Scottish
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Forum

Assessing The Potential To Reduce The Infaunal Species List
Required To Give An Indication Of Stress In Sediments

SARF042

A REPORT COMMISSIONED BY SARF
AND PREPARED BY

Plymouth Marine Laboratory

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Project Final Report Form

Project Details

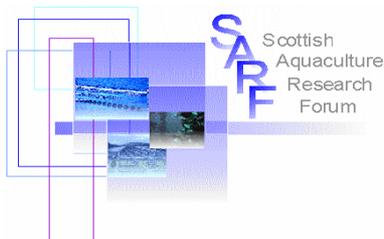|	
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Name(s) and address(es) of contractor organisation(s): Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH	
Contractor's Project Manager: Dr Paul J. Somerfield	
SARF Project Manager: Mark James	
Total SARF Project costs £15277	
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Is there any Intellectual Property arising from this project which is suitable for commercial exploitation (*This question requires a YES/NO answer only. All other details of any Intellectual Property must be included under the Scientific Report or in an accompanying Annex*). ....YES NO ☐ ☒ *Staff years of direct science effort*	

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Scientific objectives

List the scientific objectives as set out in the contract. If necessary these can be expressed in abbreviated form. Indicate where amendments have been agreed with the SARF Project Manager, giving the date of amendment.

1. Data organisation
2. Data analyses
3. Report writing

The objective of this work was to undertake a comprehensive statistical analysis of benthic data from around fish farms, held by SEPA, to determine whether analyses based on a single reduced species list can adequately reflect variation and stress in the whole benthic community, what that list should be, and how practical it would be to employ it (i.e. does it really represent the potential for savings?). We also undertook analyses using other proposed effort-reduction techniques for comparative purposes.

Milestones

List the milestones. It is the **responsibility of the contractor** to check fully that **all** milestones have been met and to provide a detailed explanation if this has not proved possible.

Milestone		Target Date	Milestone Met	
Number	Title		In Full	On Time
1	Data acquisition	07/03/08	Yes	No
2	Data analysis	07/05/08	Yes	Yes
3	Final report	06/06/08	Yes	Yes

If any milestones have not been met please give an explanation below.

Biological data in the database was not in an analysable state, and considerable effort had to be made to make it so. Although this did not delay the overall project, it did reduce the time available for analyses.

Executive Summary

The executive summary must not exceed 2 sides in total of A4 (minimum font size 10) and should be understandable to the intelligent non-specialist. It should cover the main objectives, methods and research results, together with any other significant events and options for new work (the box below will expand to accommodate the Summary).

The objective of this work was to undertake a statistical analysis of benthic data from around fish farms, held by SEPA, to determine whether analyses based on a single reduced species list can adequately reflect variation and stress in the whole benthic community, what that list should be, and how practical it would be to employ it (i.e. does it really represent the potential for savings?). We also undertook analyses using other proposed effort-reduction techniques (analyzing samples to higher taxonomic levels such as families, rather than to species) for comparative purposes.
The work discusses the extent to which benthic analysis around farms may be speeded up and made less expensive, to the benefit of SEPA and those required to monitor the benthic environment, and provides recommendations about how this work may be further developed. All objectives have been met.

The results make it very clear that there is great potential to develop a monitoring and assessment programme which can deliver statistically robust assessments of the biological status of sediments in the vicinity of aquaculture installations, with considerable reductions in the level of taxonomic discrimination and effort required. This will require the development of new sediment quality assessment criteria, as the existing ones cannot be delivered using a reduced list in such a way as to be reliably consistent with previous assessments.

There is considerable 'overengineering' in the current assessment methodology. Although 'replicate' samples are collected, this replicate information is not used in any sensible way in the course of assessments, other than to decide whether conditions are azoic close to cages. It needs to be considered whether 5 samples per station are necessary, as a reduction to, for example, 3 per station would certainly reduce the amount of effort and time required to analyse the samples. Alternatively, a suitable statistical assessment methodology which actually uses the replicate information could be developed. This would allow a probabilistic approach to assessment, with parallel assessments of the probability of error and a proper determination of the number of samples actually required to deliver robust assessments.

The current assessment procedure considers each survey independently. As demonstrated in this report there is much to be gained by considering each survey within an overall analysis based on information from previous assessments. This allows a robust intercomparison between surveys, and allows the erection of criteria based on knowledge of the actual relative effects of aquaculture activities in different places, rather than on conceptual (or numerical) models which are several steps removed from reality. Such an approach is required if management is to be adaptive, learning from experience. To deliver such an approach the existing database structure, and data-entry quality controls, should be improved and tailored. We have gone a considerable way towards developing what is needed in this project, but to deliver an operational system would require further investment.

While the focus of all of the assessment work has been abundances of organisms, arguably a better indication of the ecological status of macrobenthic communities is to be gained by examining biomass. The availability of abundance data alone precludes the application of a number of indicators of community stress that the addition of biomass data would facilitate. Such methods can be used to compare the degree of disturbance at different sites, and to place each on a continuous comparative scale, and some only require the proportions of biomass and abundance within phyla at each site to be assessed.

The acquisition of information about changes in benthic assemblages around fish farms for environmental monitoring is perceived to be time consuming, expensive, and to require expertise. It is true that the nature of the changes, conforming to a well known succession model of organic enrichment, and the nature of the community structure in marine sediments, with high levels of structural redundancy, makes it possible to focus effort with consequent savings in time and effort, and using less expertise. This will, however, require further work.

The report concludes with the following recommendations and suggestions for further work:

1. That consideration be given to developing a robust assessment methodology based on the abundances of higher taxa across large numbers of surveys. This development should include an assessment of the statistical power of different techniques to detect particular changes in the benthos associated with organic enrichment, and an assessment of the sampling requirements actually needed to deliver assessments. The focus should be on developing an assessment regime that delivers simple assessments for the minimum investment, but at the same time is ecologically defensible and efficient.

2. That consideration be given to further developing the database structures and procedures developed in this project to deliver a system to underpin the new assessment methodology, which will facilitate combined analyses of data from different surveys.

3. That consideration be given to requesting biomass data (preferably at the species level, alternatively at the phylum level) in addition to abundance data from those delivering survey information, for a limited period of time (say one year). This information should then be used to develop a robust methodology for assessing stress which does not rely on inter-station comparisons but which uses combined information from many surveys. This would have a minor short-term cost implication, which should be addressed, but the potential benefit is to move away from complete taxonomic discrimination of all organisms in samples.

4. That consideration be given to incorporating AMBI into a new assessment framework. This index is to be preferred to ITI as it is based on sensitivities of taxa gleaned from large numbers of studies across Europe. The potential for modifications of the index focusing on different aspects of community structure is recognized, and these should be assessed in relation to the effects of aquaculture to determine which modification delivers the most appropriate information for the least effort. This work should also consider developments with respect to the implementation of the Water Framework Directive to minimize duplication of effort.

Assessing the potential to reduce the infaunal species list required to give an indication of stress in sediments.

P. J. Somerfield[1], R. M. Warwick[1], M. Atkins[1], C. L. MacNeill[2], K. R.Clarke[1,3]
[1] Plymouth Marine Laboratory, Prospect Place, Plymouth PL1 3DH
[2] Plymouth Marine Laboratory Applications Ltd, Prospect Place, Plymouth PL1 3DH
[3] Primer-E Ltd, 3 Meadow View, Lutton, Ivybridge PL21 9RH

Introduction

The objective of this work was to undertake a comprehensive statistical analysis of benthic data from around fish farms, held by SEPA, to determine whether analyses based on a single reduced species list can adequately reflect variation and stress in the whole benthic community, what that list should be, and how practical it would be to employ it (i.e. does it really represent the potential for savings?). We also undertook analyses using other proposed effort-reduction techniques for comparative purposes.

The work discusses the extent to which benthic analysis around farms may be speeded up and made less expensive, to the benefit of SEPA and those required to monitor the benthic environment, and provides recommendations about how this work may be further developed. All objectives have been met.

