD compliant classification tools. If a modified assessment methodology is to be considered, it must be stressed that highly stringent testing needs to be carried out if the resultant ecological assessment is to be proved in terms of repeatability, consistency and statistical rigour. In addition, the methodology is heavily reliant upon staff with a high level of taxonomic competency.

7. Phase III

Phase III will focus on the establishment and testing of the multimetric classification tools for habitat specific benthic invertebrate communities. WFD compliant classification tools will be proposed by November 2004, and then evaluated further during Phase IV (November 2004 - November 2005).

Phase III currently deals with soft sediments only. The aims of Phase III are to:

- Establish type specific reference conditions (qualitative and quantitative) through the use of habitat specific reference conditions (EUNIS)
- Identify a suitable multimetric for use in the ecological classification of the benthic invertebrate community of specific habitats
- Define the behaviour of classification tools to pressures acting in coastal and transitional waters
- Define ecological status class boundaries
- Assess the risk of misclassification of ecological status

The Phase III R & D technical report will detail the proposed WFD compliant tools (testing April 2004 - November 2004). Conclusions from work in Phase I and II, such as Rapid Assessment, will also be incorporated.

7.1 CASE STUDY – Testing of classification tools

Introduction
The case study outlined below indicates the approach that will be taken in testing the classification tools (deriving reference conditions and boundary criteria). The preliminary study is based on a subsection of NMMP data using stations within WFD transitional waters type 4 (TW4).

As NMMP transitional water stations should be located in fine depositional sediment in the higher salinity areas of the transitional waters, it was assumed that habitat type is reasonably consistent between stations. This assumption is now known to be incorrect and NMMP data have been sent to the Institute of Estuarine and Coastal Studies (IECS) for assignment to habitat type. As such the following example should be regarded only as indicative of the process that will be followed.

Method
This example follows the multimetric method developed by Borja *et al.* (2003). The multimetric incorporates three metrics; Richness (S), Shannon Weiner (H') and AZTI Marine Biotic Index (AMBI).

NMMP benthic invertebrate abundance data from water bodies designated as transitional water type 4 were extracted from the R & D UNICORN© database. Four NMMP stations (site codes 505, 555, 565, 566) have been included in the study. Samples were collected in every year between 1992 and 2002 (total of 95 samples). Samples were taken using a standardised methodology (as defined in the Green Book) with five replicate Day grabs (0.1 m^2) taken at each station and sieved using a 0.5 mm mesh. The taxa in the dataset

were truncated following the criteria described under data truncation (Section 4.3) and the metrics, S, H' and AMBI, were then calculated. The metric values for the five replicates from each station were averaged to give a mean value per station per year, giving 19 sampling occasions in all. In the multimetric method described by Borja *et al.* (2003), ecological status class ranges are set for each of these metrics (Table 7.1). Although each index is calculated using the same data, it is possible for index values to fall into different status class categories because each index measures different characteristics of the benthic invertebrate assemblage. To take account of this variability in each metric's assessment of status, the multimetric method uses a weighting factor (equivalent to the derived status). The weighting is termed by Borja *et al.* (2003) as Equivalent Assigned Value (EAV) and is a value between 0 and 1 (Section 3.4). One is assigned to high status and then the weighting decreases with declining status (0.75, 0.5, 0.25 and 0 for good, moderate, poor and bad status respectively). The EAVs are then used to give the overall assessment of status by summing them and dividing by the number of indices incorporated into the multimetric, in this case three. This final multimetric output provides an Ecological Quality Ratio (EQR).

Table 7.1 Index ranges and Equivalent Assigned Values (EAV) for ecological status classes

Ecological Status	H'(log2)	S	AMBI	EAV
High	>4.8	>60	0-1.2	1
Good	3.6 - 4.8	45-60	1.2-3.3	0.75
Moderate	2.4 - 3.6	30-45	3.3-4.3	0.5
Poor	1.2 - 2.4	15-30	4.3-5.5	0.25
Bad	0 - 1.2	0-15	5.5-7	0

The final assessment of ecological status of the benthic invertebrate assemblage for WFD depends on the value of the EQR. It is therefore necessary for the full range of the EQR to be subdivided into intervals that equate to each ecological status class. To this end, Borja *et al.* (2003) suggested intervals for each ecological status class (EQR^{2003}). Further analysis has led to modification of the intervals by Borja (pers comm.) in 2004 (EQR^{2004}) (Table 7.2). The two methods of splitting the EQR into ecological status class intervals have been used here to investigate whether they split this dataset into appropriate classes with regard to meeting the requirements of the normative definitions.

Table 7.2 EQR intervals for ecological status classes as stated by Borja *et al.* (2003) (EQR^{2003}) and modified by Borja (pers comm.) (EQR^{2004})

Ecological Status	EQR^{2003}	EQR^{2004}
High	0.9-1	0.8-1
Good	0.7-0.9	0.6-0.8
Moderate	0.5-0.7	0.4-0.6
Poor	0.25-0.5	0.2-0.4
Bad	0 - 0.25	0 - 0.2

Results

There was a difference in the proportion of sampling occasions allocated to each status, between the two methods ((EQR^{2003} and EQR^{2004}, Table 7.3). In order to establish which (if either) was compliant with the normative definitions, the benthic invertebrate taxa behind the EQRs were investigated. An MDS analysis (using PRIMER©) of the averaged abundance data for the 19 sampling occasions was carried out using a fourth root transformed Bray Curtis similarity matrix. The sampling occasions were labelled with the status class derived for each EQR method (Figure 7.1). An ANOSIM analysis (PRIMER©) was carried out to determine how separate the groups were, on a scale of 0 (groups are indistinguishable) to 1 (all similarities within groups are less than any similarity between groups i.e. the groups are clearly defined). The ecological status groups were more clearly defined for EQR^{2004} (R = 0.41, p = 0.003) than they were for EQR^{2003} (R = 0.296, p = 0.017). Significance values were calculated at the 5% probability level. The Global R statistic is only 0.41 and therefore does not indicate distinct groups. However, it is likely that there would be a gradual transition between ecological status classes and that the groups would therefore not be clearly defined. Boundary criteria distinguish between ecological groups and Phase III will develop these criteria further. The risk of misclassification when benthic data lies close to or on the boundaries between statuses will also be established in Phase III.

Table 7.3. Comparison of Borja *et al* (2003) EQR intervals and associated ecological class status (EQR^{2003}), with modified intervals (EQR^{2004}).

	High	Good	Moderate	Poor	Bad
EQR^{2003}	0	2	6	10	1
EQR^{2004}	1	3	10	5	0

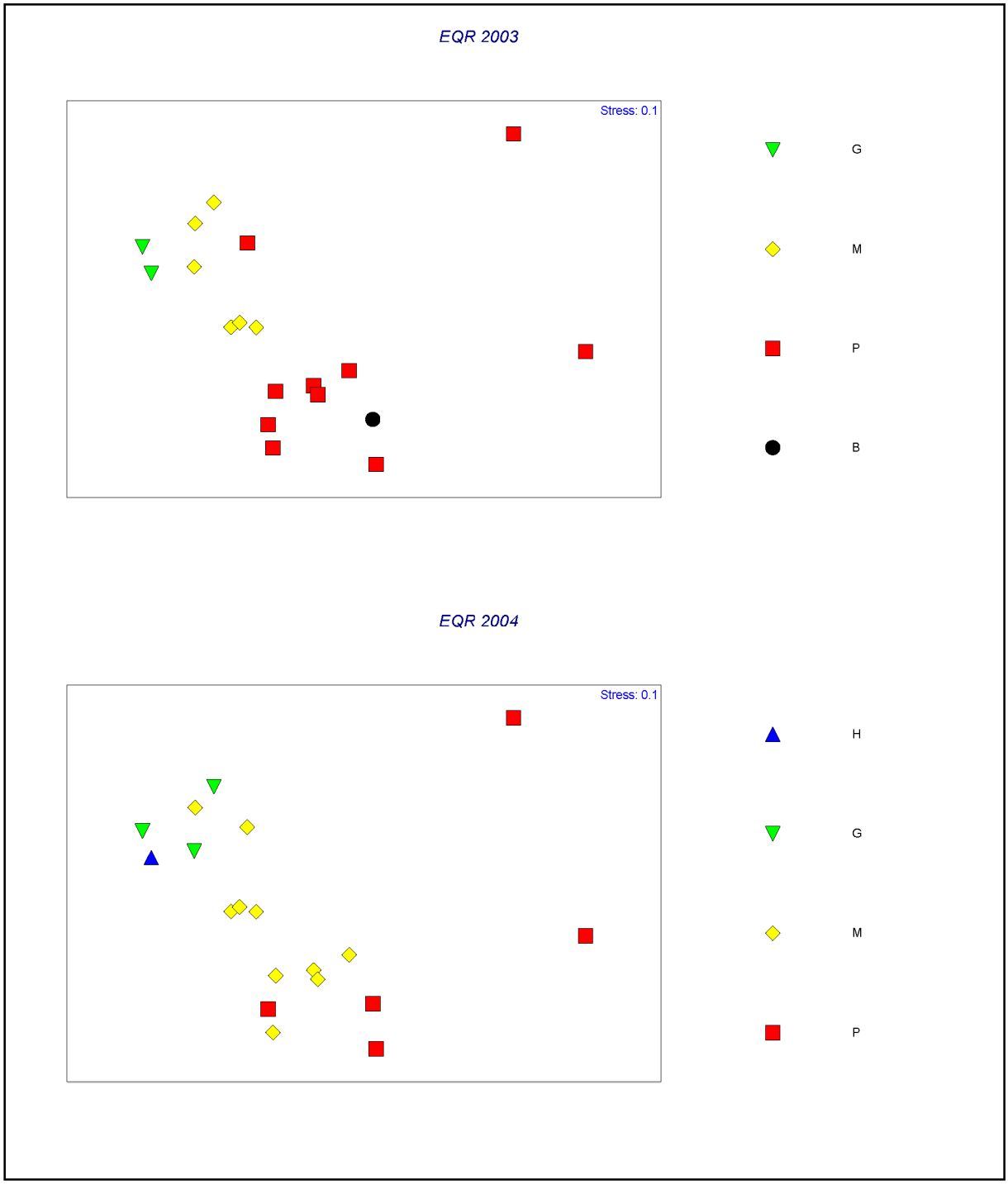

Figure 7.1: Multi-dimensional scaling plot of taxa abundance data of 19 sampling occasions taken within transitional water type 4 water bodies. Each sampling occasion is labelled according to ecological status established through application of the multimetric method described by Borja *et al.*, to derive EQR^{2003} and EQR^{2004}.

Further investigation of EQR^{2004} method was carried out. Taxonomic data for samples within each status were grouped up to phyla, with the exception of Annelida, which were split into to the classes, Polychaeta and Oligochaeta. This was done to take account of the

proportions of the Oligochaeta. Class Oligochaeta is classified as ecological group V in AMBI and in high proportions can be considered as taxa indicative of pollution (Section 5.2). Across the assigned ecological classes, there was a change in the proportions of Annelida when considered at the class level. With the transition from high to good ecological status, polychaetes decreased in occurrence, whereas oligochaetes increased in occurrence (Figure 7.2). Oligochaetes account for 37% of the taxa in the poor status class. However, the remaining assemblage is comprised from the range of other phyla. The allocation of poor status to the sample may be unwarranted because despite the high percentage of oligochaetes in the sample, there is still a diverse range of other taxa in the samples. As such the assemblage may be indicative of a more naturally stressed area of a TW2 water body, rather than a response to an anthropogenic impact. This reinforces the need for habitat-specific reference conditions relevant to salinity zones.

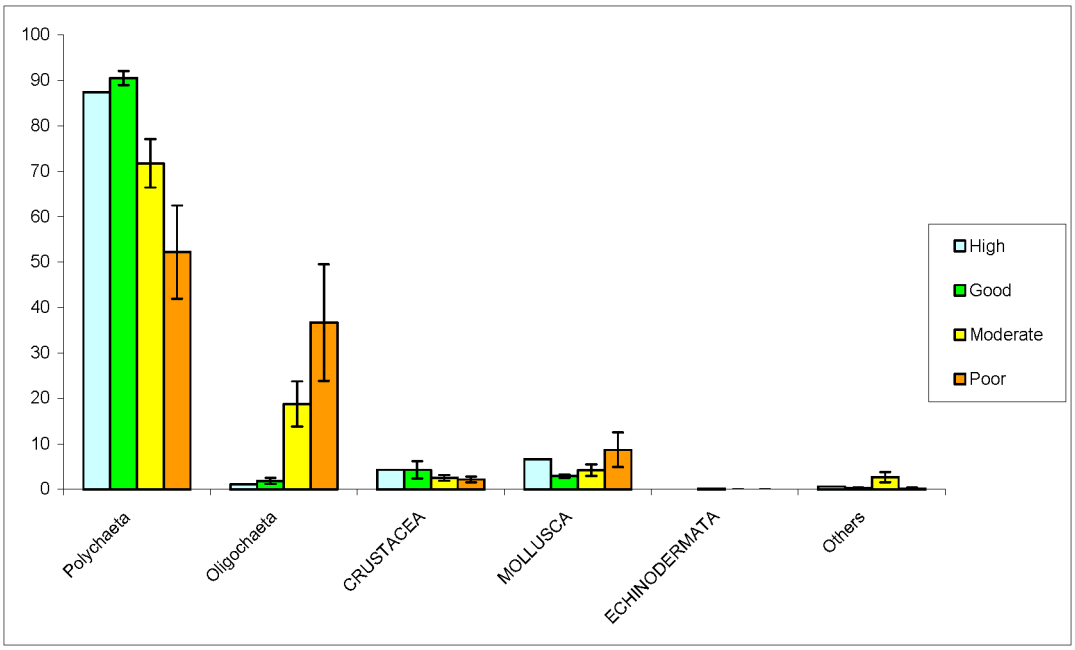

Figure 7.2 **Proportions of each phyla (Annelida split to Classes, Polychaeta and Oligochaeta) at each ecological status determined by the multimetric developed by Borja *et al.* (2003), using the revised EQR intervals (EQR^{2004}, Borja pers comm.).**

The taxonomic composition within each status class was further investigated with regard to Polychaeta, the proportions of which dominate across all ecological classes. The varying ecological status according to EQR^{2004} was further investigated by considering the orders within Polychaeta (Figure 7.3).

The change in proportions of polychaetes from samples ranging from high to poor (ecological status) was characterised by a decrease in Sabellida (AMBI GI) and an increase in Phyllodocida (AMBI GII). Spionida (AMBI GIII) are present in higher proportions but there was no pattern of change indicated with altered ecological status. The presence of spionids in such high proportions may indicate that they are characterising taxa for the particular water body type and salinity range. Capitellids, often

considered pollution indicative taxa with regards to organic enrichment (Pearson & Rosenberg, 1978), did not increase in association with degraded status but maintained relatively similar proportions for all ecological statuses.

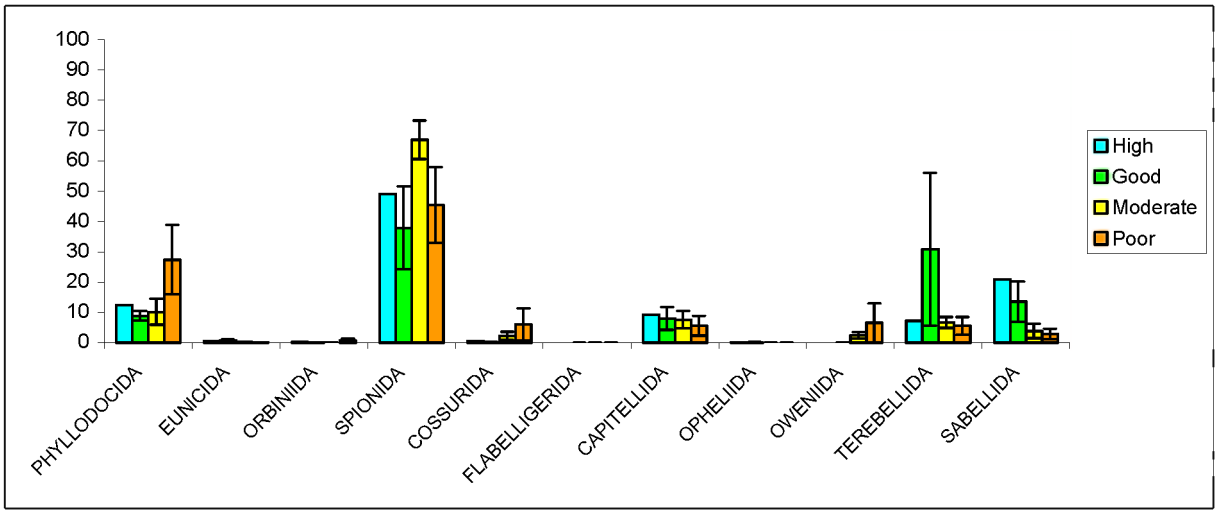

Figure 7.3 **Proportions of each Order present within the phylum Polychaeta, at each ecological status determined by the multimetric developed by Borja *et al.* (2003), using the revised EQR intervals (EQR^{2004}, Borja pers comm.).**

Discussion

This exercise represents the approach that will be undertaken in Phase III (Figure 7.4). Further study of the NMMP dataset against physico-chemical parameters needs to be undertaken to ensure that the samples falling into moderate and poor status are there due to anthropogenic impact and not through natural stress. Likewise the testing of this dataset with EUNIS habitat type allocations to the stations is necessary because the spread of status classes in this example may result from the range of habitat types in the samples considered. As such, the metric ranges and EQR intervals used within the multimetric may not be appropriate.

