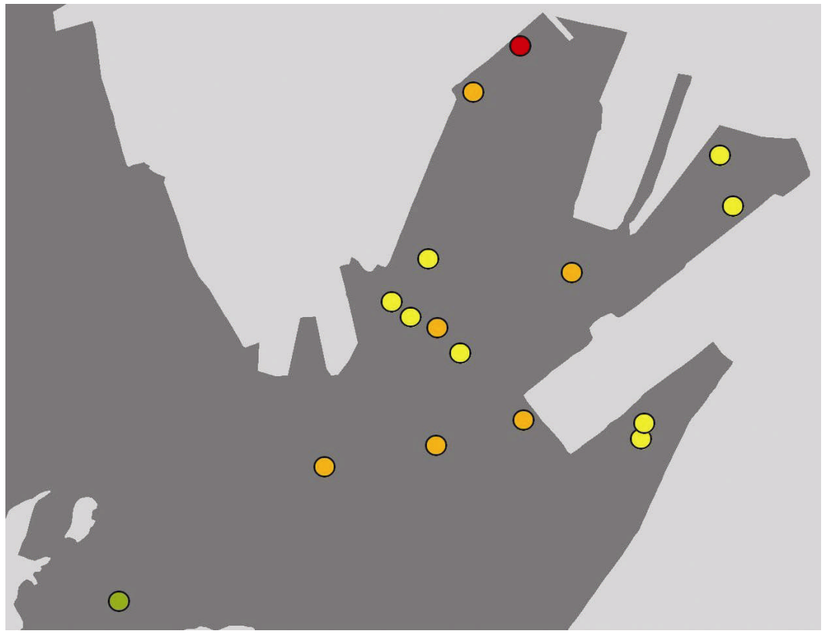
REPORT SNO 5208-2006

Developing indices for quality-status classification of marine soft-bottom fauna in Norway



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REPORT

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Abstract. As part of the implementation of the EU Water Frame Directive new multimetric indices are developed to assess the quality status based on marine soft-bottom fauna. In this report, several diversity and sensitivity indices were analysed and tested on Norwegian data in order to choose the most suited for inclusion in a multimetric index for Norwegian habitats. The data for the assessements comprised more than 1600 samples from Norwegian fjords and coastal waters. Coefficients to convert species numbers for smaller and larger sampling areas are provided. Correlations between index values and sediment grain size were analysed, accordingly, the indices were normalised to common sediment type prior to fauna classification. Two alternative multimetric indices were suggested:

Index 1 = $[0.5*(1-AMBI_{63}/7) + 0.5*(SN_{63}/ 2.7)*(N/(N+5)]$

Index 2 = $[0.5*(1-AMBI_{63}/7) + 0.5*(H_{63}/6)]$

where AMBI is a sensitivity index, SN and H diversity indices, and N the number of individuals in the sample.

Both indices can attain values between 0 and 1, where 0 will indicate bad quality status, and 1 will indicate high quality status.

4 keywords, Norwegian	4 keywords, English
1. Klassifisering av økologisk status	1. Ecological status classification
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Developing indices for quality-status classification of marine soft-bottom fauna in Norway

Preface

The Water Frame Directive requires member states to assess the Ecological Quality Status of water bodies. Assessments will be based upon i.a. the status of marine soft-bottom fauna communities. To describe quality status, multimetric indices including species sensitivity or tolerance and species diversity are established. Several countries are currently optimising indices adapted for assessing their own habitats.

The present report describes the analysis of different diversity and sensitivity indices in order to choose the most suitable ones for Norwegian habitats. Suggestions are made on how to combine them in a multimetric index. The work was based on data from Norwegian fjords and coastal waters (NIVA database). It was carried out as part of the strategic institute programme BIOKLASS, continuing previous work on soft-bottom fauna classification.

Oslo, 2 May 2006

Brage Rygg

Contents

Summary

The aim of the present work was to analyse and test diversity indices and sensitivity (biotic) indices for use as classifiers of ecological status in marine soft-bottom communities.

The empirical basis for the analysis were more than 1600 samples (sample = station*date) from Norwegian fjords and coastal waters.

Normalisation of species numbers to a standardised area is necessary when comparing sites where sampled areas are different. Because most sampling in Norway is carried out taking four grab replicates à 0.1 m^2 (a total of 0.4 m^2) per station, number of species per 0.4 m (S_{04}) was chosen as a standard parameter for species density. Coefficients for estimating the number of species per 0.4 m^2 (S_{04}) were established, based on number of species observed in smaller or larger areas at the same locality. It was found that the number of species per grab (0.1 m^2) on the average must be multiplied by 1.9 to be comparable to species number in four pooled grab samples (0.4 m^2).

The species numbers also increase with increasing number of individuals in the samples. By log-transforming both axes used in the Margalef index *d* (S and lnN), thus converting the axes to lnS and ln(lnN), an approximately linear correlation appears. The value of the transformed Margalef index does not change significantly, even if the number of individuals in the sample (e.g. at different numbers of grab samples per station) changes. Based on this, a new index for species richness, SN, is suggested: SN = lnS/ln(lnN). This can be used as an index of diversity. Another commonly used diversity index is H', which combines evenness and number of species in the sample.

One complicating factor is that diversity is dependent on sediment grain size. Heterogeneous sediments containg sand and gravel in addition to mud offer more niches to the fauna and tend to raise diversity. Therefore, fauna indices need to be normalised to sediment grain size before values from different sites can be compared or classified. Normalisation formulas were established for the diversity indices SN and H' and the sensitivity index AMBI.

The correlation between diversity and sediment grain size may be applied to testing the adequacy of different diversity indices. Will an index which more accurately detects faunal diversity gradients along sediment coarseness also be a better descriptor of diversity in general and thus better detect diversity gradients caused by pollution or other disturbance?