Background

SEPA require operators to submit environmental surveys of the sea bed around the cages. The analysis of the benthic fauna component of the gathered samples is time consuming, expensive and requires highly trained and experienced analysts. Results of a recent fish farm Data Review Project indicate that there may be potential to develop a reduced species list that might be robust enough to give an indication of stress within the sediments. If such a list could be developed then it would open the way for the analysis of benthic faunal samples to be speeded up, be less expensive and the turnaround times would be more realistic. The purpose of this work is to undertake a comprehensive statistical assessment of the SEPA dataset, primarily using methods developed at PML for this purpose, to determine whether a reduced species list can be achieved. We also examine other data-based effort reduction methods for comparative purposes, with a view to making recommendations about the best way forward.

The data source: biological monitoring

Biological monitoring is carried out in the vicinity of fish-farming installations. Sediment samples are collected by means of a grab. Generally 5 grabs are collected from each location. The sediment is sieved, and animals retained on the sieve are preserved for subsequent analysis when they are then identified to the lowest possible taxon (generally species) and counted.

Until recently, locations were determined by fixed distances from the edge of the cages. Samples were collected from under or beside the cages, 25 m away, and at distances beyond 25 m from the cages. The assumption was that no effects should be apparent at 25 m, so samples at 25 m should not differ from samples collected further away. The area within 25 m of the cages is therefore termed the 'allowable zone of effects' or AZE. We are aware that the size of the AZE may now be determined by modelling. Monitoring in these instances focuses on the edge of the AZE, with sampling at the boundary, 10 m beyond, and 10 m within. For the purposes of this report, however, we will tend to focus on the fixed-distance surveys as these provide the majority of the data.

Sediment quality criteria for the benthos differ between within the AZE and outside it (Table 1). Within the AZE it is accepted that organic enrichment effects will occur. The criteria are selected to ensure that they do not exceed an acceptable amount. Effects beyond the AZE are determined

by comparing conditions at 25 m with those further away. There is a degree of confusion here, as the action levels associated with the sediment quality criteria imply that a considerable degree of environmental degradation at 25 m is acceptable, even though it is outside the allowable zone of effects.

Table 1. Sediment Quality Criteria: Action Levels and associated requirements for taxonomic discrimination within samples.

Determinand	Action Level within AZE
Number of taxa	Less than 2 polychaete taxa present (replicates bulked)
Number of taxa	Two or more replicates with no taxa present
Abundance	Organic enrichment polychaetes present in abnormally low densities
	Action Level outside AZE
Number of taxa	Must be at least 50% of reference station value
Abundance	Organic enrichment polychaetes must not exceed 200% of reference value
Shannon-Weiner Diversity	Must be at least 60% of reference station value
Infaunal Trophic Index (ITI)	Must be at least 50% of reference station value

In the context of assessing ecological status using these criteria a number of 'measures' are used, which are derived from the species abundances in grab samples. Some of these are straightforward, namely whether there are two or more polychaete taxa present in the AZE, or whether two or more samples (these are not genuine replicates in any statistical sense, as the replicate information is not used) in the AZE have no animals in them. Others require a degree of specialist knowledge, such as whether organic enrichment polychaetes are present in abnormally low densities, or whether they exceed 200% of abundance at reference stations. Organic enrichment polychaetes are taken to be those that normally occur in the AZE, which for the purposes of calculating ITI (see below) are placed in Trophic Group 4 (regardless of their actual trophic mode, which is often unknown). Abnormally low density in the AZE is taken to be <1000 individuals.m^2. In addition to these 'measures', 3 indices (number of taxa, Shannon-Wiener, ITI) are used to assess the status of communities outside the AZE.

Species richness
Species richness (number of taxa) is a straightforward measure. It is notoriously sample-size dependent, by which we mean that larger samples, or more samples, will almost always discover further species. Although there are a range of measures which attempt to adjust for differing numbers of individuals these are not employed. They are, however, unnecessary as sampling effort (5 grabs) is kept constant.

Shannon (or Shannon-Wiener) diversity index.
Different diversity indices emphasize the species richness or equitability components of diversity to varying degrees. Equitability (or evenness) expresses how evenly individuals are distributed among the different species, and is the opposite of dominance. Shannon is one of the most commonly used:
H' = $\Sigma_i\, p_i \log(p_i)$
where p is the proportion of the total count arising from the ith species. This measure is heavily influenced by changes in evenness. Note that logarithms to the base 2 are sometimes used in the calculation, reflecting the indexes origination in information theory, but there is no natural biological interpretation of the values. The use of natural logarithms (to the base e) is preferable, and commonly used. It is easy, however, to convert values back and forth between bases. H' is, like richness, sensitive to sampling effort so values should only be compared if studies have equivalent sampling effort.

Infaunal Trophic Index (ITI)

For the calculation of the ITI invertebrates are categorised into four groups according to their feeding mode:

Group 1 – suspension feeders; Group 2 – surface detritus feeders; Group 3 – surface deposit feeders; Group 4 – sub-surface deposit feeders

The index is then: ITI = $100 - (33.3(n2 + 2n3 + 3n4)/(n1 + n2 + n3 + n4))$

where *n1* is the proportional abundance of organisms in trophic group 1, and so on. The index may take values from 0 to 100. In a monitoring context, a relationship between feeding mode and sensitivity is assumed, with relative sensitivities ranging from suspension feeders (most sensitive) to sub-surface deposit feeders (least sensitive). Thus values of the index may be used to define the status of an assemblage, as a high value implies a preponderance of sensitive organisms inhabiting the area, while a low value indicates that an area is only inhabited by insensitive organisms and sensitive ones are absent. The relationship between ITI values and the status of the community with regard to anthropogenic impacts is normally given as:

ITI >50 – little effect; ITI 20-50 – enriched; ITI <20 - degraded

SEPA, however, use a slightly different (and more conservative) scale:

ITI > 60 – community normal; ITI 30-60 – community changed; ITI <30 – community degraded. Since a considerable proportion of samples from the fish farm studies have ITI scores of <1, we felt it necessary to add a further category for this group, tentatively termed "very degraded".

We have some reservations about the use of this index, some of which have already been expressed (Levinton, 1991; Maurer et al., 1999; Dauer, 1984). Among these is the implied assumption that different trophic groups have different sensitivities. In addition, a large proportion of the species in the fish farm surveys are not included in the published list for UK waters (WRc, 1992), and we have had to make informed decisions as to which groups these species occupy. In assigning previously unassigned species we have found it difficult to distinguish between the feeding modes 2 and 3. Where ITI scores have been given, we are concerned that many genera comprise species that are given different scores from one another for no apparent reason (e.g. *Amphiura, Glycera, Malacoceros, Nephtys, Nereis, Praxillella/ura, Scololepis, Sphaerosyllis*).

Potential for a reduced species list within the current assessment framework

Each of the sediment quality criteria action levels has, associated with it, an implied level of taxonomic discrimination within samples (Table 2).

Table 2. Taxonomic discrimination within samples implied by Action Levels.

Action Level within AZE	Required taxonomic discrimination
Less than 2 polychaete taxa present (replicates bulked)	Polychaetes
Two or more replicates with no taxa present	"Taxa"
Organic enrichment polychaetes present in abnormally low densities	Organic enrichment polychaetes
Action Level outside AZE	
Number of taxa must be at least 50% of reference station value	All
Abundance of organic enrichment polychaetes must not exceed 200% or reference value	Organic enrichment polychaetes
Shannon-Wiener diversity must be at least 60% of reference station value	All
ITI must be at least 50% of reference station value	All

Within Allowable Zone of Effects

Biological monitoring within the AZE is limited to examination of samples from the cage-edge. It is a reasonable assumption that the vast majority of animals sampled at the cage edge are 'organic enrichment polychaetes', so there is no need to discriminate organic enrichment polychaetes from

other species of polychaetes in order to assess these criteria. All that is required is to determine whether polychaetes are present, if so to count enough of them to be sure that there are at least 1000 m^2 or so, and then to look at them for long enough to determine that more than one or two species are present.

