



Program participants: - Agip - Conoco Phillips - Exxon Mobil - Hydro - Petrobras - Shell - Statoil - Total



9296 Tromsø Tel. +47 77 75 03 00 Faks +47 77 75 03 01

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Sammendrag / Summary:

In connection with the development of an EIF (Environmental Impact Factor) for drilling discharges, Akvaplan-niva has carried out an evaluation of different statistical tools that are used to analyse changes in benthic communities. The statistical approach chosen must have the following characteristics: high sensitivity and objectivity, applicable for modelling and impact assessment, recognised and accepted by the regulatory authorities and the scientific community. It is concluded that CDI fullfills most of the given criteria well, however, it is recommended that further experience is gained with use of the method under varying environmental conditions.

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Prosjektleder / Project manager

Helde C. Trannum

Kvalitetskontroll / Quality control

Dohn

Hilde Cecilie Trannum

© Akvaplan-niva

Salve Dahle

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## 1 Introduction

## **1.1 Introduction to benthic communities**

Biological communities are generally comprised of a large number of species. Changing environmental conditions are often followed by a change in the structure of these communities; some species may disappear or decrease in abundance, while other species may increase in abundance or establish themselves. With regard to pollution a typical response is a decrease in the number of sensitive species, increase of tolerant species and a corresponding reduction in the biodiversity. Soft-bottom communities are particularly well suited to study the effects of environmental changes with regard to human activities as the fauna to a large extent is characterized by immobile species that are relatively long-lived. The composition of the fauna therefore reflects the environmental conditions well.

From the petroleum industry's perspective, benthic faunal communities have two main uses. Firstly, these are a major part of the ecosystem and a food source for bottom-feeding fish and marine mammals. Secondly, by their digging and feeding activities, these animals function in exactly the same way as earthworms do in gardens in that they mix the sediments, aerating them and helping to promote natural break-down processes.

Figure 1 illustrates the processes and changes in faunal communities in marine sediments, along a gradient of increasing organic enrichment (such as by oil). In stable, undisturbed sediments, usually many different kinds of animals are present, although each generally is represented by relatively few individuals. There usually is a mixture of deep-burrowing animals and smaller ones living near the surface. Some of the deep-living animals can be very long-lived. With an increased organic input, some kinds of animals thrive, and increase in number, whereas many other forms die out from the community. The burrowing depth also tends to decrease. With ever increasing organic input, the diversity and bioturbation (sediment mixing) activities of the community decrease, until only bristleworms remain. With increased pollution beyond this level, even the most "resistant" bristleworms disappear, and the sediment becomes anoxic. Usually, a whitish layer of sulphate-reducing bacteria develops on top of the sediment, under which is a black layer containing hydrogen sulphide and/or methane gas.

Using this knowledge of benthic faunal response to organic input allows us to check the status of the sediments around industrial activities at regular intervals and to monitor change. In turn, this allows remedial actions to be taken (preferably before the conditions are severely affected).

In order to analyse and assess changes in the communities a wide variety of different methods have been developed. Some of these methods are statistical, while others are qualitative. In relation to the monitoring surveys of the petroleum installation in Norwegian waters, it is required by the Norwegian Authorities to use 10 parameters for assessing the status of the benthic communities (Appendix 1 to "Aktivitetsforskriften").



Figure 1. Schematic illustration of changes in the sediments and bottom fauna along a gradient of increasing organic pollution (modified after Pearson & Rosenberg 1978). This principle still is used as a major tool for marine environmental monitoring.

## 1.2 Aims of the project

The development of an EIF for drilling discharges, which aims to come up with an integrated model system to perform environmental risk analyses for drilling operations, will partly be based on the assessing of *in situ* changes in the benthic communities, with regard to synthetic and water based muds. Here one can take advantages of the enormous amount of data arising from the monitoring surveys of the petroleum installations and also the experience of different statistical methods used in such surveys. In this context it is important to be aware of the fact that the closest stations used in the monitoring are placed 250 m away from the installation, while the EIF approach will also have to focus on closer stations. Further, the most pronounced effects recorded in the offshore monitoring arise from the earlier discharges of oil based drilling mud. Nevertheless, it is of importance for the acceptance of the EIF for drilling discharges that the chosen concept convincingly takes into account environmental knowledge gained from more than 15 years of standardised monitoring surveys in the vicinity of oil and gas installations.

The project has following aims:

- Evaluation of potential statistical methods including those required by the HSE regulations and other "newer" methods like e.g. CDI.
- A short description of the different methods, with regard to sensitivity, advantages and disadvantages, in addition to examples of use if possible.
- Evaluate which input data are being used by the different methods and the possibility to supply such data.
- Conclusion and recommendation for statistical method(s) to be used in the ERMS program for description of seafloor disturbances.

# 2 Norwegian HSE regulations for evaluating chemical pollution

As important as the monitoring of the biological communities is the monitoring of the chemical pollution. In the Norwegian sector it is required to measure total hydrocarbons (THC), selected hydrocarbons (NPD, PAH and decalines), olefins, ether, ester and the metals barium, cadmium, copper, lead, mercury, chromium and zinc. Other components may also be analysed, depending on the drilling history and the type of drilling fluid. For THC and metals there will always be a natural background level in the sediments, which has to be taken into account when evaluating the measured concentrations. Also the grain size is analysed on each station.

On the basis of the concentrations of the compounds mentioned above, the so-called LSCvalue (Limits of Significant Contamination) is calculated. This value is used to test whether the observed values on the other stations indicate significant contamination or not. From this, one can estimate the magnitude of the contaminated area. Prior to the calculation of the LSCvalue the multivariate method PCA (Principal Component Analysis, see below) is conducted on the chemical data from the regional- and reference stations from both the actual year and the previous survey to clear out whether the region should be divided into sub regions. Thereafter average background levels are calculated for the whole region, for eventual sub regions and for the single reference stations. Based on this, the values representing significant contamination is assessed.

PCA (Principal Component Analysis) is a method used to visualise the degree of intercorrelation between the measured environmental variables. As the environmental variables often are highly inter-correlated, a PCA can be expected to do an adequate job of representing these variables in two dimensions, where the first axis represents the largest part of this variation.

The concentrations in the sediment do not alone give information on the degree of impacts on the biota. Thus, chemical analysis is coupled with faunal analysis to achieve a holistic perspective on both the fate and effects of the contaminants.

## 3 Summary of methods for analysing changes in benthic community structure

Statistical methods for analysing changes in community structure fall under the three general headings of univariate, graphical/distributional and multivariate. Also qualitative methods are used, where experienced biologist assess the status of the communities on the basis of the abundance of indicator species and/or functional groups, for instance.

The parameters used by the offshore monitoring are number of species/number of individuals, diversity index (Shannon-Wiener, H'), evenness (Pielou's measure, J), expected number of species per 100 individuals ( $ES_{100}$ ), species-area curves, ten most dominant species at each station ("Top 10"), clustering analysis and multidimensional scaling. Other analyses, e.g. canonical correspondence analysis (CCA) and Bio-ENV are also frequently used. Here these methods are presented, together with other methods considered relevant with regard to an EIF approach.

## 3.1 Univariate methods – diversity indices

#### 3.1.1 Introduction

As the name implies, univariate methods integrate the information in the species list to a single variable, for example a diversity index. The idea is that this variable can give information of the status of the community, and then samples can be compared to look for gradients in faunal effects.