The diversity index which showed the highest correlation with sediment grain size was SN. SN is therefore the priority choice of diversity index for ecological status classification. The index H' has a slightly lower correlation with grain size, but more correctly describes diversity in small samples.

The sensitivity index AMBI is used by Spain, UK, Ireland and Denmark. AMBI is also the priority choice of sensitivity index in Norway.

Two alternative multimetric indices for fauna classification are suggested:

Norwegian multimetric index 1 = $[0.5*(1-AMBI_{63}/7) + 0.5*(SN_{63}/ 2.7)*(N/(N+5))]$

Norwegian multimetric index 2 = $[0.5*(1-AMBI_{63}/7) + 0.5*(H_{63}/ 6)]$

where AMBI a sensitivity index, SN and H' diversity indices, N the number of individuals in the sample. The subscript '63' indicates normalisation to standard sediment grain size.

1. Introduction

The Water Frame Directive requires member states to assess the Ecological Quality Status of water bodies. Assessments will be based upon i.a. the status of marine soft-bottom fauna communities. To describe quality status, multimetric indices including species sensitivity or tolerance and species diversity are established. Several countries are currently optimising indices adapted for assessing their own habitats. There are also intercalibrations and harmonisation efforts going on to arrive at common classification boundary settings for the North East Atlantic.

The present work was carried out as part of the strategic institute programme BIOKLASS.

The aim was to analyse and test diversity indices and sensitivity (biotic) indices for use as classifiers of ecological status in marine soft-bottom fauna communities in Norway. Suggestions were made on how to combine them in a multimetric index. A multimetric index can combine different aspects of the fauna which may be affected by environmental disturbance. Also, it will be simpler to use for classification purposes than two or more distinct unimetric indices.

The work was based on data from more than 1600 samples (sample = station*date) from Norwegian fjords and coastal waters (NIVA database). Most samples consisted of 4 separate grab samples (4 x 0.1 m^2), pooled prior to the data analysis. The samples were obtained between 1975 and 2005, mostly from southern Norway.

Previous works on soft-bottom fauna classification in Norway were reported by Olsgard et al. (2003) and Lyche et al. (2004).

In the present work, index reference values (values expected at unpolluted, reference conditions) were not established, and boundary values between high, good, moderate, poor and bad status were not defined. Suggestions for reference and boundary values will be given in a future report.

2. Description of indices, with analyses and examples

2.1 Species-area curves and species density (the number of species occurring in a defined area)

The number of species (S) present at a site fairly well indicates the health status of the fauna community. However, the numbers of species will vary with the sampled area or the total number of individuals. Samples of different sizes therefore cannot be compared directly, but should be standardised in some way before comparison.

One approach is to calculate species-area curves. These can be generated by calculating the average number of species in permutations of one, two, three, etc. grab samples from one meta sample including several grab samples. The curve describes the cumulative number of species as a function of the number of grab samples (= cumulative area). The climb rate of the curve indicates the probability of finding new species in the next sample.

As new species are added in an extended effort of sampling, the probability of finding additional species in the next sample will gradually decrease.

Figure 1 shows an example of a species-area curve and how the climb in species numbers decreases when the sampled area increases. The curve was generated using the program PRIMER (http://www.primer-e.com/).

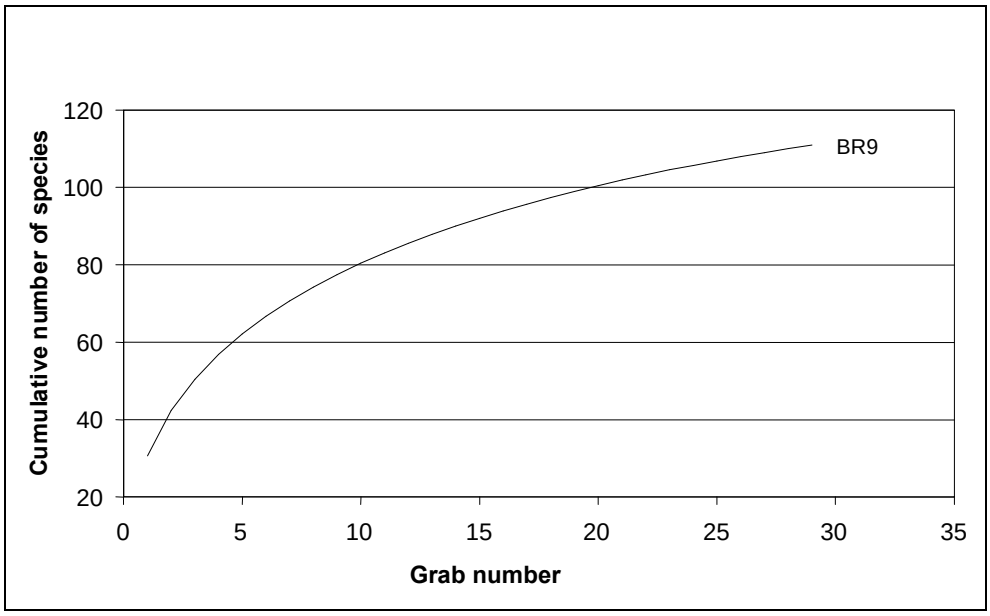

Figure 1. Species-area curve at station BR9 in outer Oslofjord (29 grab replicates).

Normalisation of species numbers to a standardised area is necessary when comparing sites where sampled areas are different. The estimation of density of species is more complicated than the estimation of density of individuals, because the simple linear conversion cannot be used. However, because most sampling in Norway has been carried out using four grabs of 0.1 m^2 (a total of 0.4 m^2) per station, number of species per 0.4 m (S_{04}) was chosen as a standard parameter for species density.