Outside Allowable Zone of Effects
Of the 4 biological sediment quality criteria used to assess compliance three, namely the number of taxa (which we assume to mean number of species), Shannon-Wiener diversity and the Infuanal Trophic Index are univariate measures that require all taxa (species) to be identified. Thus it appears that there can only be real potential for use of a reduced species list within the current assessment framework if it can be shown that patterns in values of the 3 indices calculated using a reduced list are identical to those in values calculated using the full list.

The database
Two databases in Microsoft Access format were provided to PML for this work. One was the SEPA self-monitoring database and the other was a SEPA Fish Farm database. For the purpose of our analyses, the self monitoring database was used to extract abundance data and environmental data such as sediment analyses plus any associated site/sample metadata. Additional metadata for sample sites was extracted from the Fish Farm database. A new database structure was constructed, based around a look-up table that allowed every species name in each of the survey spreadsheets in the SARF database to be matched against the UK Marine Species Directory (Howson and Picton 1997). This database structure allowed us to combine species abundance data from all surveys using a standard species list (and taxonomic hierarchy). Having omitted samples with no content, we produced a data table with abundances of 1030 taxa in 1695 samples. As there is no evidence of replicate information from different grab samples at different locations being used for statistical analyses, such as comparisons of means among locations, replicates were averaged, to give a matrix of 296 samples.

Initial analysis
A range of non-parametric multivariate methods were used in the course of this study. The methods are described in Clarke and Warwick (2001) and Clarke and Gorley (2006). Certain methods (e.g. BVSTEP) will be described in the appropriate place later in this report, but to describe our initial analyses of the data we use nonmetric multidimensional scaling (MDS). This is a method that arranges points, representing samples, in two dimensions so as to maximise the 'goodness of fit' between the distances between the points, and a measure of resemblance (similarity) between the original samples. For the purposes of the majority of MDS analyses presented in this report we used a mild (square-root) transformation of the data to downweight the influence of numerically dominant taxa, and then calculated similarity between samples using the Bray-Curtis similarity coefficient for input into the MDS algorithm. The easiest way to think about the resulting MDS plots is that they are like maps, and samples that are alike lie close to each other. With 296 points on each plot, it is impossible to compare plots by simply looking at the sample-labels. We chose to code samples using values of an index of benthic community structure specifically designed to discriminate the status of assemblages along organics enrichment gradients.

AMBI
In recent years several benthic biotic indices have been proposed for use in marine waters. One of them, named AMBI (AZTI Marine Biotic Index) has been applied to different geographical areas under various impact sources (Borja et al., 2000, 2003) and is currently being considered by the UK Environment Agency for marine monitoring in the context of the European Water Framework

Directive. AMBI scores have therefore been applied to the present data in order to assess their status in these terms.

Individual species are assigned to five ecological groups according to their sensitivity/tolerance to environmental stress gradients from the most sensitive (Group I) to the most tolerant (Group V), and the index is based on the proportions of individual abundance in each group. A full current list of AMBI species assignments (3459 taxa) to ecological groups is available. Unfortunately this list uses idiosyncratic species nomenclature and there are many spelling mistakes, so that matching it with the present data from fish farms was problematic. A similar process to that applied for the construction of the standardised species list for the data (see above) was applied, providing us with a look-up table with AMBI categories for most of the species in the data table.

To calculate AMBI total abundances of organisms within each ecological group are summed, and the totals converted to percentages of the total abundance in the sample. The overall AMBI score for a sample is calculated using the formula:

AMBI = ((1.5 %II)+(3 %III)+(4.5 %IV)+(6 %V))/100

where *%II* is the percentage of animals in ecological group I, *%III* the percentage in ecological group II, and so on. Thus it may be seen that AMBI shares many features with ITI, being a weighted average based on relative proportions of organisms in different categories. AMBI, however, is based in an objective assessment of the relationships between benthic species and levels of pollution over a wide area of Europe, whereas the assignment of species to trophic groups is often subjective and requires a detailed knowledge of the biology of the species involved, which is usually lacking.

AMBI scores may be used to describe the ecological status of the benthic assemblage: High status (0-1.2), Good status (1.2-3.3), Moderate status (3.3-4.3), Poor status (4.3-5.5) and Bad status (>5.5). We use AMBI scores to display the effects of organic enrichment gradients on community structure in MDS plots (Fig 1)
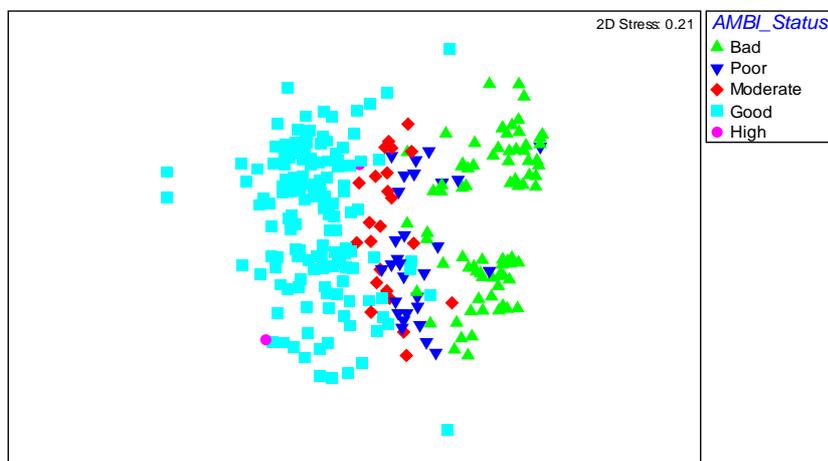

Figure 1. MDS plot of all 296 samples based on abundances of 1030 taxa in the database, overlain with AMBI scores for each sample.

Figure 1 is an interesting plot, displaying as it does the main interrelationships between all samples in the database. A clear gradient from unimpacted (generally 'good' status) samples to impacted ('bad') samples is readily apparent. We include this plot because it illustrates how crucial work to standardise and harmonise the taxonomic assignment of organisms may be in situations where data from a number of surveys are to be compared. There is a clear vertical separation between two sets of surveys in the plot. Such differences between surveys could interpreted as being important, and could also make efforts to standardise assessment protocols problematic. A detailed analysis of the data, however, showed that this major separation resulted from a simple taxonomic artefact

– in approximately half of the surveys the abundant organic enrichment polychaete Capitella capitata was identified to the species level, whereas in the other half it was identified only to genus. This fact is illustrated by overlaying the sample locations in the MDS plot with symbols scaled in size according to the abundances of Capitella capitata (Fig.2) or Capitella (Fig. 3) in the samples.

Figure 2. MDS plot of all 296 samples based on abundances of 1030 taxa in the database, overlain with symbols scaled in size with abundances of Capitella capitata.
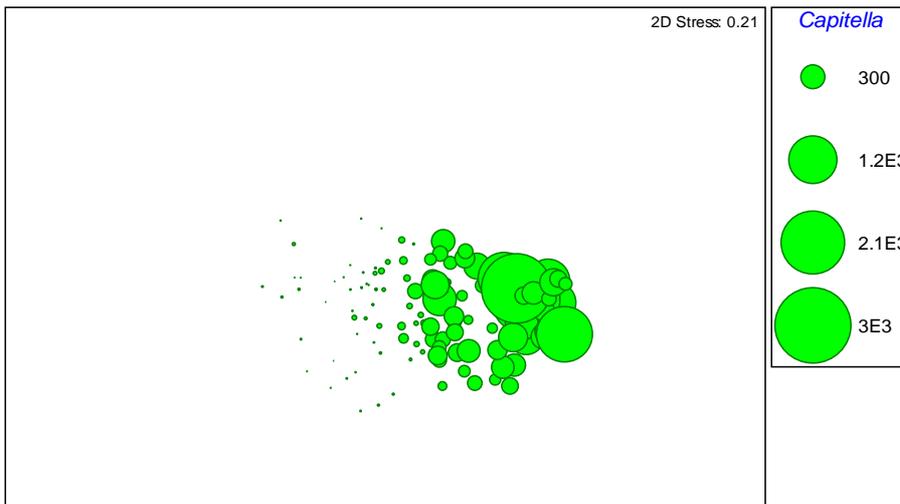

Figure 3. MDS plot of all 296 samples based on abundances of 1030 taxa in the database, overlain with symbols scaled in size with abundances of Capitella.