The simplest form of univariate measures is the number of individuals and species (species richness) in a sample. These are important characters of a sample, but do not give any information on how the individuals are distributed between the different species. Diversity indices, on the other hand, are combined measures of the number of species present and the distribution of individuals among these species. There are several different indices, where some mainly focus on the species richness, while others mainly focus on the distribution of individuals between the species. Diversity indices are classical in environmental monitoring as pollution typically is followed by a change in the composition of a community. None of the methods are species-specific, and two communities with a completely different taxonomic composition can therefore have the same indices. The diversity indices assume a random distribution of individuals in a sample, which is seldom the case in natural communities, and also that species/individuals relationships are similar in the communities being compared (Gray & Pearson, 1982).

There are natural gradients in species richness on several scales, relating to e.g. latitude on a global scale, depth and type of environment (fjord/coast/open sea) on regional scales and spatial heterogeneity (e.g. grain size) on a smaller scale. Based on that, it is difficult, if not even meaningless, to develop universal criteria for diversity indices. Indeed, when first proposed, diversity indices were used for quantifying ecological changes, e.g. to compare the diversity of different-sizes islands or tropical to boreal regions (MacArthur, 1957; 1965). As Gray & Pearson (1982) point out such indices have appeal to engineers and legislators, but obscures much information that may be useful in delimiting effects of pollution and may mislead an inexperienced observer by oversimplifying complex situations. However, in limited, homogenous areas diversity measures can represent a meaningful way of assessing pollution-induced changes in space and time. In this context it is also important to be aware the fact that slightly disturbance may in fact increase diversity ("intermediate disturbance hypothesis; Connell, 1975), which also complicates the use of diversity indices.

#### 3.1.2 Description of diversity indices

In the HSE regulations in Norway today the Shannon-Wiener and Hurlbert's diversity indices are used, together with the Pielou's evenness index.

The Shannon-Wiener index (Shannon & Weaver, 1949) takes into account both the number of species and their relative distribution, but the index is considered most sensitive with regard to the distribution of individuals between the species. The index usually ranges between 0 and 4, where a low value indicates a species-poor community and/or a community dominated by a few species, and a high value indicates a species-rich community, both with regard to species richness and the distribution of individuals between the different species.

Hurlbert's diversity index (Hurlbert, 1971), a modification of Sanders rarefaction technique (Sanders, 1968), is originally a graphical method for presenting diversity. It is based on how many species are expected to be found when randomly taking a subsample of the original

sample. From this it is possible to present curves with the expected number of species found when collecting an increasing number of individuals, and to make comparisons of different samples and communities. The diversity index is thus independent of the sample-size, and makes it possible to compare samples having different total abundance directly. For small samples the method tends to overestimate species richness, since the method is based on an even distribution of individuals among the species, which is seldom the case (Gray, 2000; 2002).

Since diversity changes both with changes in the number of species and with changes in the relative abundance of species, diversity indices are usually accompanied by some measure of evenness, most often the Pielou's evenness index. Evenness describes the relative distribution of individuals among species. Thus, a low evenness value indicates that one or a few species dominated the sample quantitatively (i.e. a high degree of dominance), while a high value indicates a more even distribution of individuals among species (i.e. a high degree of co-dominance of species where no species are particularly abundant).

In the vicinity of oilfields there is often a gradient in diversity with reduced values closest to the installations. A significant correlation between reduced diversity and high concentrations of barium, olefin and ester in the sediments was reported by DNV and Akvaplan-niva (1999). When it comes to water based drilling muds, mainly composed of benthonite clay and barite, the impacts seem to be restricted, and limited to a very small radius around the installations. Barium is generally considered to have no toxic effect on the fauna, although some studies have indicated toxic effects of barium (Cranford *et al.*, 1999). The main effects of water based muds are assumed to be related to the smothering effects of the particles. Olsgard (1993) found a significant correlation between sediment content of mine tailings and Shannon-Wiener diversity, which strengthens the potential for using the Shannon-Wiener diversity index in the EIF-approach. The sedimentation rates influencing the fauna were in the order of 4-5 cm per year. However, the sedimentation rate when discharging cuttings may be approximately 1 cm per year closest to the installation, and decrease outwards from the installation (pers. com. Henrik Rye, calculated for the test-field Sleipner Vest Alpha Nord, which is planned to be used as a test case for the development of EIF for sediments).

For readers having a special interest in the calculation of the diversity indices, their formulas are given below:

#### Shannon-Wiener index

The Shannon-Wiener index, H', is given by:

$$H' = -\sum_{i=1}^{s} p_i \log_2 p_i$$

where

 $p_i$  = proportion of the i'th species in the sample and s = the number of species.

#### Hurlbert's diversity index

Hurlbert's diversity index,  $ES_n$ , is the expected number of species in a subsample having *n* randomly collected individuals consisting of *N* individuals and *s* species, and is given by:

$$ES_n = \sum_{i=1}^{s} \left[ 1 - \frac{\binom{N-N_i}{n}}{\binom{N}{n}} \right]$$

where N = total number of specimens in the sample $N_i = \text{the number of specimens of the i'th specie}$ n = the number of specimens in the subsamples = the number of species in the whole sample

The index is usually presented as  $ES_{100}$ , i.e. the expected number of species in a sample of 100 specimens selected at random.

#### **Pielou's evenness index**

Pielou's evenness index (Pielou, 1966), *J*, which is the index used in the offshore monitoring, is given by:

$$J = \frac{H'}{\log_2 s}$$

(symbols as in the Shannon-Wiener index)

This index does not give much information alone, and is only meaningful when presented together with the diversity indices.

#### 3.2 Graphical/distributional methods

In graphical/distributional methods relative abundances and eventually biomass of different species are plotted as a curve, which retains more information about the distribution than a single index. As for the univariate methods, these methods are not species-specific, and two communities having no species in common can therefore come out as similar in these methods.

There are several methods for giving visual representations of communities:

#### 3.2.1 Log-normal plots

Log-normal plots (Gray & Pearson, 1982) is the graphical method that is most used. It is based on the fact that the log-normal distribution, where individuals per species are divided into geometric classes, has been found to give good fits to most large samples from heterogeneous communities (Gray, 1981). A typical response of organic pollution is that rare species become less abundant, while some species increase in abundance, which results in a deviation from the log-normal distribution. The log-normal plots are most often presented as a curve where number of species are plotted against number of individuals per species in geometric classes (Class I = 1 individual, Class II = 2-3 individuals, Class III = 4-7 individuals etc.). Curves from undisturbed communities typical start high on the abscissa (class I) and flattens out towards the highest classes, while curves from disturbed communities have a flatter appearance in starting lower on the abscissa and stretching towards the higher classes. In addition to the fact that the plot itself gives valuable information on the disturbance status of a community, the method can also be used for selecting indicator species objectively (Gray & Pearson, 1982). Both moderately common species and common species will generally increase in abundance under organic enrichment. Gray & Pearson (1982) proposed to focus on the moderately common species, where changes are most rapid along a spatial or temporal gradient. The indicator species vary between different areas, and when using the plot one can select species that show a response towards pollution in a specific area.

Rygg (1986) showed that the log-normal distribution was not necessarily suited to detect changes in communities disturbed by metal pollution. A response towards contaminants may be a reduction in both the number of species and individuals, resulting in unchanged diversity indices (Olsgard, 1993). The curve or line will therefore not necessarily show a deviation from the log-normal distribution in such cases, but may instead be lowered in relation to the reference curve.

The method is used in the Norwegian offshore monitoring today. Compared to the diversity indices, this method is considered more sensitive as it retains more information on the community structure. However, in only slightly disturbed communities it is not always easy to conclude about eventual effects when using this method alone. Furthermore, with regard to the EIF-approach, it suffers from the fact that it is not easily quantified.