Having established reference conditions, the ranges of each of the indices can continue to be calculated at each status. The relevance of these ranges will have to be assessed through their reflection of the normative definitions for each status. Once the ranges are set and a multimetric is established for a habitat type, the risk of misclassification can begin to be evaluated.

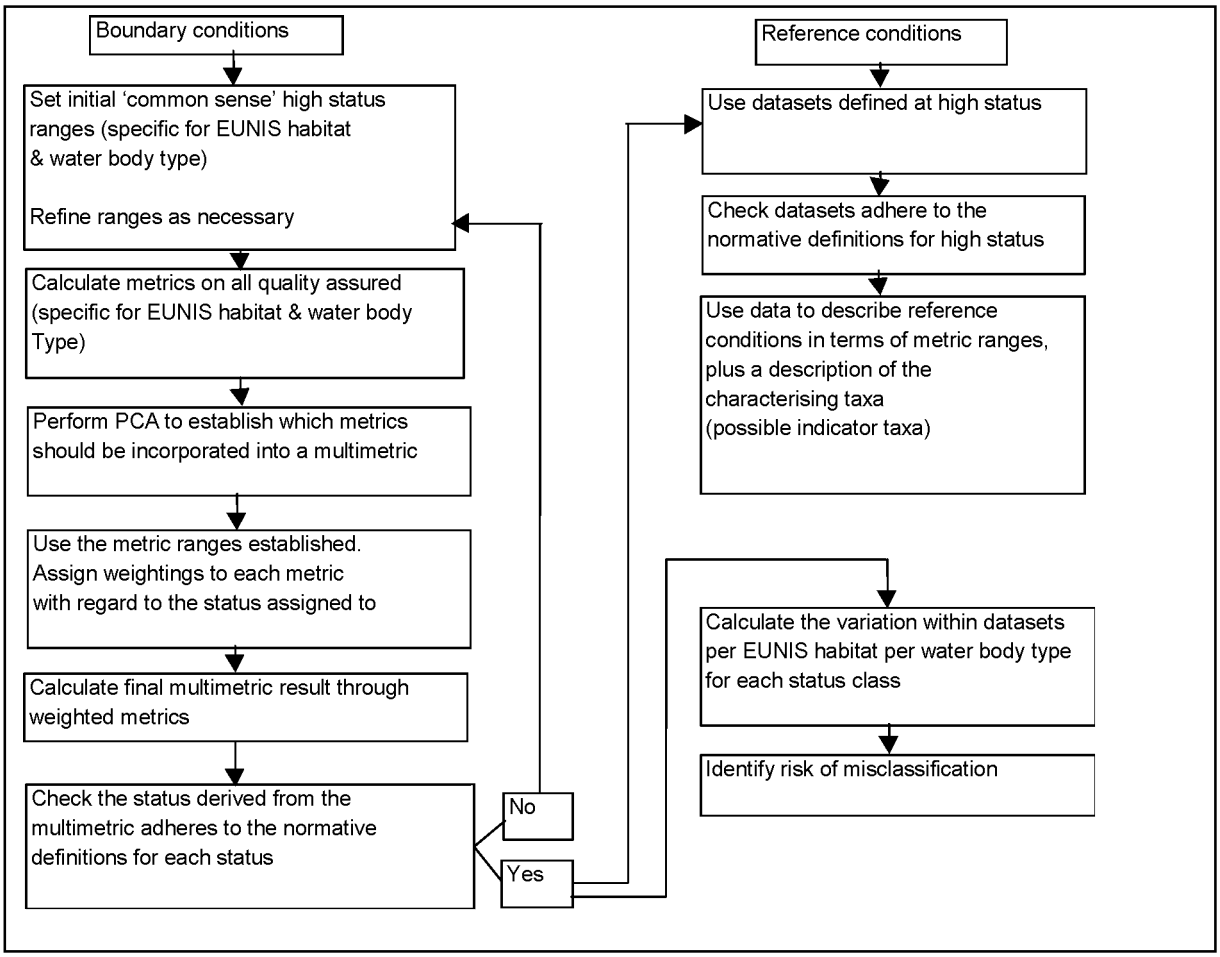

Figure 7.4 Flow diagram of stages to establish reference conditions and boundaries between ecological classes (Phase III)

Table 7.4 Timescale for Phase III

2004	Timescale for Development and Recommendation of Classification tools
April	• Draft R & D report to be circulated (Phase I and II) • Continued input of data to R & D database • Testing of Classification tools (habitat specific) Reference conditions Boundary criteria Risk of misclassification (establish statistical criteria) • Field sampling: Collection of sample for intercalibration and classification tool testing
May	• Qualitative reference conditions to be circulated • Continued input of data to R & D database (Unicorn 4 rollout (EA)) • Testing of Classification tools (habitat specific) Reference conditions Boundary criteria Risk of misclassification (establish statistical criteria) • Reporting: EA TraC WG meeting • Field sampling: Collection of samples for intercalibration and classification tool testing
June	• NMBAQC meeting - forward data truncation lists for validation • Reporting: MBITT Project Board meeting MTT: Present worked example of classification tools • Continued input of data to R & D database (Unicorn 4 rollout (EA)) • Testing of Classification tools (habitat specific) Reference conditions Boundary criteria Risk of misclassification (establish statistical criteria)
July	**Staff Contracts end (project reliant on contract renewal)** • Testing of Classification tools (habitat specific) Reference conditions Boundary criteria Risk of misclassification (establish statistical criteria)
August	• Phase III R & D report drafted • Final modifications to suggested classification tools
September	• Phase III R & D report • Reporting: MTT meeting
October	• Complete Phase III R & D report, circulate for comment • Reporting: MBITT Project Board meeting
November	• Phase III completed, WFD compliant classification tools suggested • Begin Phase IV: validation of classification tools following the Intercalibration exercise, further validation of suggested classification tools

8. SUMMARY & RECOMMENDATIONS

8.1 Summary

The following points provide a summary of the main conclusions of phases I and II:

- Within a water body type, the development of classification tools must be (EUNIS) habitat-specific to minimise the effect that natural variability will have on the metrics under consideration. The scheme will also use truncated data to address the inconsistencies in the recording of taxa and will be comparative to the normative definitions specified in the WFD.

- Classification tools will be based upon the way in which selected diversity indices respond to anthropogenic influences on benthic invertebrate communities. A multimetric approach, incorporating a range of indices, will be used. A variety of indices will provide a more accurate indication of variability within the benthic community than one index alone.

- The project has assessed two novel metrics, AMBI and AvTD, for their possible inclusion within a multimetric. These metrics are a progression from the traditional information statistics because they consider structural changes in benthic assemblages. AMBI detects changes to the proportions of sensitive taxa with an anthropogenic impact, whilst AvTD detects changes in the taxonomic structure of a community. The development of multimetrics suitable for habitat types within each water body type is in progress and will be reported in Phase III.

- The testing of AvTD has highlighted the need for the incorporation of a frequency distribution. This will be available and tested during Phase III, to ensure AvTD is a WFD compliant tool.

- Samples have been collected specifically for WFD classification tool testing. In Phase III, the sampling methodology will be developed and standardised, to reduce further variability in benthic data due to differing methods.

- An initial trial of a more rapid approach was carried out to assess benthic invertebrate communities. If this approach were to be used for WFD surveillance monitoring, further intensive trials would be needed, as well as highly trained benthic invertebrate identifiers would be required in the field teams. The cost-benefits of this method will be evaluated in Phase III.

8.2 Recommendations

Sampling

- Surveys carried out during Phases I and II have emphasised the importance of normalising sampling methodology by standardising factors such as sample type, size and season. It is imperative that habitat type is assigned at the time of sampling. Digital photography and PSA samples can be used to validate assignments.

- The trials of a more rapid approach to ecological assessment have highlighted the need for greater taxonomic expertise in the field of identification and that further, comprehensive testing is required if the resultant ecological assessment is to be proved in terms of repeatability, consistency and statistical rigour.

Data

- The development of a substantial database with which to test the metrics under consideration has been a significant task and has highlighted crucial aspects for data storage in the future:
    - a suitable database
    - associated information e.g. depth, salinity, PSA needs to be stored in conjunction with the sample data

- For WFD assessment, priority needs to be allocated to datasets that have standard methodology and complete supporting data (e.g. particle size analysis, sediment chemistry).

- Prior to statistical analysis, all datasets must be standardised due to discrepancies in the level of taxonomic identification in national datasets. Data truncation rules have therefore been established by MBITT. MBITT suggests that once these rules have been reviewed by the NMBAQC team and European AQC groups, they will be applied on a much wider scale for analytical quality control between WFD regulators and the scientific benthic community as a whole. These rules may require development, as further habitats, e.g. hard substrata, are included in testing for the establishment of classification tools. It is essential that any identification and truncation issues, be discussed with NMBAQC members. Such problems can then be addressed and solutions implemented at a national level.

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10. APPENDICES

APPENDIX I

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APPENDIX II

Qualitative Reference Conditions per Water Body Type

Please note that these reference conditions are not yet complete. Reference conditions still need to be established for several water body types and the final level at which the references will be set has not yet been finalised. These modifications will be made before the final version is prepared for UK TAG.

Water Body Type	Reference Conditions
TW1	**Upper estuary sublittoral muds (A4.326)** Upper estuary muddy sediments with very low fluctuating salinity, characterised by the oligochaetes *Limnodrilus hoffmeisteri* and *Tubifex tubifex*. Other taxa may include *Marenzelleria wireni*, *Gammarus zaddachi*, *Paranais litoralis* and *Heterochaeta costata*. The position of this biotope in the estuary may vary seasonally depending on freshwater input. **Middle/lower estuary sublittoral muds (A4.322, A4.323)** Stable muddy substrate in moderate or variable salinity conditions of the middle or lower estuary. The invertebrate community is dominated by the polychaete *Aphelochaeta marioni* and the oligochaete *Tubificoides* spp. These taxa are generally accompanied by *Nephtys hombergii* whilst the polychaetes *Capitella capitata*, *Melinna palmata*, *Mediomastus fragilis* and to a lesser extent *Polydora ciliata*, may also occur in high numbers in some areas. Other members of the cirratulid polychaete group e.g. *Caulleriella zetlandica.* and *Tharyx* spp. may also occur in high numbers, sometimes replacing *A. marioni* as the dominant polychaete. Also present may be the *Streblospio shrubsolii* (Polychaeta), *Diastylis rathkei typica* (Tanaidacea), *Hydrobia ulvae* (Gastropoda) and *Macoma balthica (*Bivalvia).
TW2	**Littoral muds (A2.3)** Variable or reduced salinity muddy substrates with a particle size of less than 0.063mm diameter typically forms extensive mudflats. Littoral muds support communities characterised by abundant polychaetes, such as *Hediste diversicolor*, *Eteone longa* and *Pygospio elegans*. Also abundant are oligochaetes (*Tubificoides spp*), the bivalve *Macoma balthica*, the spire shell *Hydrobia ulvae* and the furrow shell *Scrobicularia plana.* The biological community becomes increasingly impoverished in reduced salinity conditions.
TW3	**Muddy sand shores (A2.25)** Fine stable sands from upper to lower shores occurring in large sandy estuaries of west coast of England and Wales. Sediment contains only a small amount of silt, and muddy sand usually forms gently sloping flats supporting a low diversity biological community. The drier sediment of the upper shore is characterised by the amphipods *Bathyporeia* spp and *Corophium* spp with a limited abundance of polychaetes and bivalves. Sediment of the mid and lower shore remains saturated throughout the tidal

	cycle and supports a lower abundance of amphipods but a wide range of polychaetes commonly occur, including *Nephtys hombergii, Scoloplos armiger* and *Pygospio elegans.* The bivalves *Cerastoderma edule* and *Macoma balthica* are also common.
TW4	**Circalittoral sandy or shelly mud with *Virgularia mirabilis* and *Ophiura* spp. (A4.273)** Circalittoral fine sandy mud or cohesive muddy sand, in shallower or more exposed part of sealochs. The biological community is characterised by *Virgularia mirabilis* and *Ophiura* spp and contains mainly polychaetes such as *Goniada maculata*, *Nephtys incisa*, *Minuspio cirrifera*, *Chaetozone setosa*, *Notomastus latericeus* and *Owenia fusiformis.* *Cerianthus lloydii*, *Liocarcinus depurator* and *Pagurus bernhardus* are also usually present. *Turritella communis*, *Chaetopterus variopedatus*, *Lanice conchilega* and, less commonly, *Arenicola marina* are also found in some areas in this biotope. Bivalves can include *Myrtea spinifera*, *Lucinoma borealis*, *Mysella bidentata*, *Abra alba*, *Corbula gibba* and *Pecten maximus.* Nemerteans may also be widespread. **Seapens and burrowing megafauna in circalittoral soft mud (A4.362)** In the more sheltered basins of sealochs, at depths of 15m or greater, plains of fine mud have conspicuous populations of seapens, typically *Virgularia mirabilis* and and *Pennatula phosphorea.* These muds may be heavily bioturbated by burrowing megafauna, such as *Nephrops norvegicus.* The infauna may contain significant populations of the polychaetes *Pholoe* spp., *Glycera* spp., *Nephtys* spp., spionids, *Pectinaria belgica* and *Terebellides stroemi*, the bivalves *Nucula sulcata*, *Corbula gibba* and *Thyasira flexuosa.* The echinoderms *Amphiura chiajei, Amphiura filiformis* and *Brissopsis lyrifera* and the gastropod *Turritella communis* may also be present in large numbers, although there may be some areas where these species are absent. The burrowing anemone *Cerianthus lloydii* and the ubiquitous epibenthic scavengers *Asterias rubens*, *Pagurus bernhardus* and *Liocarcinus depurator* are present in low numbers in this biotope.
CW1	Possibilities are: A2.24 Sand shores A2.25 Muddy sand shores A4.121 Shallow water coarse sands (sparse fauna marine infralittoral clean sand) A4.132 Mobile cobbles, gravels and sands (Pomatoceros, Balanus and bryozoan crusts) A4.21 Fully marine shallow clean sands
CW2	**Sublittoral muddy sand faunal communities (A4.251)** Sheltered lower shore and shallow sublittoral sediments of sand or muddy