A simple correction for this artefact (combining abundances of Capitella, Capitella capitata and a single survey in which they were identified only to the family Capitellidae) into a single category, Capitellidae, produced an MDS in which the predominant pattern is that of the gradient from good to bad ecological status (Fig. 4). The corrected data underlying this plot, now with 1027 taxa, forms the basis for the analyses which follow.
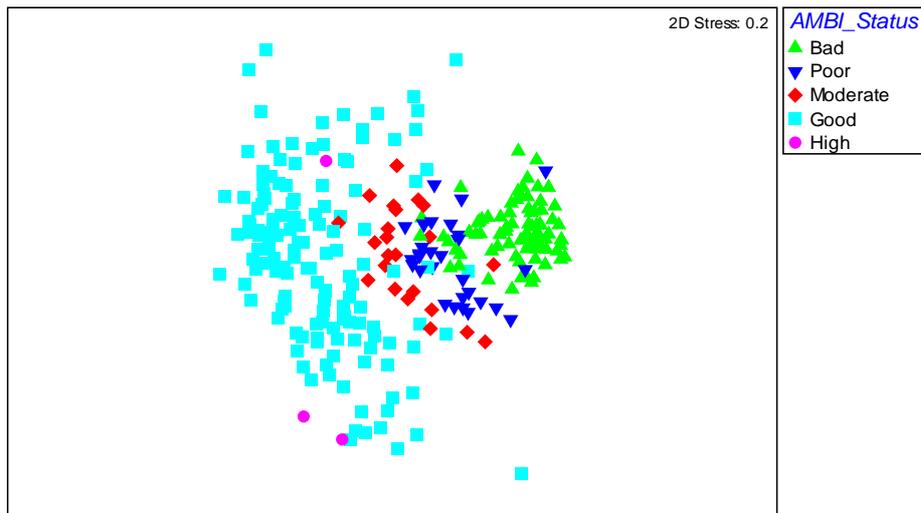
Figure 4. MDS plot of all 296 samples based on abundances of 1027 taxa in the database (Capitella, Capitella capitata and Capitellidae combined), overlain with AMBI status.

Reducing analytical effort

The MDS of Fig. 4 shows a clear signal of community change in relation to stress levels (in this case AMBI scores). The ordination is based on a matrix of 1027 species but the question naturally arises as to whether all these species are influential in forming the pattern. This cannot be the case, of course, because many species are very uncommon. The question then arises as to whether the pattern largely determined by a small number of highly influential species, or whether the pattern could be faithfully reproduced if the samples were only analysed to higher taxa, e.g. families or even phyla, rather than to the species level. If so, an MDS of that small species subset, or an analysis at higher taxonomic levels, should generate a near perfect match to Fig. 4. If this is achievable, then analysis of an appropriate subset of species, or analysis at higher taxonomic levels, could greatly improve the cost effectiveness of compliance monitoring.

Reducing the species list

Clearly, if a reduced species list is to be used in the context of compliance monitoring it needs to be a list that is applicable globally to all fish farms, and not a list specifically tailored to an individual farm. The species in the subset should also be easily identifiable. There are several possible ways of producing such a list.

BVSTEP

In this routine (Clarke & Warwick, 1998) subsets of variables (species) are selected from the full matrix such that their sample ordination matches, as closely as possible using a matching criterion, the ordination of samples from the full matrix. This is done by optimising the correlation between the elements of the underlying similarity matrices, rather than matching the respective ordinations, because of the approximation inherent in viewing inter-sample relationships in only 2-dimensions. The appropriate correlation coefficient could be Spearman or Kendall, or some weighted form of Spearman, but there is little to be gained in this context from using anything other than the simplest form, the standard Spearman coefficient (ρ). A definition of a 'near-perfect' match is needed, and this is (somewhat arbitrarily) deemed to be when ρ exceeds 0.95. Certainly two ordinations from similarity matrices that are correlated at this level will be virtually indistinguishable and could not lead to different interpretation of the patterns. The requirement is therefore to find the smallest possible species subset whose Bray-Curtis similarity matrix correlates at least at $\rho = 0.95$ with the (fixed) similarity matrix for the full set of species.

There is a major snag, however, to carrying out this procedure. A search through all possible subsets of species involves an exceedingly large number of combinations, which would be computationally impossible. One way round the problem is to search not over every possible combination but some more limited space, and the natural choice here is a stepwise algorithm which operates sequentially and involves both forward and backward-stepping phases. At each stage, a selection is made of the best single species to add to or drop from the existing selected set. Typically, the procedure will start with a null set, picking the best single variable (maximising ρ), then adding a second variable which gives the best combination with the first, then adding a third to the existing pair. The backward elimination phase then intervenes, to check whether the first selected variable can now be dropped, the combination of second and third selections alone not having been considered before. The forward selection phase returns and the algorithm proceeds in this fashion until no further improvement is possible by the addition of a single variable to the existing set or, more likely here, the stopping criterion is met (ρ exceeds 0.95). Analogously to the MDS algorithm, it is quite possible that such an iterative search procedure will get trapped in a local optimum and miss the true best solution; only a minute fraction of the vast search space is ever examined. Thus, it is important to begin the search at several, different, random starting points. Applying this (BVSTEP) procedure to the 1027 species set a smallest subset of 28 species is found (Table 3), whose similarity matrix across the 296 samples correlates with that for the full species set, at $\rho \geq 0.95$. The MDS plot based on these 28 species is shown in Fig. 5 and is seen to be largely indistinguishable from Fig. 4. Often with stepwise procedures, this may be far from a unique solution, but in this case six random starting conditions gave rise to a virtually identical species subset.
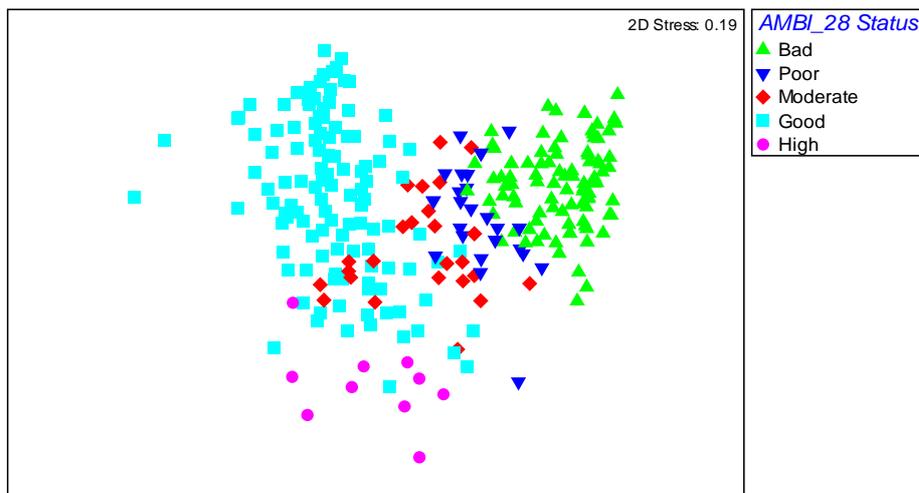

Figure 5. MDS plot of all 296 samples based on abundances of 28 taxa, selected by BVStep as providing a close match with the pattern derived from all1027 taxa.

Arguably the pattern in this MDS is even clearer than that derived from all 1027 taxa, providing a clearer discrimination between AMBI classes.

In a number of studies, disregarding the initial selection of species, and searching again for a further subset that produces a near-perfect match ($\rho \geq 0.95$) to the pattern for the full set, has shown that a second such set can be found. If the two sets are discarded, a third, then a fourth can sometimes also be identified, suggesting considerable structural redundancy in the assemblages (Clarke & Warwick 1998). In the case of the fish farm data, however, a second "peel" of species failed to achieve $\rho \geq 0.95$, and only gave peels of 11 species with a ρ of 0.484.