## 3.3 Multivariate methods

Both the biological communities and the environmental variables have a multivariate nature, which means that the information is not retained in a single coefficient. The comparison of communities is performed on the basis of both the identity of the component species as well as their relative importance in terms of abundance. Thus, in comparison with univariate and distributional techniques, more information is retained, but on the other hand, there is not calculated a single coefficient relating to the status of the communities. The focus of the multivariate techniques is therefore how the samples relate to each other and to reference stations assumed to be undisturbed, while the focus of the univariate and distributional techniques is the status of the communities based on fixed criteria.

#### 3.3.1 Cluster-analysis

Cluster-analysis uses similarities between samples and groups of samples to build a dendrogram, where samples with similar structures (distribution of individuals between species) successively are linked and plotted on a scale that reflects the similarity between them. The species data are not used directly, but instead a similarity coefficient (Bray-Curtis index) based on the species data is calculated between every pair of samples, which is then used in the clustering procedure. In order to reduce the discrepancy between large and small abundance values and to down-weight the importance of the dominant species so that the less dominant, and even rare species, play some role in determining similarity of two samples, the raw data are usually transformed before the calculation of the Bray-Curtis index. Double square root transformation is most commonly used.

#### 3.3.2 Multidimensional Scaling (MDS)

Multidimensional scaling is an ordination technique, where samples are mapped according to similarity. The plot is presented in two and/or three dimensions. The distances between samples on the plot attempt to match the corresponding dissimilarities in community

structure; nearby points have very similar communities, while points far apart have few species in common or the same species at very different levels of abundance. The so-called stress-value is used to describe how well the plot reflects the original similarity between the samples. For calculating the similarity, the Bray-Curtis index is used, based on transformed data, as for cluster-analysis.

Both cluster-analysis and multidimensional scaling are popular when evaluating effects of pollution because they provide simple illustrations on the similarity between different communities that are easily understood by non-biologists. They are used globally to assess effects of pollution, because of the power to pick out communities that differ from the control communities. However, the methods do not have a scale relating to pollution status. The analyses are therefore most often combined with univariate measures of community structure. Another drawback with the methods is that one cannot know whether isolated stations have a faunal composition that is significantly different from control stations. This is particularly the case for MDS, which does not have a scale. Thus, two community assemblages having a different degree of variation can therefore produce the same plot, as the only parameter in MDS is the relative differences.

#### 3.3.3 Correspondence analysis (CA)

Correspondence analysis is another graphical method for analysing data. An important property of CA is that joint maps can be constructed of both species and stations, which is different from the MDS. However, the method is not in use in the Norwegian monitoring today.

A fully description of the method is provided in Greenacre (1984; 1993), and here we only give a brief introduction to its rationale.

As the MDS, CA produces a plot showing the similarity of stations. However, the raw data are used directly in this method, instead of going through the Bray-Curtis similarity index. As for MDS and cluster analysis, the data are transformed prior to the analysis. The idea is to find a theoretical environmental variable that best explains the species data. In general, the first ordination axis of CA represents a constructed theoretical variable that gives the largest dispersion of the species scores, i.e. is assumed to explain the largest amount of the community variation. Further axes can be constructed which are constrained to be uncorrelated with the initial axis but also maximise the dispersion of species scores. In this way one can reduce the dimension to a few axes that represent as much as possible of the variation in the species data. Usually two dimensions are used.

Considered geometrically, each species can be thought of as a point in the multidimensional space defined by the stations, and each species is given a weight (called a "mass" in CA) proportional to the overall abundance of the species. Similarly, each station can be thought of as a point in the multidimensional space defined by the species, and each station receives a mass proportional to the number of individuals counted at that station. Dispersion is defined as the weighted sum-of-squared distances of the species points (or, equivalently, of the station points) to their average. This measure of dispersion is termed "inertia". *Inertia* in the context of frequency data is analogous to *variance* in the context of measurement data. Inertia is weighted variance, where each species point (or station point) is weighted by its mass, and the distances are all chi-squared distances. Two-dimensional maps of the species or station points can be obtained by looking for the optimal plane, and so on.

The multivariate methods described above only give information on how the different samples are related to each other, and do not say anything about the actual status of the

communities. There have been some attempts to make multivariate measures of community stress. One of these is CDI, which is discussed in the section below.

#### 3.3.4 Community Disturbance Index (CDI)

CDI (Massart et al., 1997) is a variant of a multivariate F-test for quantitatively classifying stations as disturbed/polluted or undisturbed, and was introduced as a quantitative multivariate alternative to the diversity indices, which is a less sensitive measurement of environmental stress (e.g. Gray et al., 1990). The basis for the analysis is to establish reference stations assumed to represent the natural variation in the area under study. Typically, prior knowledge is utilised in order to choose the reference set. The stations furthest away from the source of disturbance are the least affected and are thereby assumed to represent the natural variation in the area. However, if some of the reference stations turn out to be polluted, the analysis will automatically detect these as statistical outliers in the reference samples. When these stations are modelled, the natural variation in the community is modelled with local PCA (principal component analysis, see chapter 2). By local modelling, the well know "horse-shoe" associated with PCA analysis will not cause any problem. The CDI is then calculated, telling whether the stations can be classified together with the reference stations or not. By a multivariate F-test the CDI value that defines pollution (by 95% confidence interval) is given the value of 1. All samples with natural variation in the benthic fauna will be classified as samples with CDI values lower than 1. Similarly, stations with CDI values higher than 1 are classified as disturbed. Degree of disturbance is ranked according to a quantitative scale. The magnitude of the number for some oil fields has been shown to correlate very well to the concentration level of the chemical pollutants in the sediments as well as the degree of disturbance in benthic communities achieved through regular offshore monitoring (PRS, xxx; Flaaten, 2002).

Until recently CDI has not been available for independent use and evaluation. This obstacle is now overcome, and the method is commercially available. CDI is not part of the HSE regulations, and the method is not used by any of the major consultants contracted to carry out the environmental monitoring.

Thus there is a strong need to evaluate CDI on a wider scale, under different environmental conditions on the Norwegian shelf, but also in a wider geographical context. The method has to be tested both with respect to concentration of chemicals in the sediments and the benthic communities analysed by standard statistical methods. During the evaluation the methods for selection of reference stations have to be tested, ordinations of weight factors to the measured concentration of chemical elements in the sediments, how CDI relates to other variables (oxygen content, organic content of sediment, grain size etc) and to the discharges of different type of drilling fluid etc.

The test programme may be carried out at selected oil fields using existing data from the Norwegian offshore monitoring programmes, but since the closest station on most fields is 250 or even 500 m away from the platform, also especially designed programmes should be included in order to get data from locations closer to the platform.

This study is necessary in order to reach an acceptance for the use of CDI within the EIF concept, both towards the regulatory authorities and the scientific community.

#### 3.3.5 Analysis of Similarities (ANOSIM)

ANOSIM is a multivariate method that tests whether *a priori* selected groups of samples, e.g. stations, have significantly differences in faunal composition. The method is considered the multivariate parallel to ANOVA (Analysis of Variance), which is used to test for differences in univariate parameters or indices (Somerfield et al., 2002). As for cluster analysis and MDS-ordination, the method is based on the Bray-Curtis index and the ranked similarities. From this it is tested whether the similarity between groups is significantly higher than within groups.

In comparing the stations in the vicinity of an oil field with the reference station, this method could possibly be used to calculate the size of the disturbed area. This would then be the biological parallel to the calculation of LSC-values (Limits of Significant Contamination) for the chemical variables (see chapter 2). The method does not produce a value on the status of the communities, but it does tell how much the stations differ from the reference station, as the CDI. However, the method does not have a scale, as it is the relative differences that are measured. Another weakness with the method is that it requires that the reference station is representative for the rest of the stations. This can be difficult to assure, as the reference station is this context, several reference stations should be established.

The method has not been used in the Norwegian offshore monitoring.