	fine sand in fully marine conditions support populations of the urchin *Echinocardium cordatum* and the razor shell *Ensis siliqua* or *Ensis ensis*. *Lanice conchilega*, *Pagurus* and *Liocarcinus* spp., *Arenicola marina* and *Astropecten irregularis* may be occasionally found. However, a rich variety of polychaetes, and bivalves may also be found in this biotope and the precise nature of this infaunal community will be related to the nature of the substratum, in particular the quantity of silt/clay present. **Circalittoral coarse sand and gravel (A4.131)** Circalittoral gravels, coarse sands and shell gravels, often in relatively deep water, may be characterised by the presence of conspicuous venerid bivalves such as *Circomphalus casina*, *Clausinella fasciata*, *Timoclea ovata* and other robust bivalve species such as *Glycymeris glycymeris* and *Astarte sulcata*. Where the interstices of the gravel are filled by finer particles, *Spatangus purpureus*, *Gari tellinella* and *Timoclea ovata* may also be prevalent. Such communities in gravelly sediments are likely to be species-rich as they may also contain epifauna such as *Hydroides norvegicus* and *Pomatoceros lamarcki*. **Circalittoral muddy sands (A4.271)** Non-cohesive muddy sands or slightly shelly/gravelly muddy sand in sheltered or occasionally slightly reduced salinity environments may be characterised by the presence of the bivalves *Abra alba* and *Nucula nitidosa*. Other important taxa include *Nephtys* spp., *Chaetozone setosa* and *Spiophanes bombyx* with *Fabulina fabula* also being common in many areas. The echinoderms *Echinocardium cordatum*, *Ophiura albida* and *Ophiura ophiura* may also be present.
CW3	None
CW4	**Shallow infralittoral sand (A4.211, A4.212)** Fine sands in the shallow sublittoral may be characterised by *Nephtys cirrosa* and *Bathyporeia* spp. The diversity can be reduced due to physical disturbance from strong tidal streams or wave action. In more compacted sands, venerid bivalves such as *Chamelea gallina* may dominate. *Fabulina fabula* and *Magelona mirabilis* may also be characteristic. **Sublittoral muddy sand (A4.271, A4.272)** Muddy sands or slightly mixed sediments in sheltered or slightly reduced salinity environments may be characterised by the presence of the bivalves *Abra alba*, *Nucula nitidosa* and *Corbula gibba* as well as *N. nucleus*, *Lagis koreni* and *Nephtys* sp. The echinoderms *Echinocardium cordatum*, *Ophiura albida* and *Ophiura ophiura* may also be present. Where the sediment is slightly sandier, *Echinocardium cordatum* will be more dominant and *Amphiura filiformis* will also be characteristic. *Pholoe* sp., *Nephtys hombergii*, *Nucula nitidosa*, *Callianassa subterranea* and *Eudorella truncatula* are also typical of this biotope.
CW5	**Moderately exposed circalittoral rock (A3.62)** Circalittoral rock or mixed substrata in moderately exposed conditions which typically support a prominent turf of bryozoans and hydroids. The habitat has weak or moderate tidal currents which, with sand nearby, leads to some sand in suspension and influence on the fauna present. *Flustra foliacea* and to a lesser extent *Securiflustra securifrons* (or in the south *Chartella papyracea*), often form the bulk of the turf although other bryozoans, such as *Alcyonidium diaphanum* and *Eucratea loricata* may be prominent. The

	hydroids *Sertularia* spp. and *Hydrallmania falcata* are particularly characteristic of this habitat (and may dominate), although others also occur. **Circalittoral rock with brittlestars (A3.65)** Circalittoral rock or mixed substrata dominated by dense beds of brittlestars. *Ophiothrix fragilis* or *Ophiocomina nigra* may dominate separately or there may be mixed populations of the two species. When very dense, brittlestars tend to have a smothering effect on the rock, significantly reducing species diversity and biomass. Due to the mobile nature of brittlestars, some areas can appear heavily grazed. **Sublittoral muddy sand with polychaetes and bivalves (A4.251)** Sheltered lower shore and shallow sublittoral sediments of sand or muddy fine sand in fully marine conditions support populations of the urchin *Echinocardium cordatum* and the razor shell *Ensis siliqua* or *Ensis arcuatus*. A rich variety of polychaetes, such as *Notomastus latericeus*, *Mediomastus fragilis* and *Scoloplos armiger*, may occur in abundance. Bivalves such as *Mysella bidentata*, *Tellimya ferruginosa*, *Dosinia lupinus*, *Chamelea gallina* and *Gari fervensis* may also typically occur, as may the predatory worms *Pholoe inornata* and *Harmothoe* spp. *Amphiura brachiata* is common in fine sandy sediments and *Labidoplax media* in slightly muddier sediments. **Sublittoral muddy sand with echinoderms (A4.272)** Medium to fine clean / muddy sand off shallow wave- exposed coasts can be characterised by *Amphiura filiformis* and *Echinocardium cordatum*. This community is also characterised by *Pholoe* sp., *Nephtys hombergii*, *Nucula nitidosa*, *Callianassa subterranea* and *Eudorella truncatula*. Less significant taxa include *Virgularia mirabilis*, *Cerianthus lloydii* and *Chaetopterus variopedatus.*
CW6	Possible habitats (from JNCC Mermaid) Low bedrock ridges Kelp in bedrock Maerl bed Kelp in maerl bed Kelp forest Muddy sand plain overlain with gravel and shell
CW7	**Sublittoral fine sand (A4.211)** Well-sorted medium and fine shallow sands, subject to physical disturbance from strong tidal streams or wave action may be characterised by *Nephtys cirrosa* and *Bathyporeia* spp. and sometimes *Pontocrates* spp. The faunal diversity of this biotope is considerably reduced compared to less disturbed biotopes and for the most part consists of the swimming amphipods. **Tide-swept shallow sublittoral fine sand (A4.123)** Where strong tidal streams or wave action and coarse sand occur in the shallow sublittoral, dense beds of *Lanice conchilega* may occur. Several other species of polychaete also occur as infauna e.g. *Scoloplos armiger*, *Chaetozone setosa* and *Arenicola marina*.
CW8	None

APPENDIX III

Benthic invertebrate datasets held on R & D UNICORN© database from known impact gradients (TW: Transitional water, CW: Coastal water).

Pressure	Location	Water body type	Period data covers
Potash (metals and >silt clay)	Boulby Coast	CW	1998-2002
Urban conurbation	Clyde	TW	1993-2000
Industrial discharges	Tees	TW	1980-1996
Sewage effluent	Amble Northumberland Coast	CW	1996
Sewage effluent	Cambois	CW	1996
Sewage effluent	Littlehampton	CW	1995
Sewage effluent	Langbaurgh	CW	1996
Sewage effluent	Margate	CW	1996
Sewage effluent	Portobello	CW	1996
Sewage effluent	Seaton Carew	CW	1996
Sewage effluent	Seaford	CW	1995
Papermill	Severn Estuary	TW	1993-1998
Sewage effluent	Swalecliffe	CW	1996
Sewage effluent	Teign	CW	1994
Organic compounds, Sodium hydroxide, Hydrogen peroxide, Zinc and Cadmium	Caen, Taw/Torridge complex	CW	1994, 1996
Industrial discharges (Steel production)	Usk Estuary	CW	1994

Benthic invertebrate datasets from known impact gradients which are not held on the R & D UNICORN© database

Pressure	Location	Period data covers
Fish farm	Scotland/Ireland	Unknown
Disposal ground	Garroch Head	Unknown
Industrial discharges	Leven Estuary	1989-2001
Sewage effluent	Hythe	1995
Sewage effluent	Ingoldmells	1995
Sewage effluent	Mundesley	1996
Sewage effluent	Caister (Great Yarmouth)	1995
Sewage effluent	Lowestoft	1995
Sewage effluent	Clacton	1995
Sewage effluent	Jaywick	1995
Sewage effluent	Swalecliffe HNDA 1996	1996
Sewage effluent	Seaford HNDA	1996
Sewage effluent	Sandown	1995
Sewage effluent	Bognor Regis	1995
Sewage effluent	Teignmouth	1990

APPENDIX IV

Taxonomic classification of encrusting Cnidaria that should be deleted from soft-sediment data sets. Taxa in bold are those that should be removed. Those not highlighted provide an aid to the taxonomic classification of those to be truncated.

Super-Class	Class	Order	Family	Genus & Species
Hydrozoa	Leptolida	Filifera	Eudendriidae	*Eudendrium sp.*
			Pandeiidae	*Leuckartiara sp.*
			Bougainvilliidae	
			Hydractiniidae	*Hydractinia echinata*
			Clavidae	*Clava multicornis*
				Cordylophora sp.
		Conica	Phialellidae	*Phialella quadrata*
			Campanulinidae	*Calycella syringa*
				Campanulina sp.
			Lafoeidae	*Filellum serpens*
			Haleciidae	*Halecium sp.*
			Sertulariidae	*Abietinaria sp.*
				Diphasia sp.
				Hydrallmania falcata
				Sertularella sp.
				Sertularia sp.
				Tamarisca tamarisca
			Plumulariinae	*Plumularia setacea*
				Nemertesia antennina
		Proboscoida	Campanulariidae	*Campanularia hincksii*
				Rhizocaulus verticillatus
				Clytia hemisphaerica
				Gonothyraea loveni
				Laomedea sp.
				Hartlaubella gelatinosa
				Obelia sp.
Anthozoa	Octocorallia	Alcyonacea	Alcyoniidae	*Alcyonium digitatum*
	Hexacorallia	Ceriantharia	Cerianthidae	*Cerianthus lloydii*
		Actiniaria	Gonactiniidae	
			Actiniidae	*Actinia sp.*
				Gonactinia prolifera
			Metridiidae	*Urticina felina*
			Sagartiidae	*Sagartia sp.*
				Metridium senile

APPENDIX V

Classification of the Ecological Status of the Colne transitional water using AMBI

A case study of how AMBI functions was carried out on the Colne transitional water (transitional water type 4) in Essex. The aim was to investigate how ecological status as defined by AMBI relates to the expanded normative definitions and how valuable AMBI is generally as an indicator of ecological status.

Benthic invertebrate data from samples taken at ten sites along the Colne in 1992 and eight sites in 1997 were analysed. The data was based on three 0.1 m^2 Day grab replicates taken at each site. AMBI scores were assigned to each taxa and each replicate was classified with an ecological status according to the AMBI demarcations established by Borja *et al.* (2000) (Figure 1). Between-replicate, between-site and between-year differences in ecological status were studied. AMBI results were also compared to those of the other diversity indices under investigation. The relationship between the proportions of groups of taxa at each site in 1992 and the expanded normative definitions was analysed. Figure 1 shows that ecological status gradually increased from poor near the town of Colchester (sites 1-3) to moderate further downstream (sites 4-8) to high near the mouth of the transitional water (sites 9 and 10).Taxa were classified to Class level and average ecological status was calculated for each site. Figures 2 and 3 show the transition in the proportions of different classes of invertebrates along the Colne.

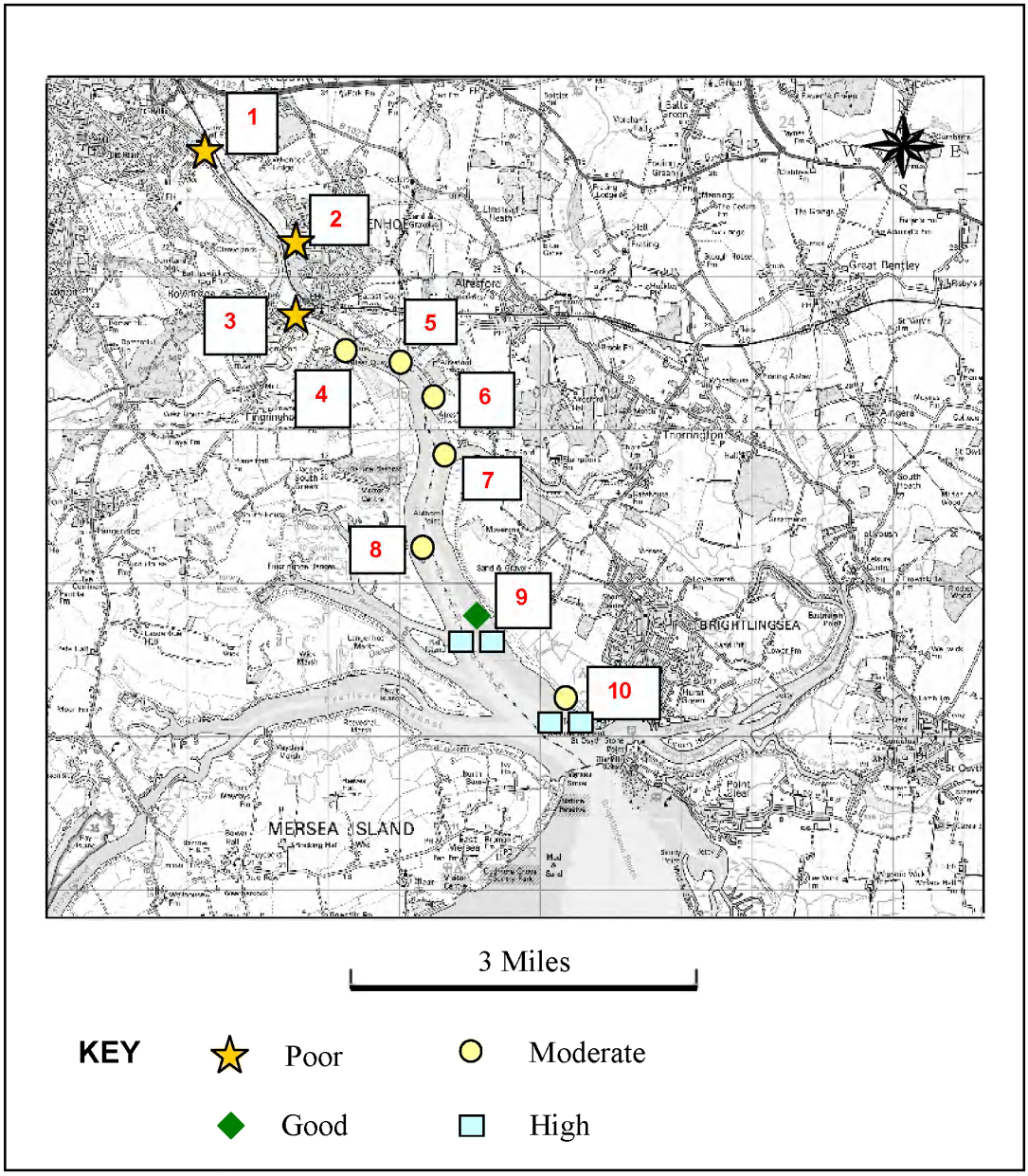

Figure 1 **Ecological status based on AMBI of 0.1 m^2 replicate samples taken at 10 sites along the Colne in October 1992. Where one symbol is displayed, all replicates had the same ecological status.**

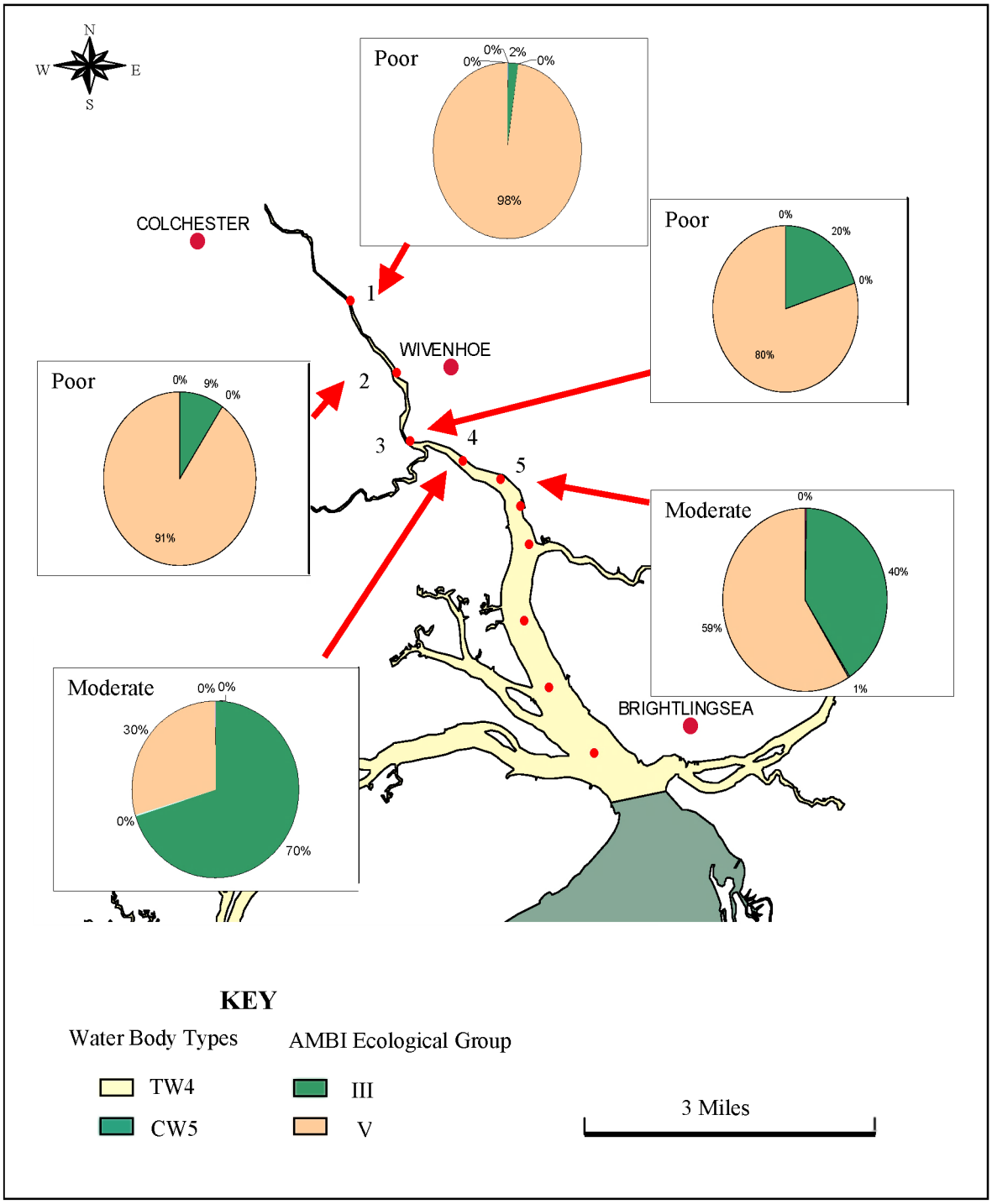

Figure 2 **Proportions of AMBI ecological groups (Borja *et al.*, 2000) at stations 1-5 surveyed along the Colne in October 1992. Abundances are based on data pooled for three 0.1 m^2 replicate samples. The ecological status of each station is indicated.**