The message is therefore clear. It is a single small set of species which is responsible for generating the observed sample patterns of Fig. 5, and there is no redundancy in this case. This

unique set of 28 species is therefore the prime candidate if a subset of species is to be selected for the evaluation of benthic environmental quality. The species in this subset are also relatively easy to identify (Table 3).

Most abundant species
An alternative way of selecting a subset of species is simply to select the most abundance species in the whole data set, i.e. a given number of species that represent a particular percentage of the species abundances in any one sample. To test the effectiveness of this method, we have selected the top 28 species (Table 3), in order to make a direct comparison with the species list derived from BVSTEP. The MDS plot for this subset is given in Fig. 6, and the correlation ρ with the full species matrix is 0.91, i.e. less than the 28 BVSTEP species.
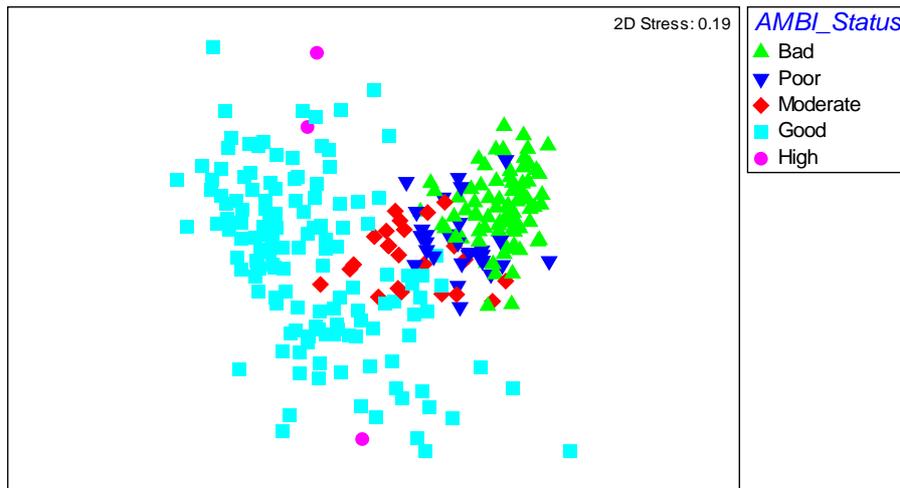

Figure 6. MDS plot of all 296 samples based on abundances of 28 taxa, selected according to percentage abundance in samples.

This is therefore a slightly inferior subset with respect to reflecting the full pattern. Interestingly, the two lists only have 12 species (43 %) in common (Table 3).

Table 3. Species occurring in reduced species lists selected by BVSTEP (28 species), by selecting the 28 'most important' species based on abundance, or by both methods.

Both	BVSTEP	Abundance
Amphipholis squamata	Abra alba	Anaitides mucosa
Amphiura chiajei	Chaetozone setosa	Angulus tenuis
Amphiura filiformis	Diplocirrus glaucus	Caulleriella alata
Capitellidae	Galathea intermedia	Cirriformia tentaculata
Malacoceros fuliginosus	Galathowenia oculata	Corophium
Mediomastus fragilis	Lumbrineris gracilis	Exogone naidina
Melinna palmata	NEMERTEA	Levinsenia gracilis
Mysella bidentata	Nephtys hombergii	Modiolula phaseolina
Prionospio fallax	Notomastus latericeus	Myriochele
Scalibregma inflatum	Owenia fusiformis	NEMATODA
Thyasira	Pholoe inornata	Ophiothrix fragilis
Thyasira flexuosa	Scoloplos armiger	Ophryotrocha
	Spio decorata	Ophryotrocha hartmanni
	Timoclea ovata	Paraonides
	Turritella communis	Tubificoides
	Urothoe elegans	Tubificoides benedii

Enrichment "indicator" species

A third alternative is to enumerate only those species that are regarded as enrichment "indicator" species, i.e. those in AMBI group V or ITI group 4. AMBI group V provides a match of 0.555 (Fig. 7) and contains only 18 species.
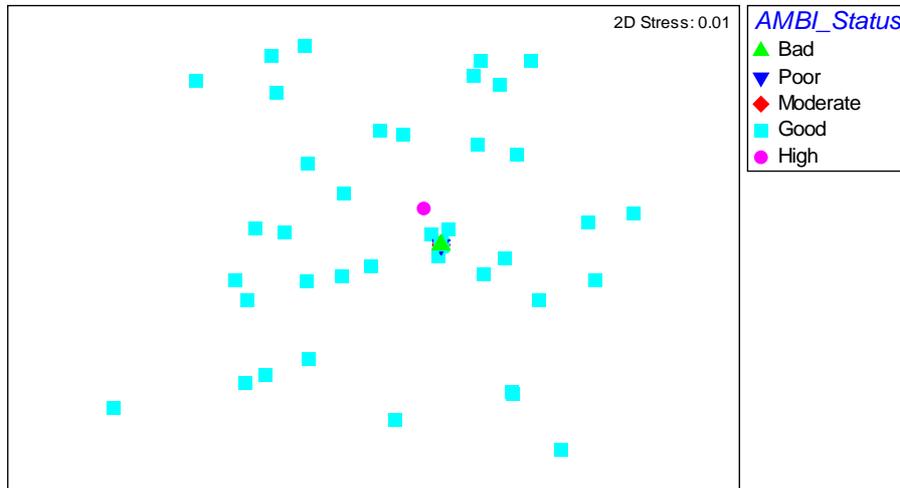

Figure 7. MDS plot of all 296 samples based on abundances of taxa within AMBI group V.

The plot (Fig. 7) is rather disappointing, as it fails to distinguish clearly between sites closest to the cages. This is largely because of the many empty samples resulting from the exclusion of most species. To improve it we used a modified form of the Bray-Curtis similarity measure (Clarke et al. 2006). The match is still low (0.535) but the modification allows us to see that the reduced set of species is still reflecting the enrichment gradient very well (Fig. 8).
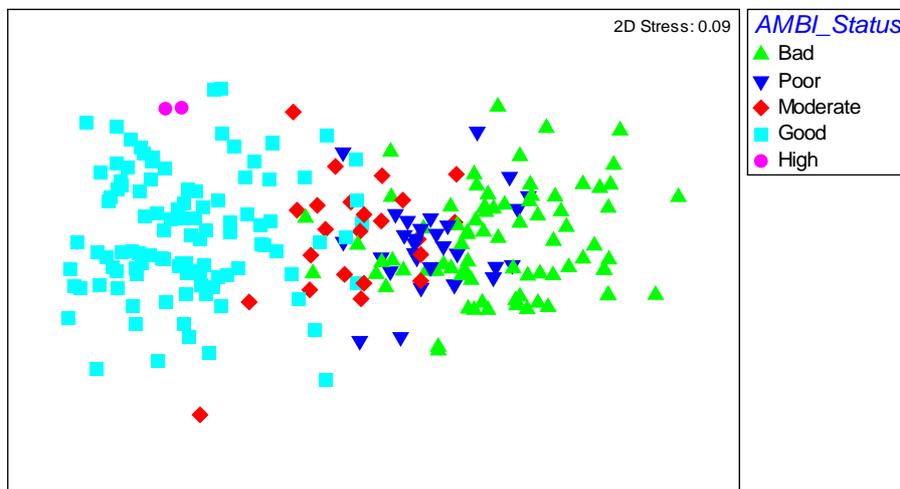

Figure 8. MDS plot of all 296 samples based on abundances of taxa within AMBI group V derived using a modified Bray-Curits similarity measure.