## 3.4 Relationship between biological and environmental variables

In monitoring context, methods that correlate biological and environmental variables play an important role. Such methods use the species data together with data of the measured environmental variables. However, it is important to be aware of the fact that they only describe how well the environmental variables are *related* to the observed biological pattern. For instance, environmental variables that are not measured, but correlated to measured variables, may also be of significance. Before concluding about a cause-effect relation, the methods should therefore be combined with experiments performed under controlled conditions, in the laboratory and/or in the field. The correlation methods can be used to pick out which variables in a particular discharge that are assumed to have the largest influence on the structuring of biological communities, and that one should focus further research on.

Both the biological and environmental data have a multivariate nature. The fact that the environmental variables usually show a high degree of inter-correlation makes it more difficult to extract the most important variables in relation to the community compositions. To treat all the multivariate biological and environmental data together and then extract the environmental variables that are assumed to be most important for the structuring of the communities, there have been developed advanced statistical tools. In some cases, however, where there are clear univariate gradients, simpler methods can also be useful.

#### 3.4.1 Regression analysis

The simplest way of studying correlations between biological and environmental variables is to perform regression analysis for one biological variable, e.g. diversity or abundance of selected species, and one single environmental variable. From this one can quantify the extent to which the dependent variable (here a biological variable) is associated with the independent variables (e.g. a contaminant). As mentioned above, such methods can be used to point out a significant correlation between reduced diversity and high concentrations of barium, olefin and ester in the sediments (DNV and Akvaplan-niva, 1999). It is also possible to conduct multiple regression analyses where several environmental variables are included. Such methods are not used in the Norwegian offshore monitoring today.

Instead of performing regression analysis on a fauna variable and one single environmental variable, one can use the first axis of the PCA (Principal Component Analysis) ordination of the environmental data.

Regression analysis can be very useful when the biotic data are best summarised by one, or a few, simple univariate measures. In most cases, however, the biotic data is best described by a multivariate summary, where methods designed to treat whole dataset of biological and environmental data together will be best suited.

#### 3.4.2 Superimposing of environmental symbols on MDS-plots

An easy way to visualise the relationship between multivariate biological data and one environmental variable is to superimpose symbols of differing size, proportional to the value of the variable, on the MDS plot of the corresponding biological samples. This method can be very informative in cases were only one environmental factor varies among the investigated samples, like in field studies where there is a gradient in only one variable or in experiments where one tests the response of one particular contaminant in different concentrations. One can also make plots for different variables and then compare these plots to evaluate which variable that is assumed to correlate best with the biological data. The method is not a part of the HSE requirements in the Norwegian offshore monitoring today, but is used by Rogalandsforskning. However, the CCA method described below is considered a more advanced tool to reveal the relationship between the environmental and the biological data, as it treats more than one environmental variable in the same operation.

#### 3.4.3 Environmental variables as supplementary variables in CA-plots

Environmental variables can be added *post-hoc* to the CA (Correspondence Analysis, see chapter 3.3.3) ordination plot by projection. The variables are shown in the plot as arrows. The analysis gives correlations between the environmental variables and the axes. Since the axes in CA are constructed to best explain species data, one can use the correlations to point out the most important environmental variables. This gives the procedure an advantage compared to MDS. The method must not be mixed up with CCA described below.

The method is not a part of the HSE requirements in Norwegian monitoring today.

#### 3.4.4 Canonical Correspondence Analysis

Canonical Correspondence Analysis (CCA) permits a direct comparison of environmental and biological parameters. Here the rationale of the method is briefly explained, but for a fully description of the analysis and the mathematical theory we refer to Jongman *et al.* (1995), Fieler *et al.* (1994) and Greenacre (1993).

The environmental variables that are included in the analysis are selected through a selection procedure. First variables that are strongly correlated to other variables are identified and removed. Then the variables explaining most of the variation of the biological data, i.e. the significant variables, are identified, through a "forward selection" procedure. Geological and chemical variables are log-transformed prior to the analysis to reduce the influence of

outliers. The second step is to analyse the species variance that can be explained by the most significant of the environmental variables listed in the forward selection procedure. The species having the highest inertia (i.e. variance) is considered being most influenced by the selected environmental variables.

The results of CCA are easily interpretable because of the visual (spatial) nature of the analytical result. Stations are plotted in a 2-dimensional space also showing the direction and magnitude of the influence of environmental variables. Species may also be included in the plot, providing information on which species are assumed to be most influenced by particular variables. The station grouping in relation to the most influential environmental variables indicates the degree of dissimilarity between the stations.

The method is not a part of the HSE requirements of the Norwegian offshore monitoring today, but has been used by Akvaplan-niva since 1994.

#### 3.4.5 BIO-ENV

As the name implies, BIO-ENV (Clarke & Ainsworth, 1993) is another method to link community structure to environmental variables. It is based on matching the MDS-plots of the biota to the MDS-plots of the environmental variables. This is done in comparing the Bray-Curtis values for the biota with the Euclidean distance of the environmental variables through a rank correlation coefficient (weighted Spearman rank correlation). The environmental variables or the combination of different variables that give the highest rank correlations, are then assumed to be the most important explanatory variables for the fauna.

An advantage with this method is that is also analyse which combinations of the different environmental variables that give the highest correlations with the variation in fauna, which may provide a more realistic picture on the disturbances, which is usually attributed to more than one factor. However, it does not visualize how stations or species are related to the environmental variables, like the CCA. It can be a useful supplement to CCA in monitoring studies (like in Olsgard, 1995), but is not alone considered having a potential in the EIF approach. The method is used by some companies in the Norwegian offshore monitoring today (Rogalandsforskning and DNV).

#### 3.4.6 Response curves

Response curves are based on plotting abundance data of selected species against a selected environmental variable. Such curves may provide important information on species' response towards specific variables.

Response curves of selected species in relation to increasing THC (total hydrocarbons) are shown in Figure 2. The data originates from 99 deep offshore stations sampled in 2002 (Snorre B, Snorre UPA/TLP, Sygna, Vigdis, Visund, Visund Nord and regional station). It is very clear that the species have different responses along the gradient of increasing THC, with tolerant and opportunistic species to the right in the figure and sensitive species to the left. The small deposit feeding polychaete *Capitella capitata* is a universal opportunist, found in huge densities in organic polluted habitats all over the world (e.g. Pearson & Rosenberg, 1978).



Figure 2. Response curve of selected species in relation to increasing concentrations of THC (total hydrocarbons). Akvaplan-niva data 2002. Abbreviations: Clym bor = Clymenura borealis (Polychaeta). Euch sp. = Euchone sp. (Polychaeta), Chaetozo = Chaetozone sp. (Polychaeta), Para jef = Paramphinome jeffreysii (Polychaeta), Thya sar = Thyasira sarsi (Mollusca), Capi cap = Capitella capitata (Polychaeta), Capi sp = Capitella sp. (Polychaeta), Hete fil = Heteromastus filiformis (Polychaeta).

It is important to be aware of the fact that the curves origin from *in situ* data, where other variables, that not necessarily are measured, also may be of importance. The results therefore have to be treated with caution. When one knows that there is a gradient in only one environmental variable, however, the curves are particularly useful. The method is not in use in Norwegian offshore monitoring today.

## 3.5 Qualitative methods

As described in the introduction, the soft bottom communities are particularly well suited to monitor impacts of environmental change, as they are mainly composed of relatively immobile species. The above methods describe how the information contained in the species list can be used statistically to evaluate the significance of such changes, however, the information on changes in the abundances of tolerant and sensitive species, which is the mechanisms beyond these "statistically visible" changes in the community, is indeed of importance when assessing pollution-induced changes. We have here used the term qualitative methods to classify methods where the experience and training of the biologists is of importance with regard to the assessment of the status of the communities. However, although these methods intuitively sound subjective, it is possible to develop objective criteria and to quantify the methods so they can be used in a classification system and eventually a modelling.