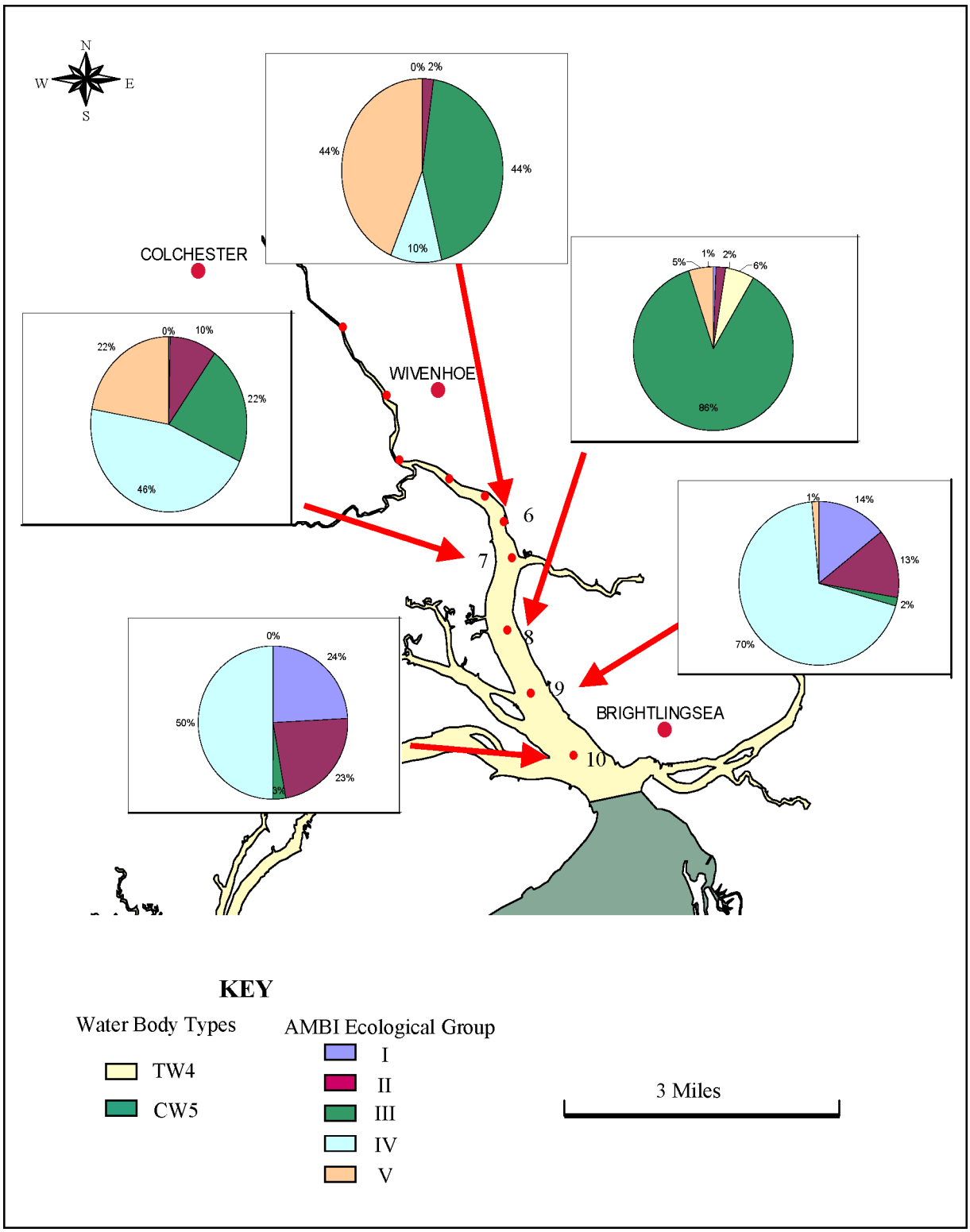

Figure 3 **Proportions of AMBI ecological groups (Borja *et al.*, 2000) at stations 6-10 surveyed along the Colne in October 1992. Abundances are based on data pooled for three 0.1 m^2 replicate samples. The ecological status of each station is indicated.**

Table 1 relates a summary of the proportions of ecological groups at each site to the descriptions of community abundance given in the expanded normative definitions (Section 2.1).

Table 1 Summary of the proportions of AMBI ecological groups at each site sampled along the Colne in 1992. The community description given in the expanded normative definitions is also shown for each ecological group.

Ecological Status	Pollution State according to AMBI	Summary of taxa proportions at each site	Community abundance description specified by expanded normative definitions
Bad	Very heavily or extremely polluted	No sites at bad status	Azoic or, if fauna present: Sensitive, Indifferent, & Tolerant Taxa (GI, GII, & GIII) absent. Opportunistic Taxa (GIV) of sub-dominant abundance. Indicator Taxa (GV) of dominant abundance.
Poor	Transitional moderately to heavily polluted	Sites 1-3: Dominated by GV taxa. Lower proportions of GIII.	Sensitive and Indifferent Taxa (GI & GII) of negligible abundance or absent. Tolerant Taxa (GIII) of sub-dominant abundance. Opportunistic Taxa (GIV) & Indicator Taxa (GV) co-dominate the abundance.
Moderate	Transitional unbalanced to moderately polluted	Sites 4-6: Greater proportions of GIII and lower proportions of GV. Site 7: Greater diversity of ecological Groups Dominated by GIV Site 8: Dominated by GIII.	Sensitive Taxa (GI) of negligible abundance or absent. Indifferent Taxa (GII) of low sub-dominant abundance. Tolerant Taxa (GIII), Opportunistic Taxa (GIV) & Indicator Taxa (GV) co-dominate the abundance.
Good	Slightly unbalanced, slightly polluted	Sites 9-10: High proportions of GIV. GI and GII in greater abundance than at other sites.	Sensitive Taxa (GI) abundance may range from high sub-dominant to absent. Indifferent Taxa (GII) of low sub-dominant abundance. Tolerant Taxa (GIII) of dominant abundance. Opportunistic Taxa (GIV) & Indicator Taxa (GV) abundance may range from negligible or low to equi-abundance with Indifferent Taxa.
High	Normal, unpolluted		Sensitive Taxa (GI) of dominant abundance. Indifferent and Tolerant Taxa (GII & GIII) absent or of sub-dominant abundance. Opportunistic Taxa (GIV) absent or of negligible abundance. Indicator Taxa (GV) absent or of negligible abundance.

The question of whether the statuses specified by AMBI correspond to those given in the expanded normative definitions was addressed:

Poor ecological status: The proportion of taxa at the sites of poor status do not fit with that given in the expanded normative definitions because groups IV and V do not co-dominate the abundance at any of the sites. Group V dominates at all three sites with poor status, which would correspond more closely with the expanded normative definition given for bad ecological status. However, groups I and II are absent and group III does have sub-dominant abundance at each site with poor status, which does correspond with the definition given for poor status

Moderate ecological status: Table 1 shows that the sites classified as moderate within the Colne represent a wide range of proportions of taxa with different ecological groups. The dominant groups at each site range from group III accounting for 86 % at site 8 and groups III and V having 44 % co-dominance at site 6. Compared to other sites with moderate status, the taxa proportions at site 7 correspond the most with the expanded normative definition because groups III, IV and V co-dominate the abundance. Generally however , it appears that the expanded normative definition does not incorporate the range of possible combinations of ecological groups within the Colne that can cause a sample or site to be of moderate status.

Good ecological status: This agrees with the expanded normative definition because groups I and II are subdominant. However the description does not incorporate group IV being in such high proportion as it specifies that these opportunistic taxa may range from negligible or low to equi-abundance with Indifferent Taxa (Group III).

For each status, the expanded normative definition partly defines the taxa proportions found at each site within the Colne. The ecological statuses of each site have been based on the demarcations set out by Borja *et al.* in 2000. In 2003, the boundaries were altered by Borja *et al.* slightly to correspond with the normative definitions (Table 2). The range of possible AMBI values for moderate status has been narrowed whilst the boundaries for poor and bad status are now based on lower AMBI scores. Therefore a site which may have been of moderate status originally may now be classed as poor. Likewise a site which may have had poor status may now be classed as bad. This alteration may mean that if re-calculated for each site, the statuses may correspond more closely with the expanded normative definitions. Ecological status based on AMBI will need to be re-calculated for the Colne based on the new AMBI demarcations. Further testing of the relationship between ecological status based on AMBI and the normative definitions will need to be carried out on other transitional and coastal waters. It is also possible that the normative definitions may have to be developed further in order to encompass every possible combination of ecological group proportions, for example, for moderate status.

Table 2 **Range of AMBI biotic coefficient values developed by Borja *et al.* (2000) and the modified ranges developed to correspond to the normative definitions (Borja *et al.*, 2003)**

Biotic Coefficient (2000)	Biotic Coefficient (2003)	Suggested WFD Ecological Status
0.0< BC ≤1.2	0.0< BC ≤1.2	High
1.2< BC ≤3.3	1.2< BC ≤3.3	Good
3.3< BC ≤5	3.3< BC ≤4.3	Moderate
5< BC ≤6	4.3< BC ≤5.5	Poor
>6	>5.5	Bad

Overall, this case study showed that AMBI is a useful indicator of both temporal and spatial change in taxa abundance along the Colne transitional water. Further analysis indicated that dominant species, such as *Heterochaeta costata*, *Streblospio shrubsolii* and *Tubificoides pseudogaster* control the AMBI classification of a sample. High abundance of Oligochaeta cause a sample to be classified as Poor, Polychaeta cause a sample to be Moderate-Good and Phoronida cause a sample to be High in status. PCA analysis indicated that diversity calculated by AMBI is comparable to diversity calculated by other diversity indices. The AMBI biotic co-efficient is influenced by the level of taxa classification (whether taxa are classified at the species, genus or family level) and it is therefore more accurate to assign scores to the lowest level of classification possible. If AMBI scores are assigned at the lowest taxa level possible and analysis of the taxa contributing to the particular ecological status is carried out, AMBI can provide a valuable indication of ecological status.

APPENDIX VI

Workshop 'Expert Judgement' (27th-28th October 2003) participant opinions of the ecological status of 36 stations sampled in the Wash (1991) (P = Poor, M = Moderate, G = Good, H = High). Agreement between workshop participants and the status assigned using the AMBI index (Borja *et al.*, 2000) is denoted by a tick (✓). A cross (×) denotes disagreement in status class. The workshop participants' judgement of status is in brackets. Notes on the reason for the allocation of a particular status is also indicated.

Station	Expert Status	Agreement with AMBI? (AMBI Status)	Notes and Reason for allocation
2	M	✓	
57	M	× (G)	*Capitella* presence
31	G /M	✓	*Chaetozone setosa* and *Mediomastus fragilis* could indicate disturbed environment.
52	M	× (G)	Although the taxa represent a healthy habitat, taxa numbers are low. There is known physical disturbance at the site but AMBI and ITI failed to describe this disturbance.
63	G		Clean sandy environment
4	M	× (G)	Site is sandy and lies within Boston Deeps. It is subject to disturbance and freshwater influence. Taxa indicate good habitat but the natural physical disturbance lowers the status.
54	G	✓	
59	P	× (G)	Mobile sediment, sand bank erosion, closest site to estuary, so most likely to be polluted. Species indicative of poor, muddy environments e.g. *Capitella* and *Tubificoides*.
62	M	× (G)	Intertidal, mixture of *Capitella* and *Nephtys* (mud) and *Fabulina* (muddy sand)
53	G	✓	Sandy habitat
7	G	✓	
14	G	✓	
6	G	✓	
21	G	✓	Shallow sandy, potentially impacted from the polluted Nene.
39	H	× (G)	Selected as monitoring site. Very deep, heterogeneous complex substrate. Good taxon list and fits biotope description well.
29	G	✓	Good physical location but *Tubificoides* lowers the status from high to good.
5	M	× (G)	*Capitella* (mud) and *Nephtys cirrosa* (sand) are present i.e. opposite ends of spectrum. Naturally physically disturbed site.
50	G	✓	Most offshore site. Deep, natural, coastal sediment.
24	G	✓	Subtidal mixed sediment.
8	M	× (G)	Edge of dredged channel. Low abundance. Low species number.
38	G	✓	Do not usually get *Mediomastus* and *Capitella* together – error with identification?
46	G	✓	
23	G	✓	
20	M	× (H)	Naturally impoverished, poor ecologically – scoured by tide? Although taxa indicate a healthy habitat, the taxon list is very

			short.
66	H	✓	*Sabellaria* reef
35	H	✓	*Sabellaria* reef
33	H	✓	*Sabellaria* reef
22	G	✓	Large taxon list. Presence of an opportunist, *Phoronis*
25	H	× (G)	Short taxon list. Status chosen because good typical coastal sandy site.
48	G	✓	Offshore, no impact, gravelly stony. Status assigned based on taxa number.
49	G	✓	Offshore, no impact, gravelly stony. Status assigned based on taxa number.
26	G	✓	Relatively representative
58	G	✓	Relatively representative
43	P	× (G)	*N. hombergii* very high whilst other taxa low in abundance. Error in recording?
40			*Chaetozone setosa* not usually found in Wash, especially not in other years – mistake with identification?
51	M	× (G)	Most impacted site. Not typical clean sand community because *N. hombergii* and *H. ulvae* present.

APPENDIX VII

Key to water body codes shown in Figure 5.9 (Section 5.3)

Number	Water Body	Number	Water Body
1	Avon	25	Loch Linnhe
2	Axe	26	Lynne of Lorne
3	Cardigan Bay	27	Lymington
4	Chichester Harbour	28	Medway
5	Clyde	29	Loch Melfort
6	Cambois	30	Mersey
7	Dart	31	Milford Haven
8	Durham Coast	32	Loch Na Keal
9	Dee	33	Newtown
10	Erme	35	Ore & Alde
11	Fal	36	Loch Craignish
12	Filey	37	Poole Harbour
13	Forth	38	Portsmouth Harbour
14	Fowey	39	Scilly
15	Gannel	40	Loch Scridain
16	Hayle	41	Seaham
17	Helford	42	Southampton Water
18	Humber	43	Swale
19	Ironotter	44	Tamar
20	Kingston Hud	45	Tay
21	Kingsbridge	46	Taw & Torridge
22	Loch Kyle	47	Wash
23	Langstone Harbour	48	Whitby
24	Loch Fyne		

APPENDIX VIII

Rapid Assessment of
Macroinvertebrate Samples

Report to The Environment Agency
and Joint Nature Conservation
Committee

Institute of Estuarine and
Coastal Studies
University of Hull

9 June, 2004

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Report: SBB117-F-2004

Client Environment Agency and Joint Nature Conservation Committee

Project Title: Rapid Assessment of Benthos Samples

9 June, 2004

Reference No: SBB117-F-2004

For and on behalf of the Institute of Estuarine and Coastal Studies	
Approved by:	_____
Signed:	_____
Position:	_____
Date:	27 February 2004 _____

This report has been prepared by the Institute of Estuarine and Coastal Studies, with all reasonable care, skill and attention to detail as set within the terms of the Contract with the client.

We disclaim any responsibility to the client and others in respect of any matters outside the scope of the above.

This is a confidential report to the client and we accept no responsibility of whatsoever nature to third parties to whom this report, or any part thereof, is made known. Any such parties rely on the report at their own risk.

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1 INTRODUCTION

The implementation of the Water Framework Directive and EC Habitats Directive requires that the ecological status of all transitional and coastal waters be described. Traditional methods of sampling and laboratory analysis are unavoidably labour intensive and hence costly, and often involve a considerable delay between the commissioning of the survey and production of the report. The development of rapid assessment techniques that will provide repeatable and relevant data in a timely and cost effective way is therefore of considerable importance.