We added AMBI group IV to the analysis. 71 species fall into groups IV and V, and the MDS (Fig. 9) bears a closer resemblance to the full species ordination, with a ρ of 0.797. Remarkably only one sample had to be omitted to produce the plot, indicating that enrichment indicator species are widespread in the vicinity of fish farms.
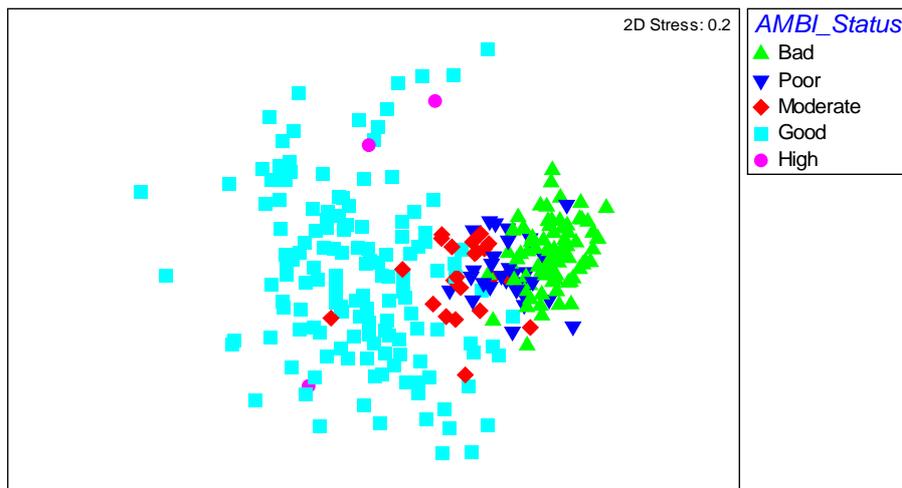
Figure 9. MDS plot of 295 samples based on abundances of 77 taxa coded as AMBI groups IV and V.

Higher taxonomic levels

The painstaking work involved in sorting and identifying samples to the species level has resulted in community analysis for environmental impact studies being traditionally regarded as labour-intensive, time- consuming and therefore relatively expensive. One practical means of overcoming this problem is to exploit the redundancy in community data by analysing the samples to higher taxonomic levels, such as family or even phylum, rather than to species. If results from identifications to higher taxonomic levels are comparable to a full species analysis, this means that:

a)   A great deal of *labour can be saved*. Several groups of marine organisms are taxonomically difficult, for example (in the macrobenthos) several families of polychaetes and amphipods; as much time can be spent in separating a few of these difficult groups into species as the entire remainder of the sample, even in Northern Europe where taxonomic keys for identification are most readily available.

b)   *Less taxonomic expertise* is needed. Many taxa really require the skills of specialists to separate them into species.

For the marine macrobenthos, aggregations of the species data to higher taxonomic levels have been made and the resultant data matrices have been subjected to several forms of statistical analysis to see how much information has been lost compared with the full species-level analysis. The results are remarkably clear in that very little information appears to be lost after a moderate degree of aggregation. There are also certain possible theoretical advantages to conducting multivariate analyses at a high taxonomic level for pollution impact studies. Natural environmental variables which also affect community structure are rarely constant in surveys designed to detect pollution effects over relatively large geographical areas. In the case of the benthos, these 'nuisance' variables include water depth and sediment granulometry. However, it is a tenable hypothesis that these variables usually influence the fauna more by species replacement than by changes in the proportions of the major taxa present. Each major group, in its adaptive radiation, has evolved species which are suited to rather narrow ranges of natural environmental conditions, whereas the advent of pollution by man has been too recent for the evolution of suitably adapted species. Ordinations of abundance or biomass data of these major taxa are therefore more likely to correlate with a contamination gradient than are species ordinations, the latter being more complicated by the effects of natural environmental variables, especially when large heterogeneous geographical areas are considered. In short, higher taxa may well reflect well-

defined pollution gradients *more closely* than species (Warwick, 1988). An additional advantage of working at higher taxonomic levels is that the enrichment "indicator" taxa often comprise a number of closely related sibling species which are very difficult to distinguish on morphological characters (e.g. Capitella, Ophryotrocha, Pontonema), so that it is actually rather difficult to determine the number of species present.

Figures 10 - 13 show MDS ordinations of the fish farm data with the species data aggregated to genera, families, phyla and the 5 most important phyla (Annelida, Mollusca, Crustacea, Echinodermata and Nematoda). Note that technically Crustacea are a sub-phylum of the Arthropoda, but as all arthropods in the samples were Crustacea we use that term. There is a remarkable good relationship with the full species MDS (0.962, 0.881, 0.605 and 0.544 respectively) and thus with benthic environmental condition, even for the five major phyla.
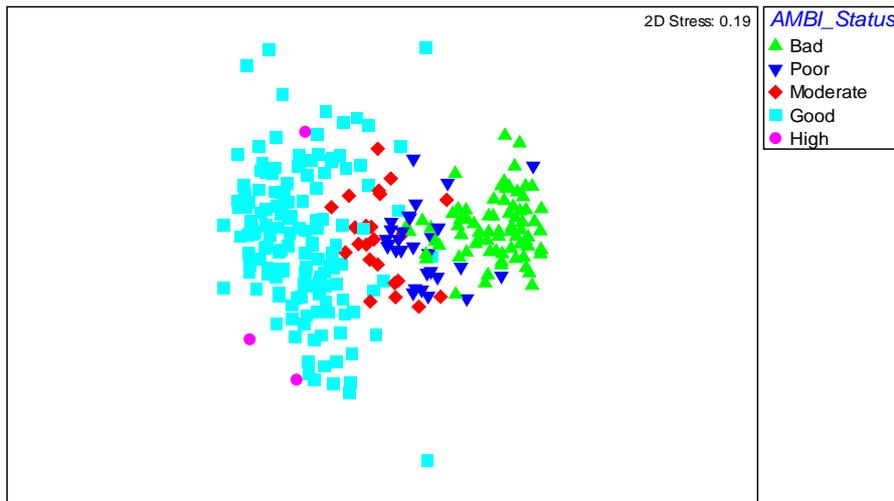

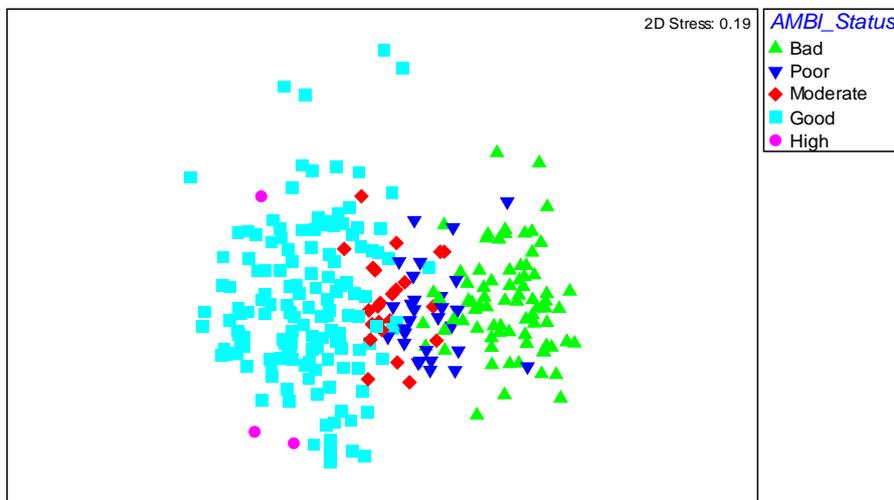*Figure 10. MDS plot of 295 samples based on abundances of genera.*

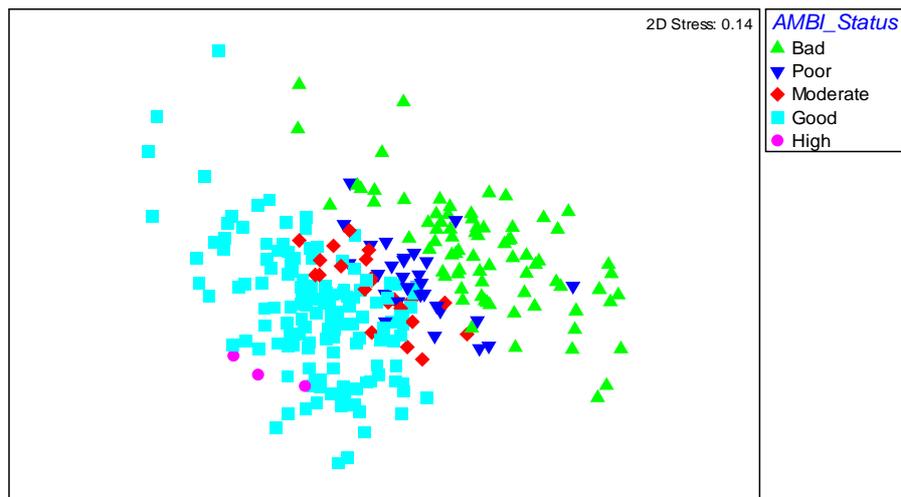*Figure 11. MDS plot of 295 samples based on abundances of families.*