#### 3.5.1 "Top ten" and indicator species

"Top ten" list is a ranking of the ten most abundant species. The % abundance and average ranking position for each species are also recorded. From this one gets information on both the composition of the communities and also on how the most abundant species dominate the communities. The so-called indicator species are here of special importance.

Indicator species are species that are particularly resistant or particularly sensitive to pollution. These are labelled "positive indicators" and "negative indicators", respectively (Keough & Quinn, 1991). The "positive indicators" are typically opportunists, while the "negative indicators" consist of species occurring relatively frequently in less polluted areas that recede and eventually disappear as the environment gets more polluted. Presence or absence of these indicators may provide a warning signal of the existence of pollution effects. With regard to the "positive indicators", the species are generally the same for physical disturbance as for organic disturbance (Olsgard, 1994). Such species may also be the same for different marine regions. Whether water-based, containing mainly inorganic particles, or synthetic-based, containing organic compounds, is used, should therefore be of minor importance with regard to these indicator species.

In pollution monitoring the most important issue is to use indicators of initial stages of pollution (Gray, 1982). For this purpose the "negative indicators" seem to be better suited than the "positive indicators". Echinoderms, like the bristle star *Amphiura filiformis* typically show a reduced abundance or disappear completely in disturbed sediments. Even within fairly discrete geographical areas in the North Sea, there are no consistent patterns of sensitive indicator species that can be suggested, according to Olsgard (1994). From this he concluded that it was not realistic using only indicator species to detect initial effects of pollution therefore. However, on the single field it should be possible to suggest such indicator taxa, which can be used in addition to other methods, eventually.

#### 3.5.2 Functioning of benthic communities

Soft-bottom communities are comprised of several species having different functions. Some species filter particles from the water column, some eat sediment particles on the sediment surface or in the sediment, while others feed on living or dead organisms. Furthermore, they differ in motility and the so-called bioturbation activity, i.e. reworking of sediments. A change in the environmental conditions, like increased input of organic particles, is usually followed by a change in the composition of the functional groups, and typically a reduction in the functional diversity (Pearson & Rosenberg, 1978). The evaluation of changes in the composition of functional groups therefore provides relevant information with regard to pollution status.

Functional redundancy is an expression used to indicate that many species can perform the same function. If one species is affected by an environmental change, another species can fill this niche. The effect of a contaminant will then not be seen on the function of the community, only on its structure (Tilman, 1997). With regard to species on higher trophic levels, i.e. foragers on the benthos, the functioning of the benthic communities is more

important than the structure. The use of functional grouping in monitoring context can therefore provide information that is more ecological relevant than the use of indicator species, but on the same time, however, the method is not necessarily as sensitive as the indicator species. Furthermore, it is not clear how contaminants influence on the functional composition. The method is not used in Norwegian offshore monitoring today. Thus this method will not be evaluated any further.

#### 3.5.3 Faunal distribution patterns

In order to get information on the size of the area surrounding a given offshore field that is influenced by the discharges, Akvaplan-niva has developed and used a classification system, where faunal distribution patterns are described by comparing fauna data (total abundance, species richness, diversity indices and abundances of the dominant species) and by using multivariate classification and ordination analyses. Four categories are used to classify the communities:

- <u>Group A</u>: Undisturbed fauna, usually with low dominance and containing a wide range of species from different taxonomic groups, including bristleworms, molluscs, echinoderms and crustaceans. Species that characteristically appear in disturbed sediments are absent or occur in very low numbers.
- <u>Group B</u>: Slightly disturbed fauna, generally with somewhat higher dominance, but still containing a wide range of species from different taxonomic groups. The faunal composition is slightly, but noticeably, changed in relation to adjacent and/or comparable stations with equivalent environmental conditions. Species that characteristically appear in disturbed sediments, including bristleworms and molluscs, show an increase in numbers, but are not usually dominant.
- <u>Group C</u>: Distinctly disturbed fauna, generally with higher dominance and lower number of species. The faunal composition is distinctly changed. Species indicative of disturbed sediments, including bristleworms and molluscs, occur among the dominant species, and echinoderms are rare.
- <u>Group D</u>: Highly disturbed fauna, totally dominated by small detritus-feeding bristleworms and particularly tolerant bivalves with symbiotic bacteria. Echinoderms and crustaceans rare or absent. Low number of species.

Natural variation can affect several of the faunal parameters within each group. The classification is therefore based on a holistic interpretation of the fauna. For example, at stations with undisturbed fauna, certain species can be present in high numbers, resulting in a lowered diversity. Amongst others, this applies to the bristleworms *Euchone* sp., *Myriochele oculata* and *Owenia fusiformis*. The distribution of these species has shown great variations, both in time and space, independent of the petroleum activities in the area.

The most usual species that appear in increased abundances in contaminated/organically enriched sediments are the bristleworms *Capitella capitata*, *Chaetozone* spp., *Ophryotrocha* sp. and *Ditrupa arietina* as well as the molluscs *Thyasira sarsi*, *T. flexuosa* and *Lucinoma borealis*. Echinoderms, such as the brittle star *Amphiura filiformis*, decrease in abundance or disappear under such conditions.

Although the method is based on fixed criteria, the person performing the classification needs to use his or hers experience and opinion. The classification is therefore subjective, and may

differ slightly between persons and/or institutions. However, as it combines different methods it gives an integrated view on the environmental conditions on a particular location.

Today, only Akvplan-niva uses this method in offshore monitoring. It may be against this method because of its subjectivity and lack of "transitional values" between the groups. However, it should be possible to develop field-specific fixed criteria to base the classification on.

## 4 Discussion

## 4.1 Use and comparison of the different methods

An evaluation of CDI has been performed for data from Vigdis (1999) and Jotun (2000) by Team consulting. Akvaplan-niva has got permission for using the results in this report. Jotun is drilled with water based drilling mud, and in 2000 there was no disturbance gradient in the fauna. On the other hand, Vigdis is drilled with synthetic drilling fluids, and there is a strong gradient in disturbance in the fauna. To give examples and to compare the different methods we have therefore used the Vigdis (1999) data consequently. The station positions are shown in Figure 3. There are three drilling wells on the field, and therefore three possible gradients with regard to faunal disturbances.



#### Figure 3. Map of stations on the Vigdis oilfield.

Univariate measures from Vigdis, 1999, are given in Table 1. At station VGIT-02 the Shannon-Wiener index is only 3,1, while at all the other stations it is over 4.

St. nr.	Distance	No. ind,	No.	H'	J	$ES_{100}$
	(m)		species			
VGIT-01	1000	2807	156	4,6	0,64	34
VGIT-02	250	1504	81	3,1	0,49	21
VGIT-03	500	3599	155	4,5	0,62	34
VGIT-04	1000	3539	166	4,5	0,61	33
VGIT-05	250	3437	176	4,6	0,62	33
VGIT-06	500	2590	150	4,6	0,64	33
VGPT2-07	1000	2336	154	5,3	0,73	40
VGPT2-08	500	2758	174	5,1	0,69	39
VGPT2-09	250	4219	147	4,7	0,65	34
VGPT2-10	500	4348	158	4,3	0,59	29
VGPT2-11	1000	3249	146	4,3	0,60	31
VGPT2-12	2000	1496	140	5,1	0,71	40
VGPT2-14	1000	3632	142	4,1	0,57	30
VGPT2-15	500	3090	153	4,8	0,66	34
VGPT2-16	250	3536	160	4,9	0,67	35
VGPT2-17	500	3444	159	4,5	0,61	31
VGPT2-18	1000	2010	158	5,5	0,75	43
VGPT1-21	500	2853	168	5,1	0,69	38
VGPT1-22	250	3345	153	5,2	0,72	38
VGPT1-23	500	3003	178	5,6	0,75	43
VGPT1-24	1000	3030	150	4,8	0,66	33
VGPT1-25	2000	2725	134	5,0	0,70	35
VGPT1-27	250	3086	170	5,5	0,74	42
VGPT1-28	500	2752	153	5,2	0,72	39
VGPT1-19	2000	1797	158	5,4	0,74	41
ref.						