S_{04} = Actual or estimated number of species per 0.4 m^2.

Figure 2 and
Figure 3 show the ratios between average species number (S) in single grab samples (0.1 m^2) and the cumulative S in four grab samples pooled (0.4 m^2) at 319 stations (NIVA database). **Table 1** and **Table 2** show ratios between S for different sampling areas at some stations in the coastal monitoring programme calculated from species-area curves. **Table 3** shows

corresponding calculations based on a station in outer Oslofjord. The coefficients in the tables can be applied for recalculating S in samples of different sizes.

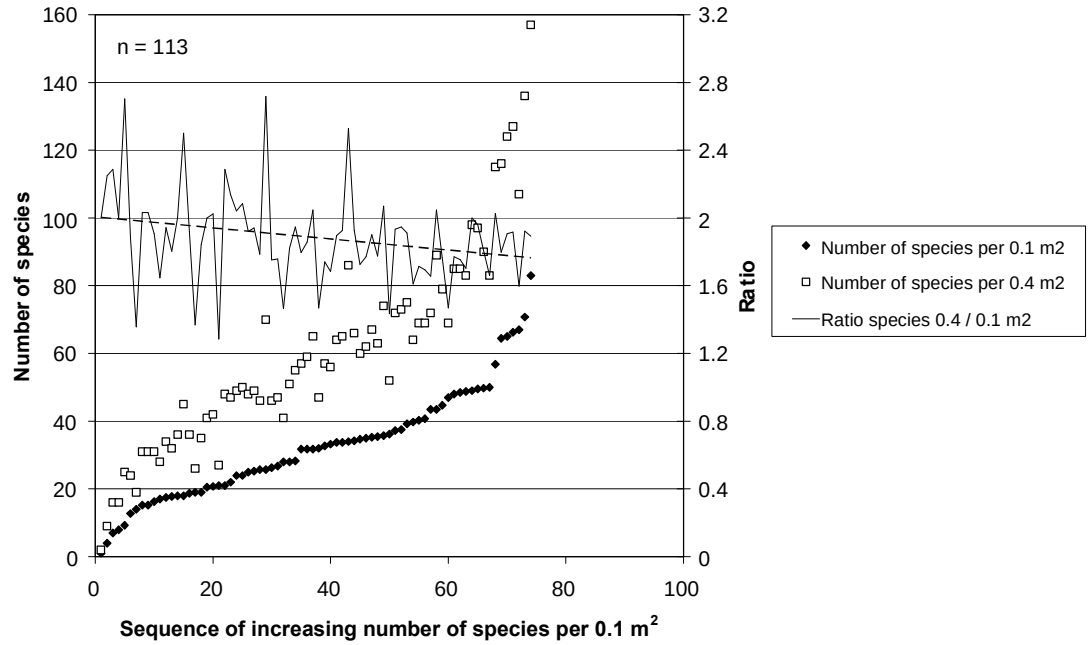

Figure 2. Average number of species per grab (0.1 m^2) in four grab samples from the same station and cumulative number of species (per 0.4 m^2). Data from Norwegian fjords and coastal waters (stations in the coastal monitoring programme excluded). Median ratio value = 1.90.

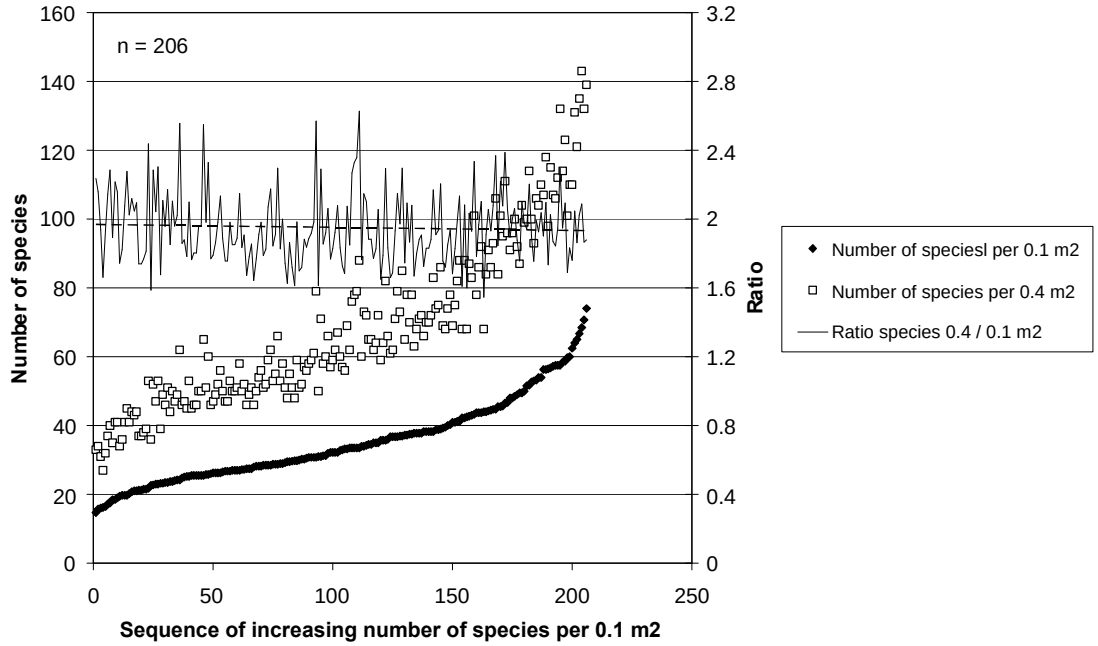

Figure 3. Average number of species per grab (0.1 m^2) in four grab samples from the same station and cumulative number of species (per 0.4 m^2). Data from the Norwegian coastal monitoring programme. Median of ratio values = 1.91.