This report details results of some possible approaches which may be adopted in the laboratory to achieve the above aims. The data originate from samples taken during an Environment Agency field workshop held on board the CSV *Vigilance* in the Fal - Ruan estuary complex in September 2002. The workshop was held as part of the Environment Agency Research and Development project (E1-116), as part of the integrated UK & Republic of Ireland approach to assessment of transitional and coastal waters. Funding was provided by JNCC for the follow-up laboratory analysis. The objectives were:

- to assess whether a subsample extracted during a pre-determined time period could be representative of the whole sample. This was addressed by Timed Sorting Analysis (TSA);
- to assess how an 'expert view' of a sieved sample assessed in the field relates to a full sample analysis (EVA - Expert View Analysis);
- to evaluate whether a sample analysed by an expert in the laboratory with basic magnification equates to the more usual microscope aided identification. This was tested using a protocol termed Restricted Laboratory Analysis (RLA);
- to validate these objectives with complete full analysis following NMBAQC protocols.

These methods were devised by Environment Agency and JNCC staff. IECS was contracted to perform the laboratory analyses.

2 MATERIALS AND METHODS

2.1 Field methods

The samples used for this project were taken from six sites within the Fal Estuary system. Tresilian samples were taken by coring (0.01m^2), Malpas, Greatwood and Messack sampling was by Day Grab (0.1m^2) and the samples from Restronguet Creek and Ruan Creek were taken with either a Van Veen grab (0.05m^2) or a core (0.01m^2). All sampling and field processing was carried out by staff of the Environment Agency, JNCC, EHS and English Nature in mid-September 2002.

Samples for Timed Sorting Analysis (TSA), Whole Sample Analysis (WSA) and Restricted Laboratory Analysis (RLA) were immediately fixed in the field using 4% formalin and later transferred to IMS. Expert View Analysis (EVA) samples were sieved (1.0 mm) in the field and examined live for 15, 20 or 30 minutes by staff who were classified as either expert, intermediate or beginner. Animals identified and

enumerated in this way were then removed to a separate labelled container and preserved for laboratory analysis. The remainder of the sample (residue) was similarly preserved. It was decided not to continue with the WSA (effectively all Greatwood and Tresilian samples) and these samples have been stored in case of future need.

At Messack the sediment consisted of subtidal gravel with shell fragments. At Restronguet Creek and Ryan Creek the substratum was estuarine muds/fine sands.

2.2 Laboratory methods

In the laboratory, samples for RLA, TSA, and residues for EVA were washed under fume extraction hood through a 0.5 mm or 1.0 mm sieve (as previously designated) to remove traces of fixative. The samples were then examined (approximately 0.25 l at a time) under a layer of water in white trays using a fluorescent 1.5x illuminated magnifier. Animals were removed using watchmakers forceps ("picking") and stored by taxonomic group in appropriately labelled containers under 70% industrial methylated spirits (IMS). With TSA samples, picking was restricted to either 15 or 30 minutes before proceeding with the full extraction. In each case the *whole* sample was examined during a 15 minute period to extract as much of the fauna as possible. This meant that, for 30 minute analyses, the samples were effectively scanned twice (for 15 minutes on each occasion) before being sorted in the usual way. These time-limited fractions were stored and analysed separately.

The invertebrates removed and identified in the field during EVA were washed as above before laboratory identification.

Identification of invertebrates was carried out using Olympus SZ30 zoom microscopes with 10x and 20x eyepieces, giving a maximum magnification of up to 80x. An additional 2x objective was occasionally used to increase the potential magnification to 160x. Compound microscopes were used for further magnification, up to 1000x. The macrofaunal animals were then identified to species level, wherever possible, using standard taxonomic keys and dissection, when necessary. Oligochaetes were cleared in lactophenol prior to microscopic examination.

For RLA treatment the sorted animals were identified and counted as far as possible using a 1.5x desk magnifier. This work was then checked "blind" (i.e. without reference to these data) by a second member of staff, but this time using microscopes as described above.

A reference collection of taxa encountered during the study was compiled.

2.3 Statistical analysis

Univariate sample statistics (Shannon diversity index) and the variables sample species richness (S) and total abundance (A) were computed to compare samples under different treatments. Cluster analysis (using PRIMER™) was also used to investigate the differences between the various sample processing methods. All cluster analyses were conducted on untransformed data using the Bray - Curtis

similarity measure (Bray & Curtis, 1957) and group average cluster mode. Further details of statistical analyses are given where appropriate in the text.

A Biotic Coefficient (Borja *et al.* 2000) was also calculated to compare the various treatments. This method assigns each taxon to one of five groups depending on its known ability to tolerate organic pollution. Group I species are very sensitive to organic enrichment and are confined to unpolluted conditions. Group II species are relatively indifferent to enrichment and their populations fluctuate independently of low levels of organic pollution. In Group III species are tolerant to excess organic matter but their abundance increases in response to organic enrichment. Group IV consists of "second-order" opportunistic species and Group V of "first-order" opportunistic species which are adapted to reduced sediments. The scores for each sample are converted to a continuous index (Biotic Coefficient) using a weighted percentage of the whole sample score (see Borja *et al.* 2000 for details).

Throughout this report sample numbers are preceded with a two-letter prefix indicating the site of origin (ME = Messack; RE = Restronguet Creek; RU = Ruan Creek).

3 RESULTS

3.1 Timed sorting analysis (TSA)

TSA was carried out on a total of 16 samples, 12 from Messack and two each from Restronguet Creek and Ruan Creek. Eleven samples from Messack were processed with a 1.0 mm mesh for a fixed period of 15 minutes (and also 30 minutes in three cases). Two of these were also analysed using a 0.5 mm screen (ME6 and ME8). The remaining Messack sample and the Restronguet and Ruan Creek samples were processed on a 0.5 mm sieve only for both 15 and 30 minutes. The 1.0 mm samples are summarised in Table 3.1.1 and the 0.5 mm samples in Table 3.1.2. These five samples were also used in RLA.

Limiting the sorting time resulted in less information being extracted from the sample. On average, for the 1.0 mm sieve fractions, a fifteen minute sorting period constituted 3.4% of total sorting time (n = 11) i.e. about 97% reduction in sorting effort. During this time it was possible to extract a mean of 8.8% of the fauna (n = 11) and 38.2% of sample species richness (n = 11).

Where two sorting periods had been applied to the same sample sieved on a 1.0 mm mesh (samples 2b, 2c & 2d), a doubling of sorting time did not result in a commensurate increase in either total fauna extracted or sample species richness. The proportion of fauna extracted was increased from a mean of 5.0% to one of 7.3% (n = 3) and the mean proportion of species per sample (species richness) increased from 24.5% to 30.1% (n = 3).

Sieving with a 0.5 mm mesh (samples ME6 and ME8) more than doubled the total sorting time (see Table 3.1.1) and reduced the mean proportion of fauna extracted in timed sorting periods from 7.1% to 2.5% (n = 2) and the mean proportion of species from 46.1% to 35.4% (n = 2). However, it should be noted that the two size fractions were supplied (and therefore sorted) separately and the 0.5 mm fraction was not

examined in the TSA. These data were subsequently added to the 1.0 mm fraction to give the 0.5 mm sample statistics as shown in Table 3.1.1. Usual practice would have been to sort the whole sample as one unit as was carried out with the 0.5 mm samples shown in Table 3.1.2. In this case a doubling of the sorting time from 15 to 30 minutes increased the mean proportion of fauna extracted (N) from 29.2% to 54.2% (n = 5) and the mean proportion of species extracted (S) from 54.8% to 74.7% (n = 5) (Table 3.1.3).

Table 3.1.2 summarises the TSA analyses using 0.5 mm mesh size. Two samples were of much higher abundance (ME7 and RU10c) resulting in a lower proportion of species and abundance being extracted during the fixed sorting times.

Figure 3.1.1 graphically displays some of the data from TSA. The generally higher percentages shown in the lower graph are a consequence of the reduced abundance in these samples.

Table 3.1.1 Extraction efficiency for 1.0 mm sieve fraction of TSA samples achieved in predetermined sorting periods (15 mins unless otherwise indicated). Samples ME6 and ME8 also show data for 0.5 mm sieve fraction.

Sample	Total N	Total S	Total sorting time (hrs)	TSA as proportion of total sorting time (%)	Fauna extracted N (%)	Species extracted S (%)
ME2b	1140	69	9.5	2.6	61 (5.4)	14 (20.3)
30 mins				5.2	93 (8.2)	16 (23.2)
ME2c	1184	66	14.7	1.7	78 (6.6)	18 (27.3)
30 mins				3.4	107 (9.0)	19 (28.8)
ME2d	1576	81	6.6	3.8	48 (3.0)	21 (25.9)
30 mins				7.6	72 (4.6)	31 (38.3)
ME5	1668	80	10.4	2.4	130 (7.8)	32 (40.0)
ME6	1653	73	11.2	2.2	101 (6.1)	32 (43.8)
0.5 mm			22.1	1.1	101 (2.5)	32 (34.0)
ME8)	1295	66	8.2	3.0	106 (8.2)	32 (48.5)
0.5 mm			18.5	1.3	106 (2.5)	32 (36.8)
ME9	1775	74	12.5	2.0	106 (6.0)	28 (37.8)
ME10	438	39	6.3	5.2	58 (13.2)	17 (43.6)
ME11	425	24	4.8	5.2	58 (13.6)	9 (37.5)
ME12	320	39	10.4	2.4	57 (17.8)	21 (53.8)
ME13	810	51	3.4	7.0	77 (9.5)	21 (41.2)

Table 3.1.2. Extraction efficiency for 0.5 mm sieve fraction achieved in TSA samples (15 mins unless otherwise indicated).

Sample	Total N	Total S	Total sorting time (hrs)	TSA as proportion of total sorting time (%)	Fauna extracted N (%)	Species extracted S (%)
ME7	1355	60	18.4	1.4	94 (6.9)	30 (50.0)
30 mins				1.6	179 (13.2)	37 (61.7)
RE8	14	6	0.7	37.5	5 (35.7)	3 (50.0)
30 mins				75.0	10 (71.4)	5 (83.3)
RE3a	17	9	1.0	25.0	10 (58.8)	6 (66.7)
30 mins				50.0	16 (94.1)	9 (100.0)
RU7a	65	14	1.0	25.0	18 (27.7)	9 (64.3)
30 mins				50.0	40 (61.5)	10 (71.4)
RU10c	443	14	13.8	1.8	75 (16.9)	6 (42.9)
30 mins				3.6	136 (30.7)	8 (57.1)

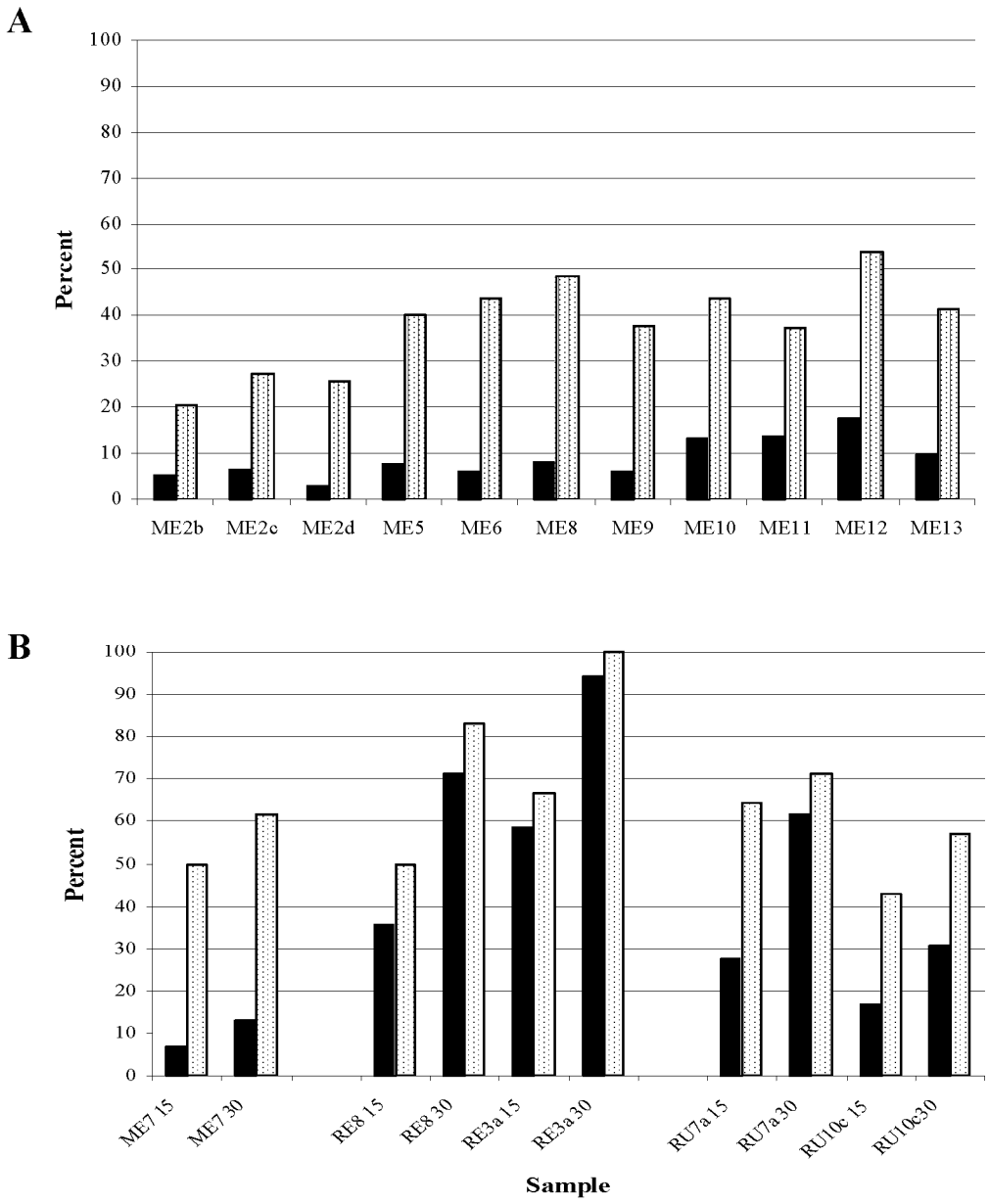

Figure 3.1.1 A Proportion of total individuals (N = solid bars) and total sample species richness (S = stippled bars) extracted from 1.0 mm mesh samples in 15 minute sorting period (see Table 3.1.1 for data). B Proportion of total individuals (N = solid bars) and total sample species richness (S = stippled bars) extracted for 0.5 mm mesh samples 15 and 30 minute sorting times (see Table 3.1.2). Sorting times indicated after sample number.

Table 3.1.3 Summary of mean percent fauna (N) and mean percent species (S) extracted by Timed Sorting Analysis.

	N		S	
	15 min. sort	**30 min. sort**	**15 min. sort**	**30 min. sort**
1.0 mm mesh	8.8 (n = 11)	7.3 (n = 3)	38.2 (n = 11)	30.1 (n = 3)
0.5 mm mesh	29.2 (n = 5)	54.2 (n = 5)	54.8 (n = 5)	74.7 (n = 5)

The species extracted from each sample during the restricted sorting period were ranked in order of abundance (Table 3.1.4). This revealed that three taxa were consistently picked more frequently: cirratulid polychaetes, the polychaete genus *Nephtys* and the bivalve *Abra alba*. These rankings did not coincide with the most abundant taxa found after full sample analysis, indicating that animals were picked for their conspicuousness and not on account of their actual abundance. However, within this subset of conspicuous fauna an attempt was made while sorting to achieve a representative collection of fauna.

Table 3.1.4. Top ten ranked species for each 1.0 mm sieve fraction (whole sample) and respective TSA data (singletons omitted). TS15 prefix = 15 minute sorting time; TS30 prefix = 30 minute sorting time; WS = whole sample.