Table 1. Univariate measures at Vigdis, 1999.

A plot of species within geometrical classes from some selected stations from Vigdis, 1999, is shown in Figure 4. The method clearly indicates that the station VGIT-02 is highly disturbed and that the stations VGIT-03, VGIT-04, VGPT2-09 and VGPT 2-10, and possibly VGPT 1-24, are slightly disturbed.



Figure 4. Plot of number of species within each geometrical class for some selected stations at Vigdis, 99.

Figure 5 shows the dendrogram from the cluster-analysis based on station data from Vigdis, 1999. Station VGIT-02 has a faunal composition that clearly separates this station from the other, at a similarity of less than 50%. The stations VGPT1-22 and VGPT2-09 are also, though to a less degree, separated in the plot.



Figure 5. Dendrogram from the cluster analysis based on station data from Vigdis, 1999.

MDS-ordination of the data from Vigdis is shown in Figure 6. Again the station VGIT-02 is separated from the other stations, which to a large degree camouflage how the other stations

are related to each other (upper plot). To investigate this in more detail the ordination was performed with VGIT-02 excluded. Now the method indicates that also stations VGPT2-09, VGPT1-22 and possibly VGIT-03 are different from the other field stations.



Figure 6. 2-D plot from the MDS analysis based on station data from Vigdis, 1999. Above: all stations, below station VGIT-02 excluded.

The result of the correspondence analysis for the same data is given in Figure 7. Again the station VGIT-02 is isolated. According to the plot, the stations VGPT2-09, VGPT1-22 and possibly VGIT-03 are also disturbed. This is exactly what was concluded from the MDS ordination.



Figure 7. CA of Vigdis, 1999.

ANOSIM was also conducted for the Vigdis (1999) data to look for differences between the reference station and the other stations. The results (not shown) were quite surprising as most stations in fact had a faunal composition significantly different from the reference station. The stations were also significantly different from each other in most cases. Despite of the fact that the fauna was strongly homogenous throughout the unpolluted part of the field (see cluster analysis in Figure 5), it was even more homogenous at each station. Thus the method does not seem to be well suited to pick out disturbed stations, at least not for this dataset. The reason for this is that the method is based on relative differences between communities.

The CDI-values from Vigdis (1999) are given in Figure 8. The station VGIT-02 has a CDI-value of 1,30, which indicates that this station is highly disturbed. Furthermore, the station VGPT1-22 has a CDI-value slightly higher than 1. However, in addition to the stations having a CDI over 1, also the stations VGPT2-09, VGIT-03, and to a lesser degree station VGIT-04, are separated in the method. This finding corresponds well with the results of the other multivariate analyses.



Figure 8. CDI-values from Vigdis, 1999 (data from Team consulting).

CCA for the Vigdis data from 1999 is showed in Figure 9. Four of the nine environmental parameters were significant; depth, total hydrocarbons (THC), cadmium (Cd) and chromium (Cr). 51.1% of the biological variation is explained by the two first axes, which again explain 84,5% of the correlation between the biological data and the measured environmental parameters. Cu and Zn had a high degree of inter-correlation with the other parameters, and were excluded from the analyses.

The first axis showed a gradient from the station VGIT-02 in the positive end to station VGPT1-27 in the negative end, which means that these stations contributed most to this axis. Cr and Cd contributed positively to this axis, while THC and depth contributed weakly negatively to it. Species having high distribution on the first axis are the bristleworms *Capitella capitata, Pholoe baltica* and *Ophryotrocha* sp. As mentioned above, *Capitella capitata is* a universal opportunist, found in huge densities in organic polluted habitats all over the world. Also the other two are resistant towards contamination. The second axis showed a gradient from station VGPT1-22 on its positive end to station VGPT2-14 on its negative end. Cr, THC and depth contributed positively to this axis. The species that contributed positively to the axis were the bivalve *Thyasira sarsi* and the bristleworms *Polycirrus norvegicus, Chaetozone setosa, Notomastus latericeus, Pseudopolydora paucibranchiata*, while *Myriochele fragilis* and *Galatowenia oculata* contributed slightly negatively. *Thyasira sarsi, Chaetozone setosa, Notomastus latericeus* and *Pseudopolydora paucibranchiata* are all commonly associated with organic pollution. The CCA thus indicates that the stations VGIT-02, VGPT2-09 and VGPT1-22 are polluted.



Figure 9. Biplots from CCA, Vigdis, 1999 for stations (upper plot) and species (lower plot). The station naming is different from the other analyses, but the numbers are the same. The species that contribute to more than 1% on four axes are included.

Faunal distribution patterns for Vigdis, 1999, are shown in Figure 10. The top-ten list of the Vigdis data, which the classification partly is based on, is given in Appendix. The community is classified as group C at station VGIT-02, as group B at VGIT-03, VGPT2-09, VGPT2-16, VGTP1-22, VGPT1-23 and VGPT1-27, as group A/B at VGIT-04 and as group A on the remaining stations.



Figure 10. Faunal distribution pattern Vigdis field, 1999.

All methods clearly indicated that the station VGIT-02 was strongly disturbed. The status of this station could even be determined during fieldwork. An EIF for sediment needs an effective tool able to identify also initial stages of pollution and to track as much as possible of the influence of a particular discharge. The Shannon-Wiener only identified the most disturbed station clearly. The log-normal distribution plot and the different multivariate analysis (cluster-analysis, MDS, CA, CCA, CDI) were more sensitive.

Of the methods used here, the faunal distribution pattern (A, B, C, D grouping) appears to be most sensitive. This is not surprisingly as it integrates both the univariate and multivariate measures, MDS and classification and the actual species data (top ten). However, even though it should be possible to develop fixed criteria with regard to the species data, it will be a major task to prepare this method to be used with the objectivity needed in a model.

#### 4.2 Conclusion

In a modelling approach one has to be aware of the fact that the highest correlation between discharges of cuttings and impacts on the seabed is in fact achieved when combining the discharge data and the chemical contamination of the sediments with the biological data for the succeeding year (Akvaplan-niva, unpublished results). Thus one can expect an approximately one-year time lag for the response. Olsgard and Gray (1995) showed that there could be up to a three-year time lag between contamination and effect. Furthermore, they showed that initial effects were correlated with THC and barium, whereas in later years the effects were correlated with heavy metals. Thus there is a shift in response over time. The monitoring is based on sampling chemical and biological data the same year, which should be borne in mind when studying existing dataset. On this background the EIF field experiment planned to be carried out by SINTEF and Akvaplan-niva (2003 and 2004) is based on studying effects of the fauna one year after the drilling activities.

To develop a tool for modelling impacts of drill cuttings, one has to focus on the methods that are best suited to quantify the relationship between cause and effect. In this respect, one should follow the conclusion of the scientific community that species dependent (multivariate) methods are much more sensitive than species independent (univariate and graphical/distributional) methods in discriminating between sites or times (e.g. Gray *et al.*, 1990; Warwick and Clarke, 1991; Olsgard & Gray, 1995). This was also the conclusion of the analyses of the Vigdis data showed above. In some cases univariate parameters may show clear correlations with environmental variables, but to focus on the transitions zones between disturbed and undisturbed areas it is of urgent importance that the methods have the sufficient sensitivity. The chosen approach in the modelling tool should therefore have a multivariate basis, and thus we cannot recommend using the Shannon-Wiener index, which has been proposed together with the CDI.