Table 1. The ratio between species number in 0.4 m^2 and species number in a smaller or larger area (0.1 m^2 to 0.8 m^2) for stations in the Coastal monitoring programme 1993-2001. The ratios are calculated from species-area curves based on 36 samples.

	Area (m^2) 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Station								
A05	2.08	1.41	1.15	1.00	0.90	0.84	0.78	0.74
A36	1.94	1.38	1.14	1.00	0.90	0.83	0.77	0.73
A46	2.10	1.41	1.15	1.00	0.90	0.83	0.77	0.73
B05	2.10	1.40	1.14	1.00	0.91	0.84	0.79	0.74
B10	2.14	1.43	1.15	1.00	0.90	0.83	0.78	0.73
B19	1.98	1.38	1.14	1.00	0.90	0.83	0.77	0.72
B20	2.18	1.44	1.16	1.00	0.90	0.83	0.77	0.73
B35	1.94	1.38	1.14	1.00	0.91	0.84	0.78	0.74
C16	2.01	1.38	1.14	1.00	0.91	0.83	0.78	0.74
C38	1.89	1.36	1.13	1.00	0.91	0.84	0.79	0.75

Table 2. The ratio between species number in 0.4 m^2 and species number in a smaller or larger area (0.1 m^2 to 0.8 m^2) for stations in the Coastal monitoring programme 2002. The ratios are calculated from species-area curves based on 7 or 8 samples per station.

	Area (m^2) 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Station								
A05	1.79	1.28	1.10	1.00	0.94	0.89	0.85	
A36	1.76	1.31	1.12	1.00	0.91	0.85	0.80	0.76
B05	1.89	1.34	1.12	1.00	0.92	0.86	0.81	0.78
B35	1.69	1.29	1.11	1.00	0.92	0.86	0.81	0.78
C16	1.92	1.36	1.13	1.00	0.91	0.85	0.80	0.75
C38	1.63	1.27	1.10	1.00	0.93	0.87	0.83	0.80

Table 3 shows the ratios between average number of species in 4 grab samples and average number in 1, 2, 3, 5, 6, 7 and 8 grab samples from a station in outer Oslofjord (BR9) where 29 replicate grab samples were taken (**Figure 1**).

Table 3. Ratio between number of species in 0.4 m^2 and number of species in smaller or larger areas (0.1 m^2 til 0.8 m^2) for station BR9. The species-area curve for the station was generated from 29 samples.

	Area (m^2) 0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
Station								
BR9	1.87	1.34	1.12	1.00	0.91	0.85	0.80	0.77

Because the curves which **Table 1** is based on shows accumulated grab samples covering a period of several years, the variations in species composition from year to year, and not merely the species numbers at the time of sampling, may have contributed to the climb of the curves. Therefore, they are slightly steeper than the curves from a single year (**Table 2** and **Table 3**).

Based on the numbers shown in **Figure 2**-**Figure 3** and **Table 1**-**Table 3**, the following coefficients for converting S to S_{04} are suggested (**Table 4**):

Table 4. Coefficients for estimating the number of species per 0.4 m^2 (S_{04}) based on the number of species observed in smaller or larger areas (0.1 – 0.8 m^2). Actually, the coefficients can be used for estimating the number of species in any area 0.1 – 0.8 m^2.

Sampled area (m^2)	Coefficient for estimating S_{04}
0.1	1.90
0.2	1.34
0.3	1.12
0.4	1.00
0.5	0.92
0.6	0.86
0.7	0.81
0.8	0.77

2.2 Species richness (number of species per number of individuals)

The species numbers increase with increasing number of individuals in the samples. As an index of species richness, the Margalef index *d* (Margalef 1958) is commonly used. Graphically, this index is the angle coefficient in the plotted (S-1)/lnN. It assumes that the number of species increases proportionally to the logarithm of number of individuals. However, in most cases the S rate of increase is higher, *d* thus underestimating species richness in smaller samples (see e.g. Ludwig and Reynolds 1988).

Figure 4 and **Table 5** show how *d* increases with increasing sample size, based on S and N from species-area curves of the coastal monitoring stations in 2002, where 8(7) grabs per station were taken.