Sample ME 2b

TS15		TS30		WS	
Phoronis sp. Indet	18	*Phoronis sp. Indet*	25	*Mediomastus fragilis*	361
Abra alba	12	*Abra alba*	17	*Phoronis sp. Indet*	233
Chaetozone gibber	12	*Chaetozone gibber*	14	*Abra alba*	107
Melinna palmata	4	*Melinna palmata*	9	*Melinna palmata*	48
Mediomastus fragilis	3	*Mediomastus fragilis*	7	*Chaetozone gibber*	43
Sthenelais boa	3	*Nephtys kersivalensis*	5	*Protocirrineris sp.*	35
Nephtys kersivalensis	2	*Sthenelais boa*	3	*Caulleriella alata*	35
		Aphelochaeta marioni	3	*Aphelochaeta marioni*	31
		Ampelisca tenuicornis	2	*Praxillella (affinis)*	25
		Praxillella (affinis)	2	*Mysella bidentata*	20

Sample ME 2c

TS15		TS30		WS	
Abra alba	26	*Abra alba*	32	*Mediomastus fragilis*	320
Chaetozone gibber	10	*Phoronis sp. Indet*	14	*Phoronis sp. Indet*	203
Nephtys kersivalensis	7	*Chaetozone gibber*	12	*Abra alba*	145
Phoronis sp. Indet	6	*Mediomastus fragilis*	9	*Chaetozone gibber*	68
Aphelochaeta marioni	5	*Nephtys kersivalensis*	7	*Aphelochaeta sp. A (unico-key)*	48
Praxillella (affinis)	4	*Praxillella (affinis)*	7	*Melinna palmata*	46
Ampelisca tenuicornis	4	*Aphelochaeta marioni*	6	*Caulleriella alata*	34
Mediomastus fragilis	3	*Ampelisca tenuicornis*	4	*Praxillella (affinis)*	33
Cirriformia tentaculata	3	*Cirriformia tentaculata*	3	*Aphelochaeta marioni*	27
Scoloplos armiger	2	*Scoloplos armiger*	2	*Monticellina dorsobranchialis*	26

Sample ME 2d

TS15		TS30		WS	
Abra alba	7	*Abra alba*	8	*Mediomastus fragilis*	617
Nephtys kersivalensis	6	*Nephtys kersivalensis*	6	*Aphelochaeta sp. A (unico-key)*	124
Chaetozone gibber	5	*Mediomastus fragilis*	6	*Protocirrineris sp.*	114
Mediomastus fragilis	4	*Melinna palmata*	6	*Chaetozone gibber*	96
Aphelochaeta marioni	3	*Chaetozone gibber*	5	*Phoronis sp. Indet*	54
Cirriformia tentaculata	3	*Aphelochaeta marioni*	3	*Abra alba*	45
Liocarcinus arcuatus	3	*Cirriformia tentaculata*	3	*Melinna palmata*	45
Praxillella (affinis)	2	*Liocarcinus arcuatus*	3	*Monticellina dorsobranchialis*	38
Phtisica marina	2	*Crepidula fornicata*	3	*Caulleriella alata*	34
Notomastus sp. (latericeus)	2	*Syllidia armata*	3	*Praxillella (affinis)*	27

Sample ME 5

TS15		WS	
Abra alba	36	*Mediomastus fragilis*	697
Nephtys kersivalensis	14	*Phoronis sp. Indet*	208
Cirriformia tentaculata	13	*Cirriformia tentaculata*	110
Phoronis sp. Indet	12	*Aphelochaeta marioni*	67
Mediomastus fragilis	12	*Tubificoides ?galiciensis?*	62
Melinna palmata	6	*Abra alba*	54
Liocarcinus arcuatus	3	*Chaetozone gibber*	53
Nematonereis unicornis	3	*Phtisica marina*	30
Sthenelais boa	2	*Microdeutopus anomalus*	29
Tapes (Tapes) decussatus	2	*Tanaopsis graciloides*	26

Table 3.1.4 (continued)

Sample ME6			
TS15		WS	
Melinna palmata	13	*Mediomastus fragilis*	565
Abra alba	10	*Melinna palmata*	168
Cirriformia tentaculata	9	*Chaetozone gibber*	164
Nephtys kersivalensis	8	*Cirriformia tentaculata*	93
Ampithoe ramondi	6	*Aphelochaeta marioni*	73
Scoloplos armiger	5	*Tubificoides benedii*	57
Mediomastus fragilis	5	*Abra alba*	49
Tanaopsis graciloides	4	*Tubificoides ?galiciensis?*	49
Euclymene (oerstedii)	4	*Galathowenia/Myriochele*	38
Aphelochaeta marioni	4	*Galathowenia/Myriochele*	38

Sample ME8			
TS15		WS	
Cirriformia tentaculata	28	*Mediomastus fragilis*	537
Nephtys kersivalensis	13	*Cirriformia tentaculata*	110
Abra alba	10	*Tubificoides benedii*	109
Tubificoides benedii	7	*Phoronis sp. Indet*	109
Cheirocratus sundevallii	5	*Chaetozone gibber*	43
Heteromastus filiformis	4	*Tubificoides ?galiciensis?*	39
Caulleriella bioculata	4	*Aphelochaeta marioni*	37
Mediomastus fragilis	4	*Abra alba*	30
Aphelochaeta marioni	3	*Microdeutopus anomalus*	30
Chaetozone gibber	3	*Monticellina dorsobranchialis*	29

Sample ME9			
TS15		WS	
Abra alba	22	*Mediomastus fragilis*	596
Nephtys kersivalensis	14	*Tubificoides benedii*	239
Cirriformia tentaculata	9	*Melinna palmata*	207
Aora gracilis	6	*Cirriformia tentaculata*	111
Mediomastus fragilis	6	*Aphelochaeta marioni*	107
Melinna palmata	5	*Chaetozone gibber*	65
Megalomma vesiculosum	4	*Aora gracilis*	57
Platynereis dumerilii	4	*Abra alba*	41
Euclymene (oerstedii)	4	*Phoronis sp. Indet*	34
Ampithoe ramondi	4	*Tubificoides ?galiciensis?*	32

Sample ME10			
TS15		WS	
Abra alba	16	*Melinna palmata*	176
Melinna palmata	12	*Mediomastus fragilis*	57
Nephtys kersivalensis	6	*Chaetozone gibber*	55
Chaetozone gibber	6	*Abra alba*	39
Nephtys hombergii	5	*Tubifex tubifex*	22
Microdeutopus anomalus	2	*Microdeutopus anomalus*	14
		Nephtys kersivalensis	6
		Nephtys hombergii	6
		Tubificoides benedii	6
		Ampelisca tenuicornis	5

Sample ME11			
TS15		WS	
Melinna palmata	21	*Melinna palmata*	197
Abra alba	13	*Chaetozone gibber*	81
Chaetozone gibber	7	*Galathowenia/Myriochele*	35
Abra nitida	6	*Abra alba*	29
Nephtys kersivalensis	5	*Mediomastus fragilis*	26
Nephtys hombergii	3	*Euclymene (oerstedii)*	10
		Abra nitida	6
		Nephtys kersivalensis	5
		Aphelochaeta marioni	4
		Tubificoides ?galiciensis?	4

Sample ME12			
TS15		WS	
Aora gracilis	12	*Melinna palmata*	104
Nephtys hombergii	11	*Mediomastus fragilis*	43
Melinna palmata	9	*Aora gracilis*	32
Abra alba	5	*Chaetozone gibber*	31
Nephtys kersivalensis	3	*Aphelochaeta marioni*	23
Chaetozone gibber	2	*Nephtys hombergii*	11
		Nephtys kersivalensis	7
		Protocirrineris sp.	7
		Abra alba	6
		Microdeutopus anomalus	6

Table 3.1.4 (contnued)

Sample ME13			
TS15		WS	
Melinna palmata	14	*Chaetozone gibber*	233
Chaetozone gibber	11	*Melinna palmata*	192
Euclymene (oerstedii)	9	*Mediomastus fragilis*	108
Abra alba	8	*Galathowenia/Myriochele*	67
Cirriformia tentaculata	5	*Protocirrineris sp.*	22
Nephtys hombergii	4	*Aphelochaeta marioni*	18
Ampelisca tenuicornis	4	*Euclymene (oerstedii)*	15
Aphelochaeta marioni	4	*Aora gracilis*	13
Galathowenia/Myriochele	4	*Tubificoides ?galiciensis?*	13
Ampelisca brevicornis	2	*Cirriformia tentaculata*	12

The results of multivariate analyses for the 1.0 mm sieve fraction from Messack are shown in Figure 3.1.2. Two main groups of data (with subgroups designated by capital letters) can be seen in the dendrogram (Figure 3.1.2), one with all the TSA data (A+B+C) and the other containing the whole sample analyses (D+E). These two "treatments" (partial analysis by restricting sorting time and full analysis) produced sufficiently dissimilar sample data for them to be separated unambiguously. Analysis of whole samples creates two clear groups (D and E) whereas TSA of the same samples identifies three less defined groups (A, B and C). TSA in effect removed samples ME2b, ME2c and ME2d from their association with ME5, ME6 ME8 and ME9 to form their own cluster (C). Sample ME12 did not appear in any cluster under TSA analysis but was grouped with samples ME10, ME11 and ME13 when the whole dataset was analysed (Cluster E).

The 15 minute and 30 minute data in samples ME2b, ME2c and ME2d are sufficiently similar to group each sample together within cluster C indicating that there was no qualitative difference between the 30 minute and 15 minute sort results.

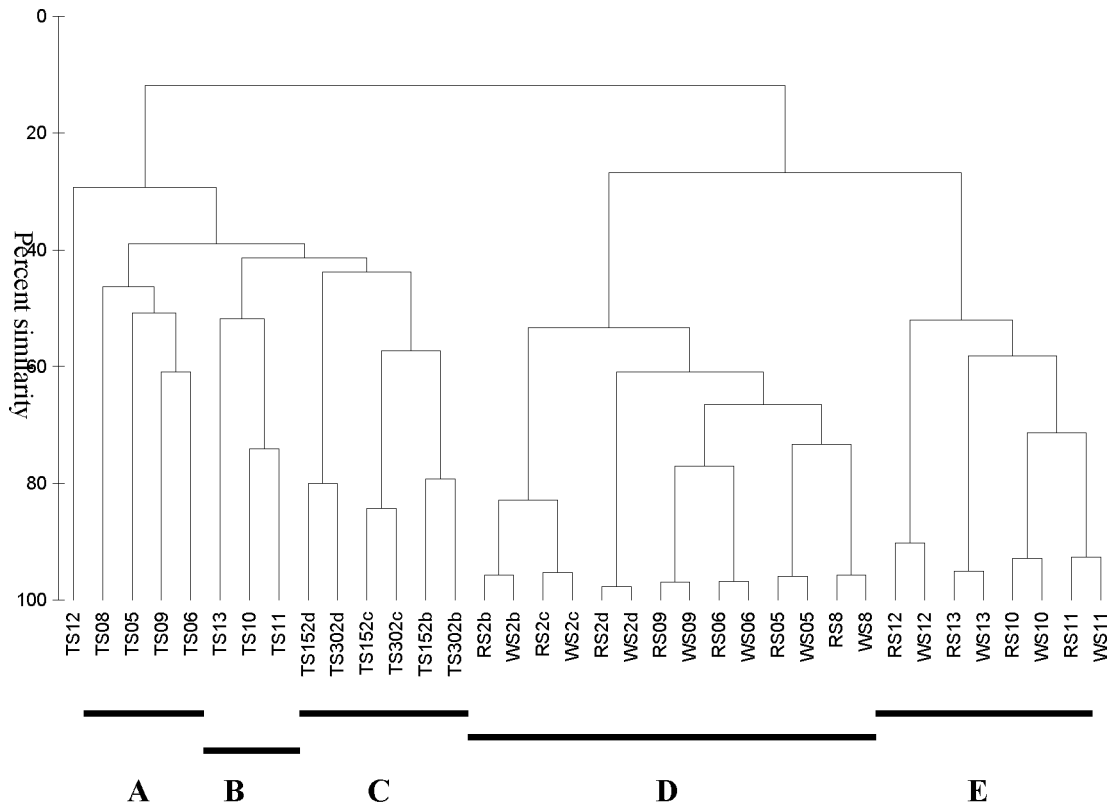

Figure 3.1.2 Cluster analysis using untransformed 1.0 mm mesh sample data. Each sample (Messack) number is prefixed as follows: TS = Timed sample (15 mins); TS15 = Timed sample 15 mins; TS30 = Timed Sample 30 mins; RS = Sample Residue; WS = Whole sample (TS + RS).

Samples processed through a 0.5 mm screen were also subjected to cluster analysis and the resulting dendrogram can be seen in Figure 3.1.3. In each case the whole sample data were closely associated with their respective TSA data. The high abundance samples (RU10c and ME7) clustered together and in each one the 15

and 30 minute sorting times were more similar to each other than to the full data. In contrast, although the low abundance samples again formed discrete clusters, in this instance the 30 minute data were closer to the full data than to the 15 minute subset.

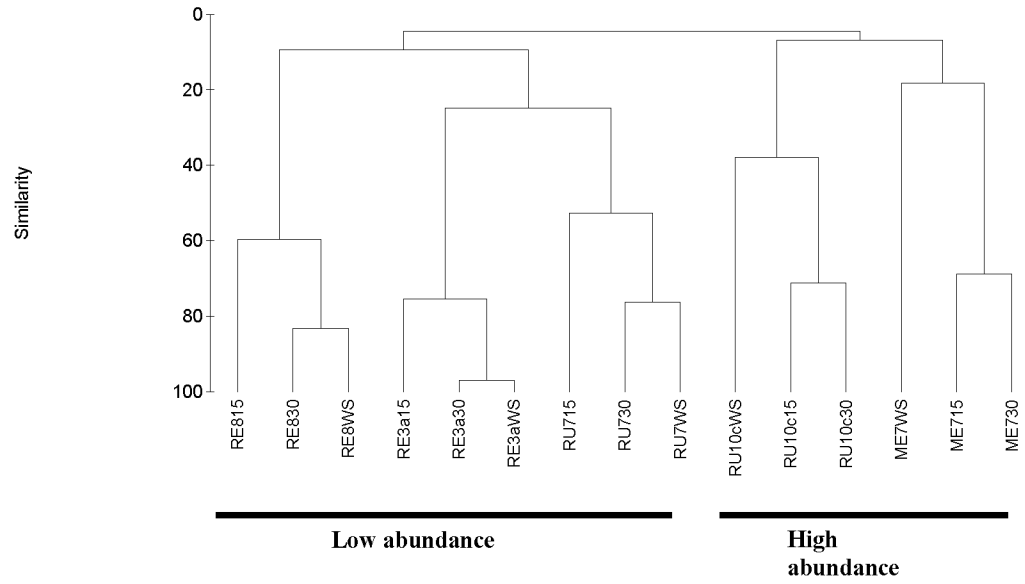

Figure 3.1.3. Dendrogram showing results of cluster analysis of samples analysed for TSA using a 0.5 mm mesh. Sorting times of 15 and 30 minutes. The -15, -30 and -WS suffixes denote 15 minute sort, 30 minute sort and Whole Sample data respectively.

Borja's Biotic Coefficient (Borja *et al.* 2000) was calculated for 1.0 mm sieved samples. This revealed very little difference between pollution classifications for sub-samples identified after time-restricted sorting and for those analysed in full. Only ME9 was re-classified following full analysis. In this case the data from the timed sorting indicated a slightly polluted environment whereas the full dataset indicated further degredation and a classification of moderately ("meanly") polluted (see Table 3.1.5). In 9 of the 11 samples compared, analysing the full sample resulted in a higher Biotic Coefficient.

Table 3.1.5 Results from Borja's Biotic Index analysis for Messack samples processed on 1.0 mm sieve. BC = Biotic coefficient.

	Timed sorting (15 mins)		Whole sample analysis	
	BC	Pollution classification	BC	Pollution classification
ME2b	2.02	Slightly polluted	2.22	Slightly polluted
ME2c	2.46	Slightly polluted	2.32	Slightly polluted
ME2d	2.41	Slightly polluted	2.96	Slightly polluted
ME5	2.38	Slightly polluted	2.74	Slightly polluted
ME6	2.33	Slightly polluted	3.21	Slightly polluted
ME8	3.08	Slightly polluted	3.26	Slightly polluted
ME9	2.28	Slightly polluted	3.31	Moderately polluted
ME10	2.56	Slightly polluted	3.09	Slightly polluted
ME11	2.87	Slightly polluted	2.91	Slightly polluted
ME12	1.55	Slightly polluted	2.61	Slightly polluted
ME13	3.14	Slightly polluted	3.05	Slightly polluted

The TSA samples processed on a 0.5 mm screen produced a more variable situation (Table 3.1.6). RE8 and RU10c gave the same results (slightly polluted and heavily polluted respectively) regardless of time spent extracting the fauna. The other samples were designated as slightly polluted on the basis of a 15 minute timed subsample, but would be classed as moderately polluted following full analysis.

Table 3.1.6 Results from Borja's Biotic Index analysis for samples processed on 0.5 mm sieve. BC = Biotic coefficient.