The statistical approach chosen for the development of an EIF for drilling discharges must have the following characteristics: high sensitivity and objectivity, applicable for modelling and impact assessment, recognised and accepted by the regulatory authorities and the scientific community. An overview of advantages and drawbacks, in addition to suitability in the EIF approach, of the different methods is given in Table 2.

Tool	Advantages	Drawbacks	Suitability in EIF
Chemical analyses			
LSC (Limit of Significant Contamination)	Calculates specific value for contamination limits.	Dependent of the selection of stations included in the calculation. Does not give the status for the biological condition.	Unsuitable alone
Univariate methods			
Shannon-Wiener H'	Internationally well-known and used statistic. Calculates a specific value relating to disturbance.	Not accepted as a measurement of effects of pollution.	Unsuitable alone
Hurlbert's ES <sub>100</sub>	Internationally well-known and used statistic. Calculates a specific value relating to disturbance.	As above.	Unsuitable alone
Pielou's J	Internationally well-known and used statistic. Calculates a specific value relating to disturbance.	As above.	Unsuitable alone
Graphical/distribution al methods			
Log-normal plots	Internationally well-known and used statistic.	Might indicate disturbance, but needs detailed information on dominant species. Difficult to quantify.	Unsuitable alone
Multivariate methods			
Cluster analysis	An internationally well- known and well-used method for many years.	Shows faunal differences between stations, but gives no information on why such differences occur and what the differences are.	Unsuitable alone. Could possibly be used together with other methods.
MDS	An internationally well- known and well-used method for many years.	As for the cluster analysis. However, combined with the results from other analyses the reason for the gradient can be	Unsuitable alone. Could possibly be used together with other methods

Table 2. Overview of advantages and drawbacks, in addition to suitability in the EIF approach, of the statistical tools evaluated in this report.

		identified. The scale of the method is relative, which makes it difficult to assess the significance of the observed differences between samples in the plot.	
CA	An internationally known and used method.	As for the Cluster analysis and MDS.	Unsuitable alone
CDI	Combines biological and environmental variables. Calculates a specific value relating to disturbance.	The method has until recently not been available. Not peer- reviewed nor internationally known or used.	Best developed for EIF so far. Needs further evaluation and practical use by external groups
ANOSIM	Calculates whether group of samples are significantly different from each other.	Based on relative differences between samples, as MDS.	Unsuitable alone
Relationship between biological and environmental variables			
CCA	Combines biological and environmental variables and shows the correlations between significant environmental factors and the faunal distribution. Internationally well-known and used.	Does not have a scale relating to disturbance.	Could possibly be used. Needs further development
BIO-ENV	Combines biological and environmental variables. Internationally well-known and used.	Does not have a scale relating to disturbance. Does not indicate how particular stations or species are related to disturbance.	Could possibly be used. Needs further development
Qualitative methods			
Top ten and indicator species	Gives specific information on the dominant species. Can be quantified.	Has not been scaled earlier. Needs to be adjusted for each specific field.	Unsuitable alone. Could possibly be used together with other methods.
Faunal groups	Evaluate the results from all analyses carried out (univariate-, multivariate- and correlation analyses). Can be quantified.	Subjective and dependent on the person/institution carrying out the evaluation.	Unsuitable alone. Could possibly be used together with other methods.

It is concluded that at present CDI is the only multivariate approach which fullfills most of the given criteria. It is however, necessary to carry out some further studies in order to document how robust the method is under varying environmental conditions, and the method has to be trimmed towards more existing offshore monitoring data. So far, it has only been used for a few dataset, and is not yet fully calibrated or validated. To ensure that the modelling of drill cuttings is based on the most powerful statistical tools we also recommend including other multivariate analyses in follow-up studies.

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## 6 Appendix

#### Table 3. The ten most dominant species at Vigdis, 1999.

Н	No.	Cum	VGIT-02 (138/250 m)	No. ind.	Cum	VGIT-03 (135/500 m)	No.	Cum	VGIT-04 (138/1000 m)	No.	Cum	VGIT-05 (70/250 m)	No. ind.	Cum
	ind.						ind.			ind.				
Galathowenia oculata	818	29%	Capitella capitata	787	52%	Galathowenia oculata	1350	37%	Galathowenia oculata	1230	35%	Galathowenia oculata	992	29%
Euchone sp.	387	43%	Galathowenia oculata	197	65%	Polydora coeca	187	43%	Euchone sp.	416	46%	Euchone sp.	441	42%
Myriochele fragilis	163	49%	Polydora coeca	79	71%	Euchone sp.	184	48%	Eclysippe vanelli	179	51%	Eclysippe vanelli	214	48%
Amythasides macroglossus	162	54%	Ophryotrocha sp.	77	76%	Chaetozone sp.	150	52%	Amythasides macroglossus	170	56%	Amythasides macroglossus	204	54%
Eclysippe vanelli	125	59%	Paramphinome jeffreysii	43	79%	Amythasides macroglossus	130	56%	Myriochele fragilis	100	59%	Polydora coeca	189	59%
Parvicardium minimum	72	61%	Chaetozone sp.	23	80%	Eclysippe vanelli	130	59%	Polydora coeca	84	61%	Parvicardium minimum	160	64%
Polydora coeca	60	64%	Nemertini indet.	21	82%	Pseudopolydora paucibranchiata	100	62%	Notomastus latericeus	80	64%	Myriochele fragilis	89	66%
Levinsenia gracilis	55	66%	Pholoe assimilis	21	83%	Notomastus latericeus	81	64%	Pseudopolydora paucibranchiata	78	66%	Thyasira ferruginea	53	68%
Thyasira ferruginea	55	67%	Spiophanes wigleyi	20	84%	Myriochele fragilis	67	66%	Myriochele danielsseni	57	67%	Levinsenia gracilis	51	69%
Thyasira obsoleta	53	69%	Pholoe baltica	17	85%	Capitella capitata	66	68%	Parvicardium minimum	49	69%	Pseudopolydora paucibranchiata	50	71%
									Thyasira obsoleta	49	70%			
VGIT-06 (70/500 m)	No.	Cum	VGPT2-07 (318/1000 m)	No. ind.	Cum	VGPT2-08 (318/500 m)	No.	Cum	VGPT2-09 (138/250 m)	No.	Cum	VGPT2-10 (138/500 m)	No. ind.	Cum
	ind.						ind.			ind.				
Galathowenia oculata	767	29%	Galathowenia oculata	419	18%	Galathowenia oculata	553	20%	Galathowenia oculata	901	21%	Galathowenia oculata	1125	26%
Euchone sp.	303	41%	Euchone sp.	232	28%	Polydora coeca	336	32%	Pseudopolydora paucibranchiata	729	39%	Euchone sp.	885	46%
Amythasides macroglossus	167	47%	Amythasides macroglossus	148	34%	Parvicardium minimum	189	39%	Euchone sp.	351	47%	Amythasides macroglossus	319	53%
Polydora coeca	149	53%	Eclysippe vanelli	139	40%	Euchone sp.	168	45%	Amythasides macroglossus	185	51%	Eclysippe vanelli	272	60%
Parvicardium minimum	120	58%	Polydora coeca	122	45%	Amythasides macroglossus	147	50%	Thyasira sarsi	174	55%	Polydora coeca	181	64%
Eclysippe vanelli	119	62%	Parvicardium minimum	96	49%	Eclysippe vanelli	125	55%	Paramphinome jeffreysii	130	58%	Pseudopolydora paucibranchiata	176	68%
Myriochele fragilis	69	65%	Thyasira obsoleta	88	53%	Thyasira croulinensis	54	57%	Myriochele danielsseni	128	61%	Thyasira croulinensis	109	70%
Thyasira croulinensis	57	67%	Thyasira croulinensis	60	55%	Levinsenia gracilis	42	58%	Polydora coeca	93	64%	Myriochele fragilis	91	72%
Thyasira ferruginea	48	69%	Tmetonyx cicada	52	58%	Yoldiella phillippiana	40	60%	Owenia fusiformis	86	66%	Myriochele danielsseni	81	74%
Onchnesoma steenstrupi	32	70%	Levinsenia gracilis	47	60%	Myriochele fragilis	39	61%	Abra nitida	84	68%	Thyasira obsoleta	70	76%
VGPT2-11 (138/1000 m)	No.	Cum	VGPT2-12 (138/2000 m)	No. ind.	Cum	VGPT2-14 (228/1000 m)	No.	Cum	VGPT2-15 (228/500 m)	No.	Cum	VGPT2-16 (70/250 m)	No. ind.	Cum
Galathowenia oculata	1145	35%	Galathowenia oculata	423	28%	Galathowenia oculata	1485	41%	Galathowenia oculata	848	27%	Euchone sp.	938	26%
Euchone sp.	326	45%	Eclysippe vanelli	73	33%	Euchone sp.	338	50%	Euchone sp.	306	37%	Polydora coeca	237	33%
Polydora coeca	233	52%	Euchone sp.	67	38%	Eclysippe vanelli	211	56%	Amythasides macroglossus	190	43%	Amythasides macroglossus	235	40%
Amythasides macroglossus	169	57%	Thyasira croulinensis	64	42%	Amythasides macroglossus	168	60%	Eclysippe vanelli	182	49%	Eclysippe vanelli	224	46%
Eclysippe vanelli	144	62%	Amythasides macroglossus	63	46%	Myriochele danielsseni	134	64%	Parvicardium minimum	146	54%	Pseudopolydora paucibranchiata	175	51%
Myriochele fragilis	125	66%	Parvicardium minimum	46	49%	Pseudopolydora paucibranchiata	126	68%	Myriochele danielsseni	96	57%	Galathowenia oculata	170	56%
Myriochele danielsseni	78	68%	Timoclea ovata	46	52%	Levinsenia gracilis	78	70%	Thyasira obsoleta	92	60%	Myriochele fragilis	123	59%
Thyasira croulinensis	48	70%	Yoldiella phillippiana	36	54%	Thyasira obsoleta	64	72%	Myriochele fragilis	91	63%	Thyasira croulinensis	116	63%
Onchnesoma steenstrupi	45	71%	Thyasira obsoleta	35	57%	Thyasira croulinensis	56	73%	Thyasira croulinensis	84	66%	Chone longocirrata	91	65%
Thyasira ferruginea	45	72%	Nematoda indet.	34	59%	Spiophanes kroyeri	41	74%	Pseudopolydora paucibranchiata	67	68%	Paramphinome jeffreysii	70	67%