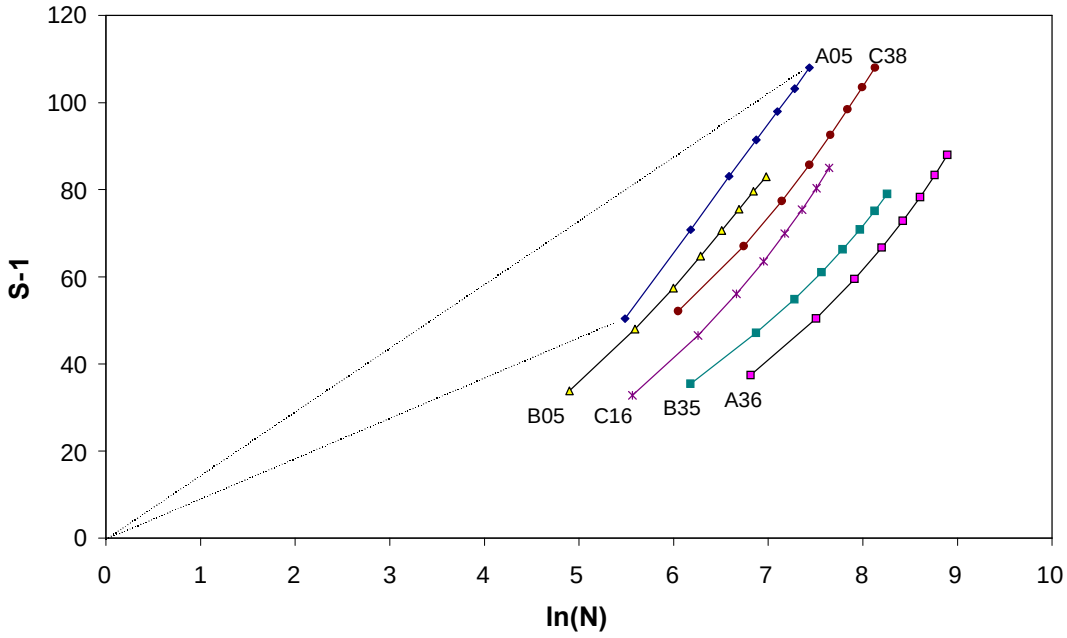

Figure 4. Plot of number of species vs. logarithm of number of individuals at six coastal monitoring stations in 2002. The angle coefficient of the line through origo and the respective point = Margalef species richness (*d*). The value of the index *d* is higher for large samples than smaller samples.

The value of *d* increases considerably with ln(N) within the same station (see also **Table 5**).

Table 5. Values of Margalef species richness (*d*) at different numbers of grab samples (same data as in **Figure 4**).

	Cumulative number of grab samples							
Station	1	2	3	4	5	6	7	8
A05	9.18	11.46	12.62	13.30	13.80	14.18	14.53	
A36	5.49	6.72	7.52	8.14	8.65	9.10	9.52	9.90
B05	6.90	8.58	9.57	10.31	10.86	11.29	11.64	11.90
B35	5.74	6.86	7.54	8.07	8.51	8.89	9.24	9.57
C16	5.89	7.43	8.42	9.14	9.75	10.25	10.70	11.12
C38	8.62	9.94	10.83	11.53	12.09	12.56	12.95	13.29

Figure 5 shows the plot of S vs. ln(N) for 1310 stations (NIVA database). It seems to be a general trait of the fauna communities that S increases faster than ln(N). This means that *d* is not independent of sample size, but increases with increasing number of individuals.

It was therefore necessary to find a more correct index for species richness, based on N and S. By log-transforming both axes used in the Margalef index (S and lnN), thus converting the axes to lnS and ln(lnN), an approximately linear correlation appears (**Figure 6**). The angle coefficient (= the value of the new index) does not change significantly, even if the number of individuals in the sample (e.g. at different numbers of grab samples per station) changes. Based on this, a new index for species richness, SN, is suggested:

SN = lnS/ln(lnN))

Like *d*, SN is dependent on S and N, but (mathematically) independent of evenness.

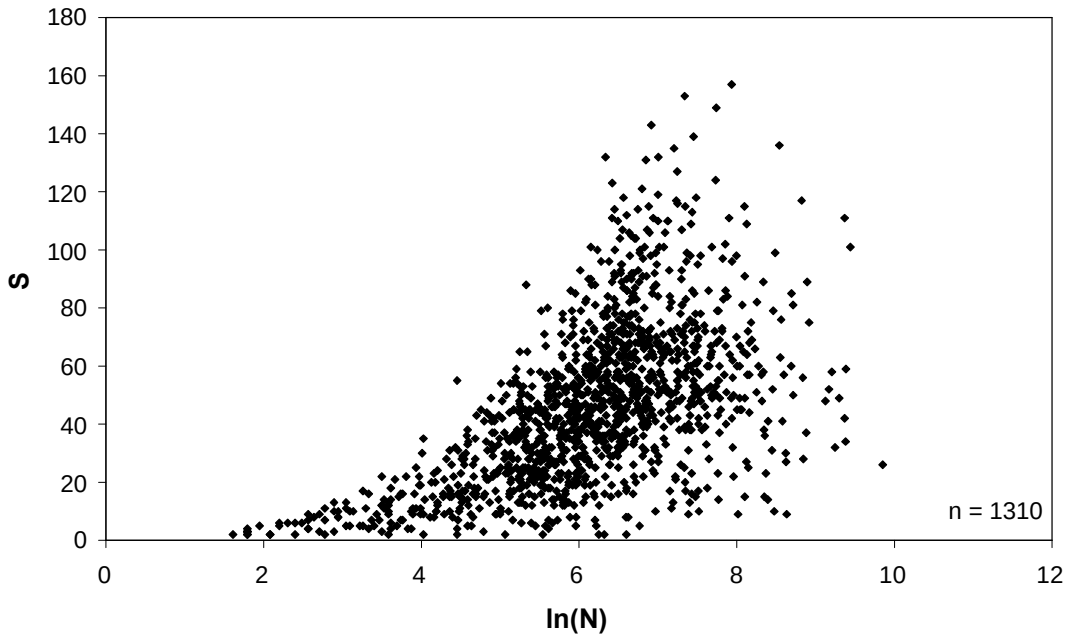

Figure 5. Correlation between S and ln(N) in 1310 samples from Norwegian fjords and coastal waters.