	Timed sorting (15 mins)		Timed sorting (30 mins)		Whole sample analysis	
	BC	Pollution classification	BC	Pollution classification	BC	Pollution classification
RE3a	3.30	slight	3.94	moderate	4.06	moderate
RE8	1.87	slight	1.50	slight	1.85	slight
RU7	3.25	slight	3.35	moderate	3.75	moderate
RU10c	5.42	heavy	5.44	heavy	5.37	heavy
ME7	3.18	slight	3.14	slight	3.50	moderate

3.2 Expert view analysis (EVA)

3.2.1 Laboratory check of field - derived data

This section effectively constitutes a quality control check of the expert field analyses. Comparison with the whole sample as subsequently determined in the laboratory is reported in Section 3.2.2.

Various techniques were used in the field to identify and enumerate part of or the complete sample. These included a restriction on the time for analysis (15, 20 or 30 minutes), splitting into different size fractions (1.0 mm, 0.5 mm) and the use of staff with varying degrees of experience (beginner, intermediate or expert). The results are summarised in the following sections according to the sampling location. This is followed by a general overview of these results.

3.2.1.1 Messack

Identification of the fauna in samples ME8, ME9, ME10, ME11 and ME13 was not attempted in the field. In these cases only one taxon was identified and this, together with animals removed during a 15 or 20 minute in-field "picking" session, was analysed by staff at IECS. Primary community variables are shown in Table 3.2.1. Field data for samples ME2b-15 min, ME2d and ME6 are presence/absence so comparisons of abundance are not possible. Sample 2a was split into two fractions; data from field identification are (mostly) numeric and a comparison of these is shown in Table 3.2.1 and included in the general overview. In this sample additional animals were extracted in the field but not identified and these are shown in Table 3.2.1 on a separate line.

Table 3.2.1. Summary statistics for Messack field identification. Proportion of total species richness (S) and Abundance (A) are calculated wherever possible.

			No. of taxa (S)			Abundance (A)		
Lab ref	**Level of experience**	**picking time (mins)**	**Field**	**Lab**	**%**	**Field**	**Lab**	**%**
ME2a	Expert	?	14	18	77.8	21	28	75.0
ME2a	n/a	n/a		33			93	
ME2b	Intermediate	15	4	14	28.6	n/a	61	
ME2b	Intermediate	30	1	10	10.0	n/a	32	
ME2d	Intermediate	15	5	21	23.8	n/a	48	14.6
ME2d	?	30	?	15		n/a	24	
ME6	Expert	?	12	32	37.5	n/a	101	9.9
ME8	Beginner	15	1	32	3.1	7	106	6.6
ME9	Expert	15	1	29	3.4	2	106	1.9
ME10	Expert	20	1	17	5.9	~100	58	
ME11	Expert	15	1	9	11.1	~100	58	
ME13	Expert	15	1	21	4.8	~100	77	

Note - IECS was supplied with sample for 2d (30 mins) but field data are not available

3.2.1.2 Malpas

The Malpas samples were analysed using a 0.5 mm screen. The 0.5 mm fraction was saved in the field and combined with the 1.00 mm fraction before laboratory analysis. In both samples the smaller fraction was noted as containing *Corophium*? and oligochaetes. Gross comparative statistics using combined size fractions are shown in Table 3.2.2. As with most Messack samples the field data were predominantly presence/absence and therefore not amenable to comparisons of abundance. The sample picking was not timed, the whole sample being worked up in the field.

Table 3.2.2. Summary statistics for Malpas field identification.

			No. of taxa		Abundance	
Lab ref	Level of experience	picking time (mins)	Field	Lab	Field	Lab
M2	Intermediate	n/a	5	3	1	36
M5	Intermediate	n/a	5	5	<100	18

3.2.1.3 Restronguet Creek

Two samples were processed from this site (Table 3.2.3). One specimen of *Abra* sp. was found in field analysis of RE10 and three taxa were recorded as present in RE12. Picking time is not known for these sites.

Table 3.2.3. Summary statistics for Restronguet Creek field identification.

			No. of taxa		Abundance	
Lab ref	Level of experience	picking time (mins)	Field	Lab	Field	Lab
RE10	Intermediate	unknown	1	3	1	18
RE12	Intermediate	unknown	3	4	0	13

3.2.1.4 Ruan Creek

Field data for three samples are available for comparison (Table 3.2.4). Most of the fauna from sample RU2 appears to be missing as only 1 bivalve was found in the laboratory analysis. Sample RU4 was picked initially by an intermediate level taxonomist, re-combined and then picked again by an expert. Large numbers of mysids were found in sample RU8 but not included in the container for analysis. IECS has analysed sample RU11 but no field data are available.

Table 3.2.4. Summary statistics for Ruan Creek field identification.

			No. of taxa		Abundance	
Lab ref	Level of experience	picking time (mins)	Field	Lab	Field	Lab
RU2	Expert	?	4	1	25	1
RU4	Expert	15	8	8	123	106
RU4	Intermediate	15	6	n/a	14	n/a
RU8	Expert	?	4	3	17	15
RU11	?	?	?	1	?	9

3.2.1.5 General overview

The foregoing sections briefly compare samples worked up in the field with the same material analysed in the laboratory. The majority of samples produced non-count data thus preventing comparisons of numerical abundance. Those samples with numerical data are compared at the end of this section.

There were 11 samples in which field identified species richness (i. e. number of taxa) can be compared with laboratory data. Five of these were identified at expert level (ME2a, ME6, RU2, RU4 and RU8) and six at intermediate level (ME2b-15 min, ME2d, M2, M5, RE10, and RE12).

Expert level identification achieved a mean of 83% (n = 5) of species richness against that determined in the laboratory. Two expert level samples found fewer species than revealed by later laboratory analysis (see Table 3.2.1). These were Messack 2a and Messack 6 which found 78% and 37% of laboratory determined taxa respectively. Missed taxa were as follows:

- Polychaeta: *Praxilella* sp. (from sample 2a), *Sthenelais boa, Hypereteone foliosa, Aphelochaeta marioni, Chaetozone gibber, Mediomastus fragilis* and *Euclymene* sp (all from sample 6)

- Oligochaeta: *Tubificoides benedii* and *T. cf. galiciencis* (both sample 6)

- Crustacea: *Cheirocratus sundevallii* (sample 2a), *Apherusa ovalipes, Ampelisca sp. Ampithoe ramondi, Corophium sextonae, Astacilla longicornis, Leptochelia dubia, Tanaopsis gracilioides* (sample 6)

- Mollusca: *Moerella pygmaea, Venerupis senegalensis* (sample 2a)

- Phoronida: *Phoronis* sp. (sample 6)

The converse situation applies to the other expert level samples taken in Ruan Creek where more (or the same number of) species were identified in the field than in laboratory analysis (see Table 3.2.4). However, most material from RU2 (*Nereis* sp., *Polydora* sp. and *Hydrobia ulvae*) was missing from the container (as can be seen by the lower abundance detected in the laboratory), thus indicating a problem created by sample handling in the field. In RU8 the presence of *Neomysis integer* was noted during fieldwork but none was included in the sample.

Expert workers were successful in identifying annelids such as *Nematonereis unicornis, Scoloplos armiger, Scalibregma* spp. *Melinna palmata, Cirriformia tentaculata* and *Polydora* spp. but, understandably, other taxa proved more difficult. There were four species of nereid polychaetes in the samples (*Neanthes irrorata, Perinereis cultrifera, Platynereis dumerilii* and *Hediste diversicolor*) but these were recorded as *Nereis* sp. Nephtyidae is a closely related family which is also difficult to identify to species level in the field. Two species were present in expert level samples (*N. hombergii* and *N. kersivalensis*). In one instance (RU4) a *Nephtys* sp. appears to have been mistaken for *Nereis* sp. Two species of sabellid polychaete

(*Megalomma vesiculosum* and *Sabella pavonina*) were identified at family level only, reflecting the difficulty of identifying these in the field.

Small crustaceans (amphipods, isopods and tanaids) were problematical and this is reflected in the list of missing taxa shown above. In sample ME2a an amphipod was recorded as *Ampelisca* sp. but only *Cheirocratus sundevallii* was found during laboratory inspection. *Ericthonius* spp. were successfully identified in sample ME6 but these may have included two species - *Ericthonius punctatus* and *Aora gracilis*. *Melita palmata* was correctly assigned in sample RU4. The larger crustaceans should be easier to identify and in these samples the shrimp *Crangon* sp. was identified correctly in the field.

Molluscs (*Hinia reticulata, Littorina littorea, Hydrobia ulvae, Cerastoderma edule, Parvicardium exiguum, Abra alba, Scrobicularia plana* and *Tapes decussatus*) were correctly identified. In one Messack sample (sample ME6) *Mysia undata* had been misidentified as *Chamelea gallina.*

Intermediate level identification achieved a mean of 60% (n = 6) species richness when compared to laboratory analysis. One sample from Malpas (M2) had more field identified taxa than found in the laboratory with *Crangon*, *Cerastoderma* and a Sand Goby being absent from the sample container.

The taxa missed in the field were as follows:

- Polychaeta: *Pholoe balthica* (2b-15 min), *Pholoe inornata* (2d), *Sthenelais boa* (2b-15 min, 2d), *Eteone longa/flava* (2d), *Nephtys hombergii* (RE10), *Nephtys kersivalensis* (2b-15 min, 2d), *Nematonereis unicornis* (2b-15 min, 2d), *Aphelochaeta* spp. (2b-15 min, 2d), *Cossura longocirrata* (M5), *Caulleriella alata* (2d), *Cirriformia tentaculata* (2d), *Chaetozone gibber* (2b-15 min, 2d), *Monticellina* sp. (2d), *Mediomastus fragilis* (2b-15 min, 2d), *Notomastus latericeus* (2d), *Praxilella* sp. (2b-15 min, 2d), *Melinna palmata* (2b-15 min, 2d)

- Crustacea: *Ampelsica* spp.(2b-15 min, RE10), *Maera grossimana* (2b-15 min), *Cheirocratus* spp.(2d), *Phtisica marina* (2d), *Leptochelia dubia* (M2)

- Mollusca: *Abra alba* (2d)

- Phoronida: *Phoronis* spp. (2b-15 min)

No annelids were successfully identified to genus or species by intermediate level workers. *Perinereis cultrifera* was identified as *Nereis* spp. (sample 2d) and worms identified as juvenile *Nereis* spp. in the field were in fact juvenile *Nephtys* spp. (M5 and RE12). In the Malpas samples oligochaetes were recorded in both size fractions but were not found to be present under microscope examination. These were mostly *Tharyx* spp.

The few small crustaceans in the intermediate level samples were mostly missed. *Corophium* was recorded (sample M2) but cannot be safely assigned to species in the field. Three crabs from Messack sample 2d were thought to be *Carcinus*

maenas but were found to belong to the closely related (and, as juveniles, morphologically similar) *Liocarcinus arcuatus*.

The molluscs *Crepidula fornicata* and *Parvicardium exiguum* were accurately identified in the field (Messack 2d). The bivalve genus *Abra* was correctly identified in Messack 2b-15 min and RE10. In the former sample this was subsequently identified as *A. alba* and in the latter as *A. nitida*. *Cerastoderma* sp. in sample RE12 was later assigned to *C. edule* and a juvenile *Cerastoderma* sp. in sample M5 was later ascribed to *Parvicardium ovale* in the laboratory. Phoronids were missed in Messack 2b-15 min.

Three expert level samples were amenable to basic numerical analysis but there was insufficient replication for significance testing between field-derived data and laboratory checks. Results can be seen in table 3.2.5.

Table 3.2.5 Comparison of field-derived data with subsequent laboratory analysis

	S	N	Shannon (log_2) (H′)	Bray-Curtis similarity
Messack 2a (Field)	14	25	3.67	41.5
Messack 2a (Lab)	18	28	4.01	
Ruan RU4 (Field)	8	123	1.51	95.0
Ruan RU4 (Lab)	8	115	1.42	
Ruan RU8 (Field)	3	17	1.25	0.0
Ruan RU8 (Lab)	3	15	1.29	

Species richness (S), and the diversity statistics H′ were similar for expert level field identification and subsequent laboratory analysis. These univariate measures do not retain the identity of the species involved and so qualitatively different samples may produce similar results. As an indication of how similar the analyses were in terms of species identified the Bray-Curtis similarity index was calculated (using untransformed data). Results were variable, RU4 (8 taxa) showing a high degree of concurrence but RU8 (only 3 taxa) showing no similarity.

3.2.2 Comparison of field - derived data with whole sample laboratory data

Field-derived data can be compared with whole sample data in six instances, two of which (Messack 2a and Ruan Creek RU4) have numerical data from field analysis. Data from all these samples are summarised in Table 3.2.6.

Table 3.2.6. Comparison of field data with whole sample analysis

Sample	H′	No. of taxa (S)	Abundance (N)
Messack 2a whole sample	3.84	72	1119
Messack 2a field data	3.67	14	21
Messack 2b whole sample	n/a	70	1140
Messack 2b field data	n/a	4	n/a
Messack 2d whole sample	n/a	84	1577
Messack 2d field data	n/a	5	7
Messack 6 whole sample	n/a	73	1653
Messack 6 field data	n/a	12	n/a
Malpas M2 whole sample	n/a	18	1599
Malpas M2 field data	n/a	5	n/a
Ruan RU4 whole sample	2.74	23	566
Ruan RU4 field data	1.51	8	123

Field data consistently underestimated sample species richness, on average finding 18.3% (n = 6) of whole sample species richness. Comparison of abundance data does not apply as there was no attempt to quantify abundance in the field.

Output from cluster analysis can be seen in Figure 3.2.1. Three main clusters were distinguished on the dendrogram. Whole sample data from Messack sites ME6, ME2b, ME2d and ME2a clustered at a similarity of 59.6 (group B). The remaining whole sample analyses paired at a similarity of 48.8 despite being from different locations (Cluster A). Two field samples from Messack clustered at similarity 53.8.

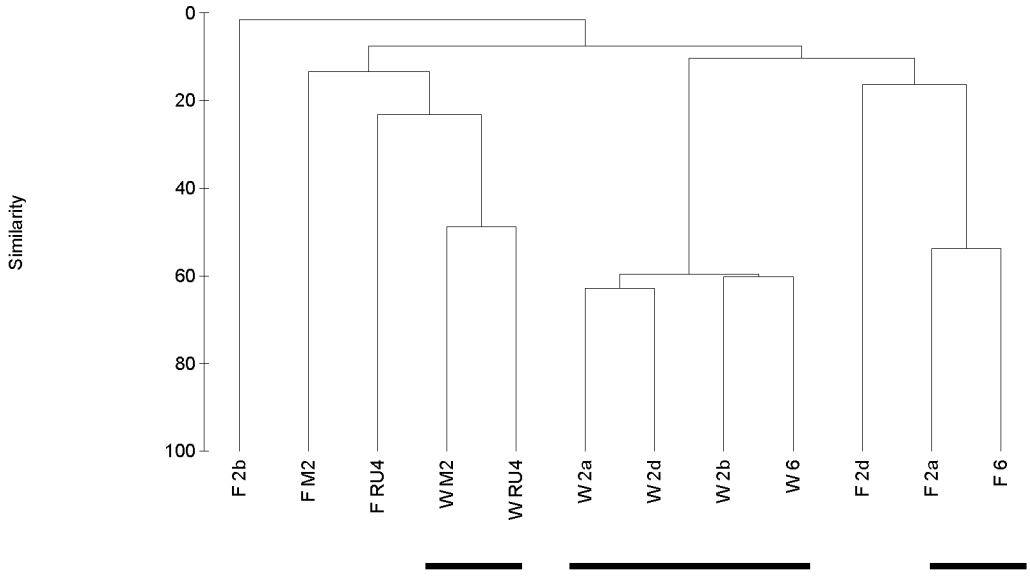

Figure 3.2.1 Dendrogram comparing field- derived data (F prefix) with laboratory analysis of the whole sample (W prefix). Bray-Curtis similarity on presence-absence data, group average clustering

Where numerical data were available for field identifications the Biotic Coefficient (Borja *et al.* 2000) was calculated and compared with the data from the full sample analysis. In each case the coefficient was higher when the full sample was analysed but in no case was the pollution classification altered (see Table 3.2.7).

Table 3.2.7. Results from Borja's Biotic Index analysis for data produced in the field and subsequently by laboratory analysis. BC = Biotic coefficient.

	Identification in field		Laboratory analysis	
	BC	Pollution classification	BC	Pollution classification
ME2a	2.05	Slightly polluted	2.61	Slightly polluted
ME2d	2.78	Slightly polluted	2.96	Slightly polluted
RU4	2.96	Slightly polluted	3.18	Slightly polluted

3.3 Restricted laboratory analysis (RLA)

Results for the five RLA samples are given in Table 3.3.1. There was a large range in abundance and species richness, two samples (ME7 and RU10c) having at least 7x greater abundance than the rest. The extraction efficiency of the two sorting periods has been dealt with in section 3.1.