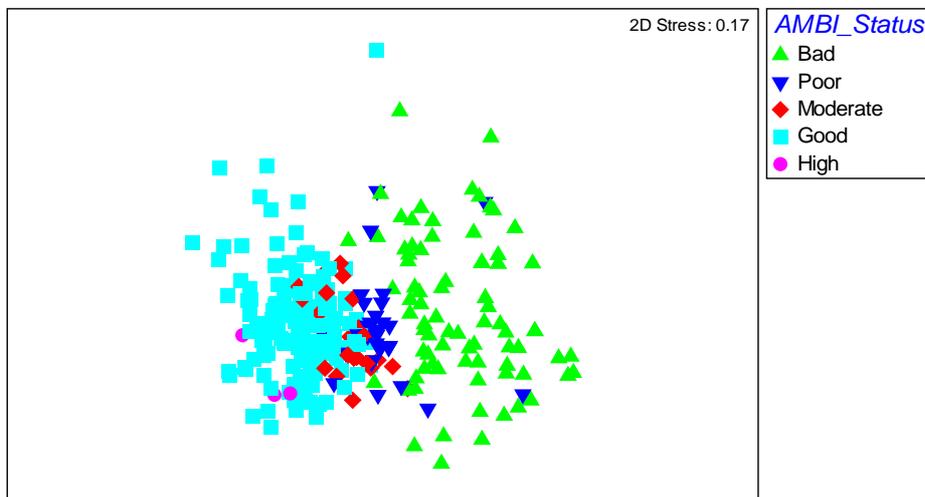*Figure 12. MDS plot of 295 samples based on abundances of phyla.*

Figure 13. MDS plot of 295 samples based on abundances of mayor phyla (Crustacea, Annelida, Mollusca, Echinodermata, Nematoda) only.

Assessing the potential to reduce the infaunal species list required to give an indication of stress in sediments.

The potential for reducing the infaunal species list required to give an indication of stress in sediments is very great. Multivariate analyses based on a subset of less than 3% of the taxa recorded at some stage in the surveys used to construct the database produce similar (or possibly better) results than analyses based on all 1027 species. Similarly, major trends in stress in sediment are revealed by analyses based only on identification to, and counting of, 5 major phyla.

To deliver this potential, and to convert it into a potential for savings in resources, a number of factors need to be addressed.

1. There is little potential benefit in restricting monitoring requirements to a subset of taxa (e.g. the 28 species identified by BVSTEP) if the effort required to detect, select and identify them is about the same as the effort required to identify everything in samples. With this in mind, there appears to be little potential saving in employing a reduced species list containing a taxonomically diverse set of taxa. To determine whether taxa in samples are those that belong to the reduced set, or something else, requires the same level of taxonomic expertise required to identify all taxa. All

taxa still need to be examined to determine whether they belong to the reduced set. The assumptions that by using a reduced species list savings in time may be made, and less expertise required, are unlikely to be true. We consider it far more likely that a focus on higher taxonomic levels (up to the level of phyla) will deliver savings in time, and reduce requirements for expertise. This approach has the added benefit of reducing the potential for taxonomic artefacts to influence assessments.

2. There is little potential benefit in restricting monitoring requirements to a subset of taxa if current methods of assessment are to be maintained. For analyses based on a reduced species set to be of use in the current monitoring framework it must be that assessments based on the full data are unlikely to lead to a different interpretation of the status of a site to that derived from the reduced list. For this to be the case there has to be close agreement in patterns of species numbers, Shannon diversity and ITI derived using the full data and the reduced set.

For Shannon diversity (Fig. 14) it is clear that although there is a reasonable overall relationship between the index calculated from the taxa selected by BVSTEP there is considerable scatter about the line. Thus criteria such as 'must be at least 60% of reference value' are unlikely to be delivered consistently by samples identified according to the two lists. For example, a sample for which Shannon calculated from the full list has a value of 2 to 2.5 could have a value calculated from the reduced list anywhere between 0.4 and 2.5.

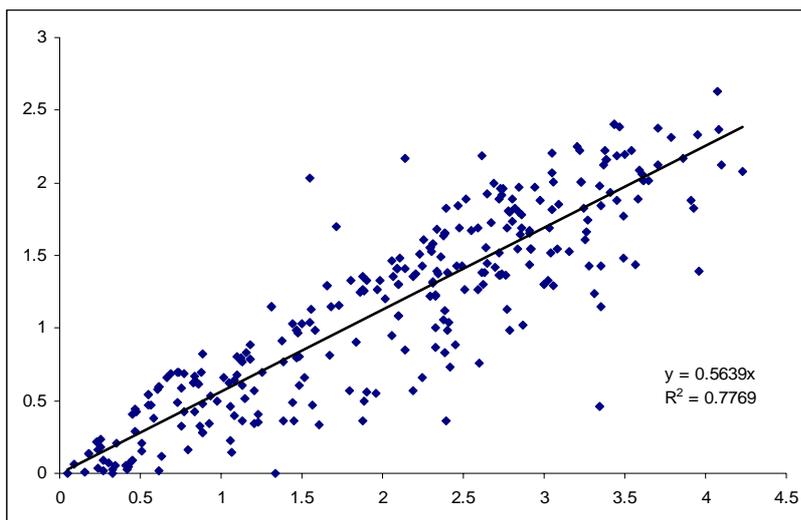The chart contains the equation:

$$y = 0.5639x$$
$$R^2 = 0.7769$$

Figure 14. Shannon diversity calculated using the 28 species selected by BVStep (y-axis) plotted against Shannon diversity calculated using all taxa (x-axis).

The situation is even worse using a reduced list based on the most abundant species (Fig. 15) as the relationship is much weaker and the spread of points about the line is much greater.
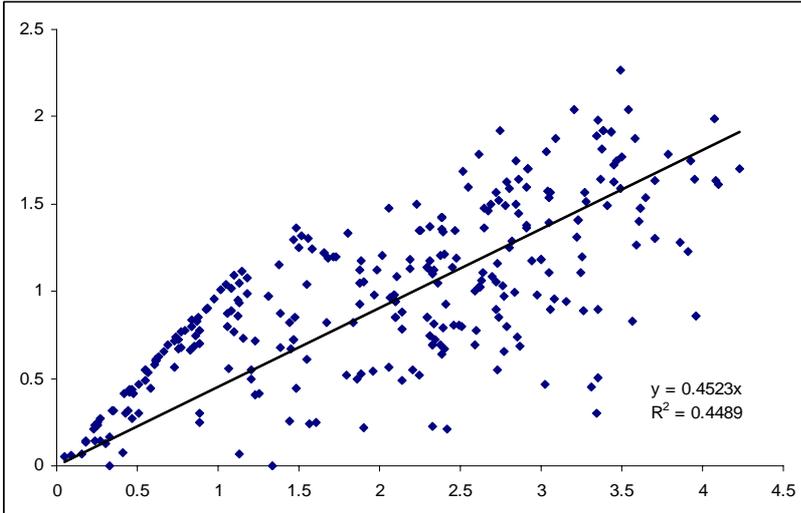
Figure 15. Shannon diversity calculated using the 28 'most important' species based on abundance (y-axis) plotted against Shannon diversity calculated using all taxa (x-axis).