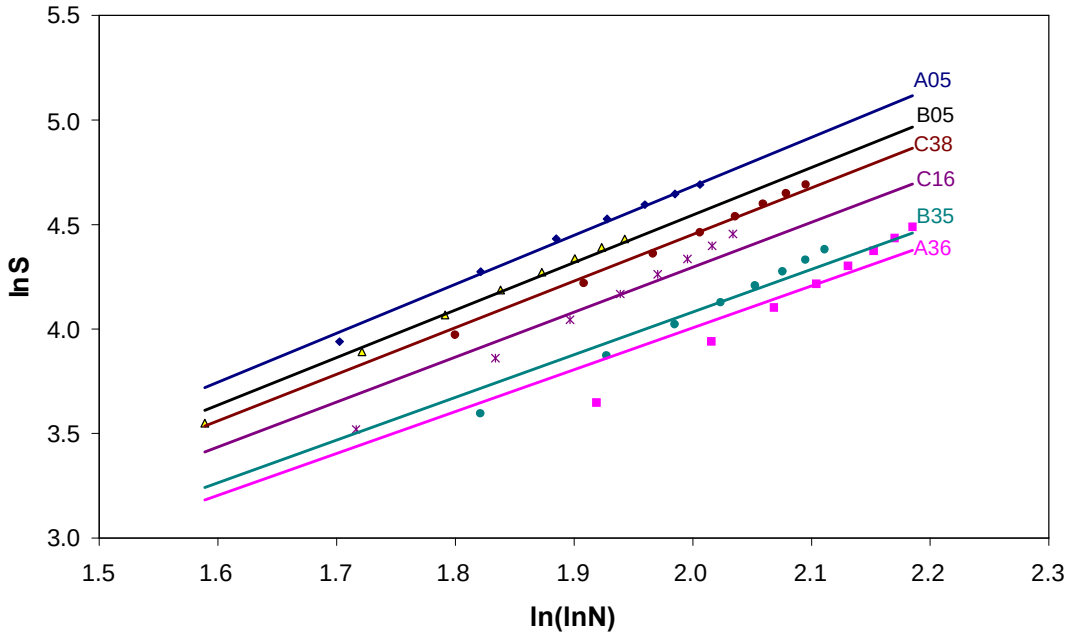

Figure 6. Plot of the species richness index SN for coastal monitoring stations 2002. Regression lines through origo for each station are shown. The values of SN are shown in **Table 6**.

Table 6. Values of the index (SN) at different number of grab samples (same data as in **Figure 6**).

	Cumulative number of grab samples							
Station	1	2	3	4	5	6	7	8
A05	2.31	2.35	2.35	2.35	2.34	2.34	2.34	
A36	1.90	1.95	1.98	2.00	2.02	2.03	2.04	2.05
B05	2.23	2.26	2.27	2.28	2.28	2.28	2.28	2.28
B35	1.98	2.01	2.03	2.04	2.05	2.06	2.07	2.08
C16	2.05	2.11	2.13	2.15	2.16	2.17	2.18	2.19
C38	2.21	2.21	2.22	2.22	2.23	2.23	2.24	2.24

An increase in the number of individuals has a much smaller effect on SN than on *d*. The separation of the stations SN-values are well retained independent of number of individuals (in the example above only station B35 and A36 show some overlap, if the index value is based on few samples at B35 and many samples at A36). The ability of the index to retain such separation is crucial when comparing species richness in different samples.

2.3 Evenness (J')

Evenness describes the distribution of individuals among species. Evenness is most commonly expressed by Pielou's Index J' (Pielou 1966) which is calculated from the Shannon-Wiener H' (see below) by the relation $J' = H'/\log_2 S$. The denominator $\log_2 S$ is equal to the maximum possible diversity which is the case when all taxa are represented by the same number of individuals (= H'max); thus, J' = H' /H' max.

2.4 Diversity index H' (Shannon-Wiener)

H' is one of the most commonly used diversity indices. H' is calculated as $-\sum (p_i)*(\log_2 p_i)$, where p_i = proportion of individuals of the total sample belonging to $_i$th species (Shannon and Weaver 1963). The index may also be expressed by $H' = J'*\log_2 S$, hence combining Pielou evenness (J') and $\log_2$number of species in one index of diversity. The index value is dependent on evenness and number of species, but not (mathematically) on number of individuals (N) in the sample.

The index H' is used in the Norwegian SFT classification system (SFT, 1997). It is also used as the diversity element in the Danish multimetric index (Borja et al. 2006).

2.5 Diversity index ES (Hurlbert index)

Another common measure of diversity is to calculate the expected number of species (ES*n*) among *n* individuals in a subsample of *n*/N from the main sample (Hurlbert 1971). By calculating ES*n* for different values of *n*, a species-individuals curve is generated. Normally this curve closely follows the species-area curve (although calculated in a different way).

The value of ES*n* for a specified number of individuals (e.g. 10, 50 or 100 individuals) can be used as an index. In Norway, ES_{100} is used in the SFT classification system (SFT, 1997).

2.6 Diversity index $\log_{10}(S+1)$

$Log_{10}(S+1)$ is used as the diversity element in the Swedish multimetric index (Blomqvist et al. 2006). This index is only dependent on the number of species present and is equivalent to the Shannon-Wiener index with the evenness factor removed.

2.7 Diversity index 1-Lambda'

1-Lambda' = $\sum[n_i*(n_i-1)/N*(N-1)]$ (Simpson 1949).

Where n_i = the number of individuals of species *i*; N = total number of individuals in the sample.

The index 1-Lambda' is used in the UK and Ireland multimetric index (Borja et al. 2006).

3. Dependency of diversity indices to evenness and species numbers

Diversity indices are dependent on both evenness and species numbers, but respond differently to the two components. In this study, the dependencies of different indices on evenness (J') and species numbers (S) were tested using empirical data. The tests were performed by calculating Spearman rank correlations. Since the 'x'-data (J' or S) in the analyses were the same, correlation differences among the indices implied different ranking of their values along the x-values. A rank correlation test was therefore chosen for comparative analysis.

The data used for the analysis were from 1665 samples from the NIVAs marine soft-bottom fauna database. The samples (stations*dates) had been obtained from fjords, archipelagos and open coastal waters along the Norwegian coast, but the majority of the samples were from southern Norway. In order to avoid artefacts in the index values, due to small number of animals, only samples with at least 6 individuals were included.

Table 7 shows correlations of selected indices to evenness and species numbers.

Table 7. Rank correlation coefficients (Spearman) of selected diversity indices vs. evenness (J') and number of species (S) in the samples. The indices are sorted high-to-low in the table, according to their correlation with S.