Table 3.3.1 Summary sample statistics for Restricted Laboratory Analysis showing results from analysis with 1.5x illuminator and subsequent microscopic analysis.

		Microscope		Illuminator			
Sample	Sorting time	N	S	N	%	S	%
ME7	15 mins	94	30	95	> 100	21	70.0
	30 mins	179	37	180	> 100	31	83.8
	Whole sample	1355	60	587	43.3	45	75.0
RE3a	15 minutes	10	6	11	> 100	5	83.3
	30 minutes	16	9	17	> 100	6	66.7
	Whole sample	17	9	16	94.1	6	66.7
RE8	15 minutes	5	3	4	80.0	2	66.7
	30 minutes	10	5	9	90.0	4	80.0
	Whole sample	14	6	13	92.9	5	83.3
RU10c	15 minutes	75	6	75	100.0	5	83.3
	30 minutes	136	8	142	> 100	5	62.5
	Whole sample	443	14	593	> 100	10	71.4
RU7	15 minutes	18	9	15	83.3	7	77.8
	30 minutes	40	10	29	72.5	8	80.0
	Whole sample	65	14	49	75.4	10	71.4

In classification analysis the high abundance samples differed from the low abundance ones in the pattern of relationships between their various “treatments” or sub-components. The basic pattern was imposed by the initial sorting period. This can be seen in Figure 3.3.1 where high and low abundance samples resolved into separate groups (i.e. the high abundance cluster A + B and the low abundance cluster C + D). In both cases these clusters were defined with very low similarity. However, within the low abundance samples the longer sorting period converged towards the whole sample data (i.e. 30 minute data paired with whole sample data in the dendrograms) whereas in the high abundance samples the two restricted sorting periods were more similar to each other than the whole sample data. With high abundance samples the results of both sorting periods were sufficiently similar to cluster each sample as a single entity, i.e. as cluster A (ME7) and cluster B (RU10c) (see figure 3.3.1). However, in the case of samples with low species richness and abundance all illuminator analyses were combined as one group (Group C Figure 3.3.1) distinct from the microscope analyses which formed Group D. This implies that illuminator data from low abundance samples bear less resemblance to the actual sample data (i.e. microscope data) than in high abundance samples (ME7 and RU10c).

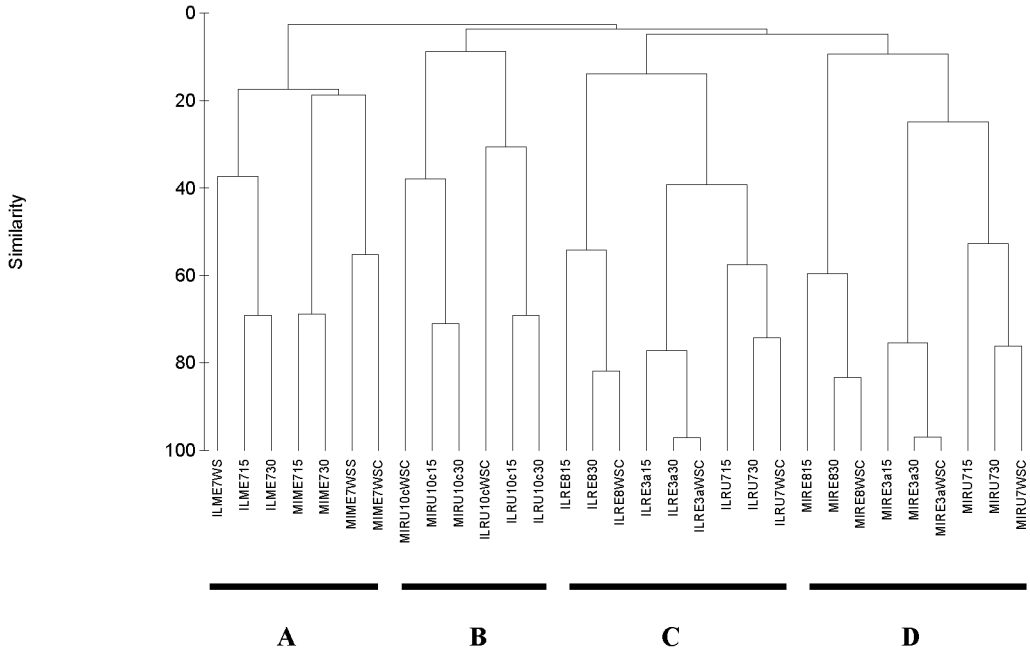

Figure 3.3.1. Cluster analysis of RSA samples. IL prefix designates data from magnifying illuminator and MI prefix denotes data from microscope analysis. "Treatments" are either 15 minute sort, 30 minute sort or whole sample analysis using compound microscope when required (15, 30 and WSC suffixes respectively). The suffix WSS in sample ME7 denotes that only a stereo microscope was used in production of the dataset.

In many cases the use of a 1.5x magnifying lens prevented identification to species level and was therefore generally similar to identification at genus or family level. Examples of such "conservativeness" include the designation of *N. hombergii* and *N kersivalensis* as *Nephtys* spp (RE3a), *Tubificoides ?galiciensis* and *T. benedii*, as Oligochaeta spp (RE3a), *Aphelochaeta marioni*, *Caulleriella alata*, *Monticellina cf dorsobranchialis* as Cirratulidae (ME7) and *Polydora cornuta*, *Pygospio elegans* and *Streblospio shrubsolii* as Spionidae spp. (RU10c). For this reason microscope analysis invariably revealed more species than identification by illuminator (see Table 3.3.1). Samples containing large numbers of cirratulids proved difficult to analyse under low power because of the difficulty in distinguishing between tentacular filaments or branchiae and smaller worms.

Some species were sufficiently distinctive to identify with the magnifying lens: *Nematonereis unicornis, Melinna palmata, Abra alba* (ME7); *Abra nitida* (RE8); *Hydrobia ulvae, Cerastoderma edule* (RU10c); *Cyathura carinata* (RU7).

In sample ME7 a microscopic identification was carried out first using a stereo microscope only (MIME7WSS in Figure 3.3.1). This gave results similar to the subsequent analysis during which further taxonomic resolution was obtained with a compound microscope. The compound microscope allowed the different species of

Pholoe, Ampithoe and *Corophium* to be identified and also gave higher precision in the identification of species in the Cirratulidae, Capitellidae and Tubificidae.

The Biotic Coefficients of samples, using magnifying lens and microscopes, are shown in Table 3.3.2. In each case, when the whole sample was analysed, the method of identification did not affect the assignment of pollution classification. However, with a 30 minute timed subsample the low power magnification data classified ME7 as "moderately polluted" when microscope analysis would have indicated only slight pollution. The same re-classification occurred in samples RE3a, RU7 and ME7 with the smaller, 15 minute subsample.

Table 3.3.2 Results from Borja's Biotic Index analysis for samples in which invertebrates were identified using a 1.5 x magnifier and then with full use of microscopes. Subsamples from 15 and 30 minute timed sorting are also shown. BC = Biotic coefficient.

		Timed sorting (15 mins)		Timed sorting (30 mins)		Whole sample analysis	
		BC	Pollution classification	BC	Pollution classification	BC	Pollution classification
RE3a	Magnifier	3.67	Moderate	4.20	Moderate	4.31	Moderate
	Microscope	3.30	Slight	3.94	Moderate	4.06	Moderate
RE8	Magnifier	1.87	Slight	1.50	Slight	1.85	Slight
	Microscope	1.87	Slight	1.50	Slight	1.85	Slight
RU7	Magnifier	3.50	Moderate	3.37	Moderate	3.66	Moderate
	Microscope	3.25	Slight	3.35	Moderate	3.75	Moderate
RU10c	Magnifier	5.38	Heavy	5.52	Heavy	5.55	Heavy
	Microscope	5.42	Heavy	5.44	Heavy	5.37	Heavy
ME7	Magnifier	3.67	Moderate	3.37	Moderate	3.54	Moderate
	Microscope	3.18	Slight	3.14	Slight	3.50	Moderate

4 DISCUSSION

Many published papers have addressed ways of streamlining the processing of marine and estuarine benthic samples (see, for instance, James *et al.* 1995; Thompson *et al*. 2003 and references therein). These generally adopt two approaches:

- to show that a large mesh (usually 1.0 mm) is as discriminative as a small mesh (0.5 mm), so enabling an investigator to do away with the extra time and effort involved with sorting and identifying smaller species and juveniles.

- to perform the analysis at a higher taxonomic level (e.g. genus or family) thus avoiding the necessity of identifying each individual to species level (e.g. Warwick, 1988; Somerfield & Clarke, 1995).

The choice of mesh size is primarily dictated by the purpose for which the data are being collected or the size of any specific target taxon (Kingston & Riddle, 1989; Bachelet, 1990; Schlacher & Wooldridge, 1996). Analyses at higher taxonomic levels give results which vary with the classification of the group of animals under consideration and the habitat type. There appear to be no ground rules for choosing an appropriate taxonomic level *a priori*.

The three methods reported here (TSA, EVA, RLA) are somewhat different in approach and, as far as is known, have not been previously described in the mainstream scientific literature. Time restricted sampling has been developed for lotic freshwater environmental assessment (Predictive System for Multimetrics or PSYM), but here the restriction is on sampling effort and not on sample processing (Anon. 2000). TSA and EVA address the problem by imposing a strict time limit on sample sorting (in the field and laboratory) and by placing constraints on identification by prohibiting the use of microscopes. The former creates non-random, fixed-size subsamples (as opposed to random, proportional subsamples) and the latter will tend to produce data at high taxonomic levels.

TSA greatly reduced the sorting time that would otherwise be needed to extract 95% of the fauna from the sample (the level stipulated in NMBAQC standards). Subsamples produced in this way generally did not resemble their 'parent' when the parent sample had high abundance and species richness. With these samples, the brevity of sorting time inevitably led to large differences between subsample size and sample size. This effect can be seen in the TSA of the Messack samples in Figure 3.1.2 where TSA samples formed a completely separate group (A + B + C) to the fully analysed samples, and in the high abundance cluster in Figure 3.1.3 where the time-restricted samples were more similar to each other than to their respective 'parent'. With a reduced sample abundance and fewer species, time-restricted subsamples more closely resembled the full sample (see here the low abundance cluster in Figure 3.1.3 where the 30 minute sort was closer to the 'parent' than the 15 minute sort). This is because a fixed sorting time will more closely approximate the actual time required for full sorting when there are fewer organisms to extract.

How closely a timed sample will come to resemble traditional data will depend on the species richness and abundance of the sample. In species-rich areas TSA may lead

to differences in interpretation of community and habitat boundaries when compared to full analyses because a more restrictive analysis will produce different relative abundances and a reduced species richness by overlooking rarer species. For example, in the Messack area, TSA effectively removed sample ME12 from Cluster E and samples ME2a, ME2b and ME2c from cluster D (Figure 3.1.2) producing clusters B and A respectively and thus altering the shape of a "traditionally" defined assemblage as it would have been mapped on the ground.

Borja's Biotic Coefficient was resilient to timed subsampling and only one sample sieved on a 1.0 mm mesh was re-classified (as more polluted) following a full analysis. With a smaller mesh three out of five samples were given more polluted status by full analysis. However, these were from different locations and so these re-classifications may not be a function of sieve size but may reflect differences in habitat type or pollution status.

Restricting time for sorting also has other practical implications. The sorter must have sufficient experience to be able to assess the sample and rapidly pick out a representative selection of fauna. Even with experience there is always the possibility that some taxa may be preferred over others, thus making it very difficult to achieve repeatability in future analyses. In this study most of the fauna appears to have been selected on the basis of conspicuousness (either size or an interest feature, such as an easily recognised tube) which has led in some cases to highly abundant animals being ignored (for instance *Tubificoides benedii* in MS9 and *Mediomastus fragilis* in ME10 and ME12 - see Table 3.1.4). Unfamiliar species may also be missed (e.g. *Protocirrineris* - ME2d). In any future investigations (and before analysing samples from a new area or habitat) it would be advisable to sort a series of abundant samples for successive periods of (say) 15 minutes until all the fauna has been removed. This would create data resembling a species - area curve from which the most acceptable sorting time could be estimated.

The field data of EVA have necessarily been treated in a more qualitative way. The potential for accuracy in the field was seen in a Ruan Creek sample (RU4) where a Bray – Curtis similarity of 95% with the laboratory check analysis was achieved. This degree of accuracy is unlikely to be seen in the majority of cases, (especially in estuarine muds) and will depend greatly on the experience of the field worker and the diversity of the sample. Field conditions are not always conducive to detailed observational work which may be hindered by poor lighting and adverse weather. Although in this case the field identification of selected animals was accurate, there was still little resemblance to the whole sample when analysed in the laboratory (Table 3.2.6). There were insufficient EVA samples with numerical data in this study to perform any meaningful analysis or to detect any trends. However, the exercise is worth repeating to determine the potential accuracy of field evaluation. As with laboratory TSA there will be problems with the repeatability of results (among individual workers, from year to year and from place to place) and also, because these are again non-random, fixed size subsamples, their representatitiveness will depend on the species richness and abundance of the 'parent' sample.

Accurate identification using low power magnification (either in the field or laboratory) is dependent on the experience of the biologist concerned. Basic invertebrate morphology is taught in universities but the emphasis has moved away

from detailed comparative zoology with the result that biologists with strong taxonomic knowledge and skills are in short supply. Even someone experienced at microscope identification of preserved material may not be sufficiently competent on live animals (which are seldom studied) without appropriate training. The outcome here suggests that using a 1.5x magnifier in the laboratory was sufficient to identify relationships between samples (Figure 3.3.1) if sufficient expertise was available. The differences between high and low abundance samples as noted in Section 3.3 were probably the result of the statistical sampling effect already alluded to. Field identification was inconclusive and bore less resemblance to fully analysed samples (Figure 3.2.1). The Biotic Coefficient was again resilient to the identification method used. In this case the sample differences were due to the reduced taxonomic resolution achievable with low power magnification, and this did not result in major differences in Biotic Coefficient as congeners are frequently assigned to the same pollution category. For instance, all species of *Ampelsca* (and hence also the genus) are assigned to Group I (even though there is evidence that *A. sarsi* might be more resistant to oil spills than other species – Dauvin *et al.* 2003)

It would not be appropriate to draw firm conclusions about the use of a magnifier without further replication and attention to experimental design. For instance, in this exercise much of the microscope analysis of TSA samples was undertaken before trying to identify fauna with the magnifier in RLA. The operators therefore had prior knowledge of precisely which species to expect and this may have improved results considerably.

Some form of restricted sorting combined with a less rigorous (microscope - free) approach to identification may have potential as a rapid assessment tool in areas of low abundance and diversity (the limits of which are yet to be defined and which may be difficult to assess *a priori*). It may also be used in situations where abundance and diversity are higher but with the understanding that results will reflect traditional analyses less faithfully (although it is recognised that a rapid assessment analysis is not intended to accurately describe a community).

The main drawbacks of restricting analysis time and eliminating microscopes are poor repeatability or consistency and lack of statistical rigour. Repeatability may be improved by confining analyses to one laboratory or group of experts, or by instituting a training schedule or series of workshops (similar to those set up under the NMBAQC scheme) through which consistency could be improved. However, the simple imposition of standard sorting times will not produce random subsamples. The ultimate aim of TSA is to reduce the size of the sample to be analysed. This is better achieved through conventional, well-established subsampling techniques (e.g. Elliott, 1977) in which the subsample size could be adjusted according to the total sample abundance (or to habitat or biotope) so keeping the processing time down to a minimum. Analysed in the conventional way, these subsamples will provide accurate species level data free from bias introduced through timed sorting. This small sampling unit would probably be insufficient to estimate the populations from which it was taken but this is often also the case with the 'parent' sample (and any replicates).

An alternative approach to "sample volume" subsampling would be to investigate random sequence techniques as advanced by Cairns *et al.* (1968) and adopted for

nematode work by Moore *et al.* (1987) In this technique a fixed number of randomly selected animals is removed from the sample. Each is compared with its predecessor and runs of similar species are then analysed statistically.

In many cases an experienced worker will have difficulty identifying animals with a 1.5x magnifying lens especially with difficult groups such as cirratulids and spionids, or when specimens are damaged. Often, only the family or genus level can be assigned tentatively. The quality of the data may be improved, however, with the use of a stereo microscope with which genus can often be rapidly determined without recourse to reference works or a compound microscope. Further investigation of scaled, random subsampling followed by genus (or family) identification using a stereo microscope may therefore be appropriate for rapid assessments.

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