For ITI a somewhat different pattern emerges. Samples with high ITI scores based on identification of all taxa tend to have higher estimates based on the BVSTEP list (Fig 16) and samples with lower scores from the full list have even lower scores using the reduced list. Having selected species that 'most closely match' the overall gradient associated with organic enrichment this should not be surprising, as the overall gradient in the data is now 'stronger' in the reduced list. This will tend to make assessments more conservative, as it is more likely that a criterion such as 'must be at least 50 % of reference value' will be failed.
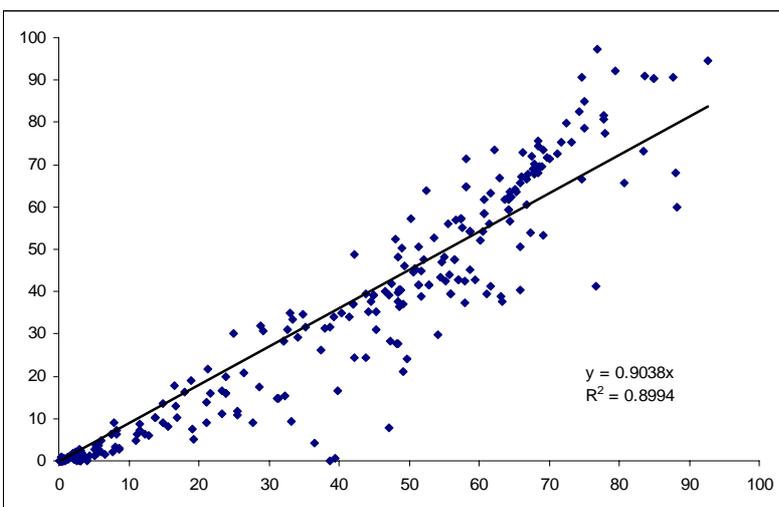

Figure 16. ITI calculated using the 28 species selected by BVStep (y-axis) plotted against ITI calculated using all taxa (x-axis).

While a similar trend is apparent using a reduced list based on abundances (Fig. 17), there is again more scatter about the line. Thus a station with an ITI score of 60 to 70 could have a score of 0 to 80 in an assessment based on this reduced list.
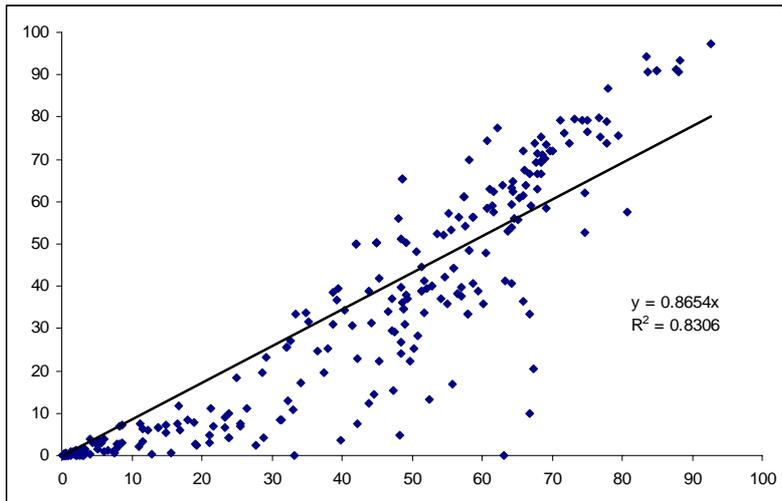
Figure 17. ITI calculated using the 28 'most important' species based on abundance (y-axis) plotted against ITI calculated using all taxa (x-axis).

Conclusions

It is very clear that there is great potential to develop a monitoring and assessment programme which can deliver statistically robust assessments of the biological status of sediments in the vicinity of aquaculture installations, with considerable reductions in the level of taxonomic discrimination and effort required. This will require the development of new sediment quality assessment criteria, as the existing ones cannot be delivered using a reduced list in such a way as to be reliably consistent with previous assessments.

There is considerable 'overengineering' in the current assessment methodology. Although 'replicate' samples are collected, this replicate information is not used in any sensible way in the course of assessments, other than to decide whether conditions are azoic close to cages. It needs to be considered whether 5 samples per station are necessary, as a reduction to, for example, 3 per station would certainly reduce the amount of effort and time required to analyse the samples. Alternatively, a suitable statistical assessment methodology which actually uses the replicate information could be developed. This would allow a probabilistic approach to assessment, with parallel assessments of the probability of error and a proper determination of the number of samples actually required to deliver robust assessments.

The current assessment procedure considers each survey independently. As demonstrated in this report there is much to be gained by considering each survey within an overall analysis based on information from previous assessments. This allows a robust intercomparison between surveys, and allows the erection of criteria based on knowledge of the actual relative effects of aquaculture activities rather than on conceptual (or numerical) models which are several steps removed from reality. Such an approach is required if management is to be adaptive, learning from experience. To deliver such an approach the database structure, and data-entry quality controls, should be improved and tailored. We have gone a considerable way towards developing what is needed in this project, but to deliver an operational system would require further investment.

While the focus of all of the assessment work has been abundances of organisms, arguably a better indication of the ecological status of macrobenthic communities is to be gained by examining biomass. The availability of abundance data alone precludes the application of a number of indicators of community stress that the addition of biomass data would facilitate. It is a relatively small additional step, once the fauna has been sorted into species or higher taxa, to obtain the

blotted wet weight of each taxon, e.g. on a top-loading balance. In particular, two additional analytical techniques that utilise this information would be of value in assessing fish farming impacts on the macrobenthos. The ABC (Abundance-Biomass Comparison) method (Warwick, 1986) involves the plotting of separate *k*-dominance curves for species abundances and species biomasses on the same graph and comparing the forms of the two curves relative to each other. There is clear pattern of change in such graphs associated with organic enrichment and, unlike the methods currently employed for assessment, comparisons between sites are not required to assess the status of a particular site. As with the analysis of species abundances, the method relies on a painstaking and time-consuming (and hence costly) analysis of samples in which all the species must be separated, counted and weighed. However, the ABC method has also proved to be encouragingly robust to analysis at the family, or even phylum, level for macrobenthos and very little information appears to be lost. A second approach to the use of biomass and abundance data for the assessment of disturbance to macrobenthic assemblages is phylum-level meta-analysis (Warwick & Clarke 1993). This can be used to compare the degree of disturbance at different sites, and to place each on a continuous comparative scale. This method only requires knowledge of the proportions of biomass and abundance within phyla at each site.

The acquisition of information about changes in benthic assemblages around fish farms for environmental monitoring is perceived to be time consuming, expensive, and to require expertise. It is true that the nature of the changes, conforming to a well known succession model of organic enrichment, and the nature of the community structure in marine sediments, with high levels of structural redundancy, makes it possible to focus effort with consequent savings in time and effort, and using less expertise. This will, however, require further work.

Recommendations and suggestions for future work

1. That consideration be given to developing a robust assessment methodology based on the abundances of higher taxa across large numbers of surveys. This development should include an assessment of the statistical power of different techniques to detect particular changes in the benthos associated with organic enrichment, and an assessment of the sampling requirements actually needed to deliver assessments. The focus should be on developing an assessment regime that delivers simple assessments for the minimum investment, but at the same time is ecologically defensible and efficient.

2. That consideration be given to further developing the database structures and procedures developed in this project to deliver a system to underpin the new assessment methodology, which will facilitate combined analyses of data from different surveys.

3. That consideration be given to requesting biomass data (preferably at the species level, alternatively at the phylum level) in addition to abundance data from those delivering survey information, for a limited period of time (say one year). This information should then be used to develop a robust methodology for assessing stress which does not rely on inter-station comparisons but which uses combined information from many surveys. This would have a minor short-term cost implication, which should be addressed, but the potential benefit is to move away from complete taxonomic discrimination of all organisms in samples.

4. That consideration be given to incorporating AMBI into a new assessment framework. This index is to be preferred to ITI as it is based on sensitivities of taxa gleaned from large numbers of studies across Europe. The potential for modifications of the index focusing on different aspects of community structure is recognized, and these should be assessed in relation to the effects of

aquaculture to determine which modification delivers the most appropriate information for the least effort. This work should also consider developments with respect to the implementation of the Water Framework Directive to minimize duplication of effort.

References to published material

This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project (the box below will expand).

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