Index	J'	S
	Spearman rs-statistic	Spearman rs-statistic
d	0.36	0.97
SN	0.63	0.77
H'	0.72	0.75
ES100	0.83	0.74
ES50	0.83	0.70
1-Lambda'	0.83	0.63
ES10	0.83	0.60
ES5	0.84	0.58

S was used here instead of S normalised to area (S_{04}), because the other indices were calculated on the same sample (same area) as S.

The correlation coefficients indicate that Margalef species richness (*d*) is strongly dependent on S and weakly dependent on J'. SN is slightly more dependent on species number (S) than on evenness (J'), but also evenness is important. H' is almost equally dependent on S and J'. ES and 1-Lambda' are more dependent on J' than on S, the dependency of ES*n* on S decreasing with decreasing *n*, probably due to loss of precision.

4. Correlations between index values and sediment grain size

Diversity is usually higher in heterogenous sediments containing coarser grains such as sand and gravel than in sediments purely consisting of mud. This effect of sediment type should be taken into consideration when comparing diversity from sites with different sediment types.

Because a heterogeneous sediment (containing both mud and fine and coarse sand) will have more niches, a higher diversity is expected. The theory is confirmed by several authors (e.g. Etter and Grassle 1992; Moy et al. 2002).

The relationship between diversity and sediment grain size may be used to examine the responses of different diversity indices to environmental gradients in general. It may be asked whether an index which more accurately detects faunal diversity gradients along sediment coarseness, also will be a good descriptor of diversity in general and better detect diversity gradients caused by pollution or other disturbance. If so, the most suitable index could be revealed by analysis of the empirical data.

The relationships were examined using the same data as in the study of correlations between indices and evenness and species numbers, but excluding the samples lacking sediment grain size data. In order to establish the diversity-sediment relationships under natural conditions,

the analyses were made separately on the two groups of samples, (1) not significantly polluted or disturbed stations, and, (2) possibly polluted or disturbed stations.

The criteria for **"**not significantly polluted or disturbed" were:

1. Not situated in areas assumed to be polluted (e.g. harbours, recipients for industry, etc.)
and
2. *Capitella* representing no more than 10 individuals per m^2
and
3. *Malacoceros* representing no more than 5 individuals per m2
and
4. Diversity H' = 3 or higher
and
5. Number of species S_{04} = 20 or higher
and
6. TOC (total organic carbon in the sediment) not higher than 50 mg/g

The polychaetes *Capitella* and *Malacoceros* are pollution-indicating species, especially when abundant.

Analyses were performed on the indices and their correlations with % mud (grain size < 0.063 mm) in the sediment.

The following indices were tested: 1-Lambda' (Simpson 1949), H' (Shannon and Weaver 1963), ES_5, ES_{10}, ES_{50}, ES_{100} (Hurlbert 1971), J' (Pielou 1966), *d* (Margalef 1958), S_{04} (present report), $\log_{10}(S+1)$ (Blomqvist et al. 2006), SN (present report), ISI (Rygg 2002) and AMBI (Borja et al. 2000).

Correlation plots of SN, H' and 1-Lambda' vs. % mud in samples from non-polluted stations are shown in **Figure 7** and **Figure 8**. The plots in **Figure 7** indicate that SN more precisely describes the diversity dependency on sediment grain size than 1-Lambda'.

Of the two indices based on sensitivity of species, AMBI was found to be better correlated with sediment grain size than was ISI at non-polluted stations (**Figure 9**).

SN and 1-Lambda for polluted stations are shown in **Figure 10**. The very low correlation between diversity and sediment grain size at polluted stations indicates that other factors, probably related to pollution, are more important to diversity.

The graphs also indicate that normalisations of index values, depending on sediment grain size, are important before comparing index values from different samples.

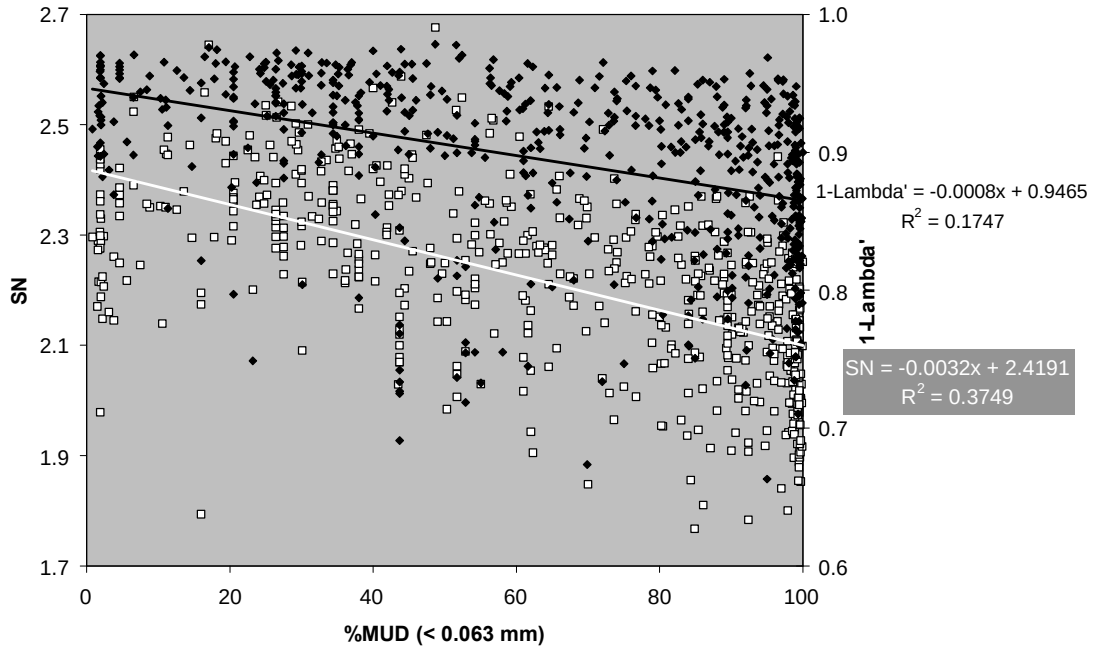

Figure 7. Correlations between SN (□) or 1-Lambda (♦) vs. % mud at non-polluted stations.