#### Cont. Table 3.

VGPT2-17 (70°/500 m)	No.	Cum	VGPT2-18 (70°/1000 m)	No.	Cum	VGPT1-21 (318°/500 m)	No.	Cum	VGPT1-22 (138°/250 m)	No.	Cum	VGPT1-23 (138°/500 m)	No.	Cum
	ind.													
Euchone sp.	821	24%	Polydora coeca	300	15%	Galathowenia oculata	549	19%	Notomastus latericeus	520	16%	Euchone sp.	425	14%
Galathowenia oculata	646	42%	Galathowenia oculata	273	28%	Euchone sp.	369	32%	Pseudopolydora paucibranchiata	396	27%	Galathowenia oculata	274	23%
Polydora coeca	315	52%	Myriochele fragilis	109	34%	Myriochele fragilis	198	39%	Chaetozone setosa	194	33%	Amythasides macroglossus	173	29%
Eclysippe vanelli	232	58%	Euchone sp.	101	39%	Amythasides macroglossus	145	44%	Galathowenia oculata	137	37%	Polydora coeca	154	34%
Amythasides macroglossus	136	62%	Parvicardium minimum	71	42%	Eclysippe vanelli	124	48%	Polydora coeca	136	41%	Myriochele fragilis	130	38%
Parvicardium minimum	110	65%	Eclysippe vanelli	66	45%	Polydora coeca	110	52%	Polycirrus norvegicus	128	45%	Notomastus latericeus	119	42%
Chone longocirrata	94	68%	Amythasides macroglossus	58	48%	Spiophanes kroyeri	84	55%	Chaetozone sp.	126	49%	Eclysippe vanelli	93	45%
Myriochele fragilis	56	70%	Levinsenia gracilis	47	51%	Levinsenia gracilis	69	58%	Euchone sp.	110	52%	Thyasira obsoleta	91	48%
Abra nitida	55	71%	Thyasira ferruginea	46	53%	Thyasira obsoleta	68	60%	Glycera lapidum	101	55%	Tmetonyx cicada	87	51%
Paramphinome jeffreysii	52	73%	Myriochele heeri	42	55%	Thyasira ferruginea	53	62%	Spiophanes wigleyi	88	58%	Paramphinome jeffreysii	64	53%
VGPT1-24 (138°/1000 m)	No.	Cum	VGPT1-25 (138°/2000 m)	No.	Cum	VGPT1-27 (70°/250 m)	No.	Cum	VGPT1-28 (70°/500 m)	No.	Cum	VGPT2-19 (318°/2000 m)	No.	Cum
	ind.			ind.			ind.			ind.			ind.	
Euchone sp.	555	18%	Euchone sp.	461	17%	Euchone sp.	410	13%	Galathowenia oculata	443	16%	Galathowenia oculata	289	16%
Myriochele fragilis	460	33%	Galathowenia oculata	350	30%	Galathowenia oculata	355	25%	Euchone sp.	386	30%	Amythasides macroglossus	149	24%
Galathowenia oculata	377	46%	Myriochele fragilis	231	38%	Notomastus latericeus	180	31%	Myriochele fragilis	224	38%	Parvicardium minimum	139	32%
Amythasides macroglossus	200	52%	Amythasides macroglossus	206	46%	Amythasides macroglossus	173	36%	Amythasides macroglossus	161	44%	Euchone sp.	94	37%
Thyasira ferruginea	100	56%	Eclysippe vanelli	127	50%	Eclysippe vanelli	114	40%	Eclysippe vanelli	93	47%	Eclysippe vanelli	93	42%
Eclysippe vanelli	99	59%	Spiophanes kroyeri	97	54%	Myriochele fragilis	97	43%	Parvicardium minimum	69	50%	Levinsenia gracilis	71	46%
Polydora coeca	91	62%	Polydora coeca	84	57%	Abra nitida	83	46%	Thyasira croulinensis	64	52%	Pseudopolydora paucibranchiata	62	50%
Pseudopolydora paucibranchiata	91	65%	Thyasira ferruginea	77	60%	Levinsenia gracilis	74	48%	Myriochele danielsseni	58	54%	Thyasira ferruginea	57	53%
Spiophanes kroyeri	57	67%	Heteroclymene robusta	74	63%	Parvicardium minimum	72	50%	Levinsenia gracilis	57	56%	Spiophanes kroyeri	51	56%
Levinsenia gracilis	56	69%	Pseudopolydora paucibranchiata	64	65%	Pseudopolydora paucibranchiata	72	53%	Thyasira obsoleta	56	58%	Nematoda indet.	50	58%