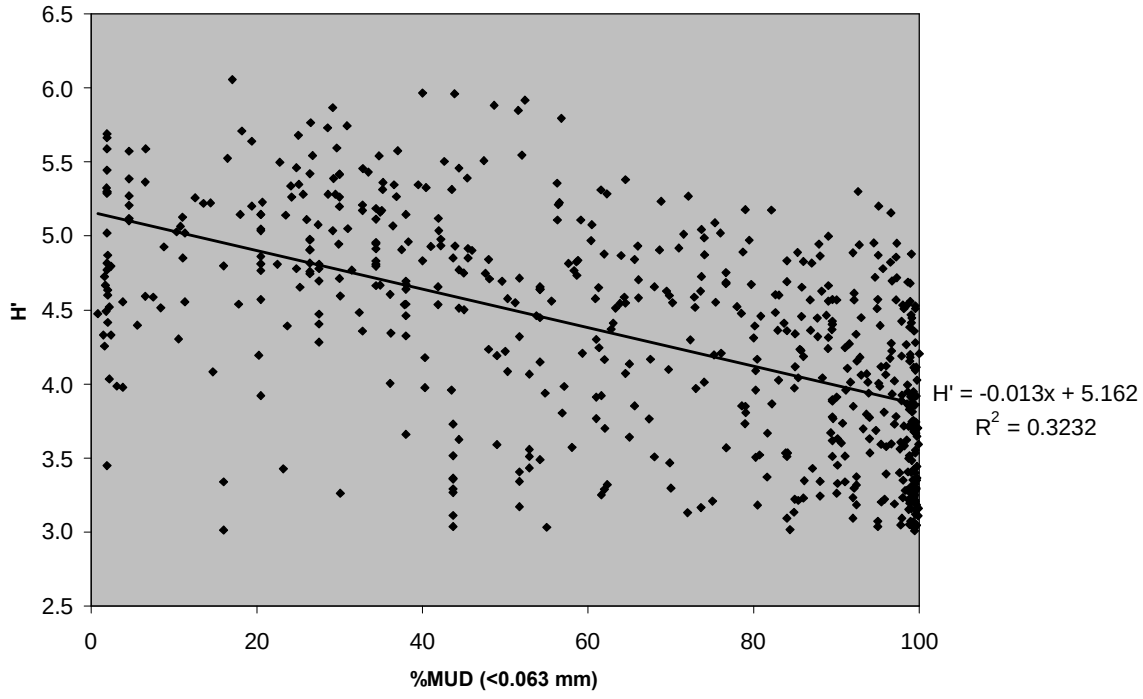

Figure 8. Correlation between H' (♦) vs. % mud at non-polluted stations.

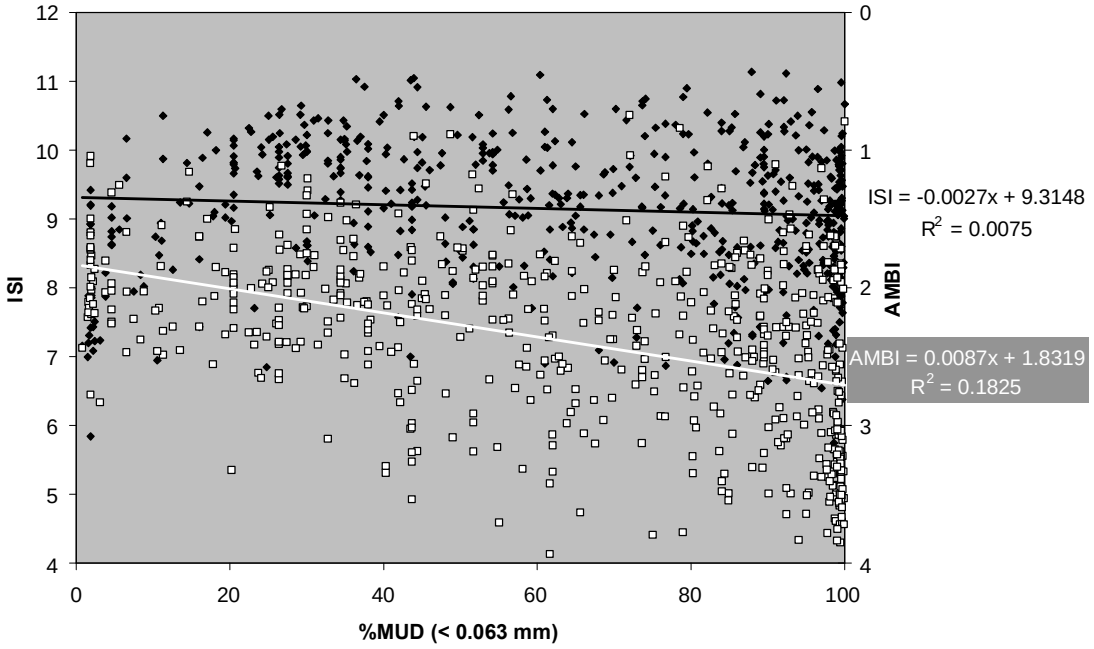

Figure 9. Correlations between ISI (♦) or AMBI (□) vs. % mud at non-polluted stations. AMBI values in reversed order.

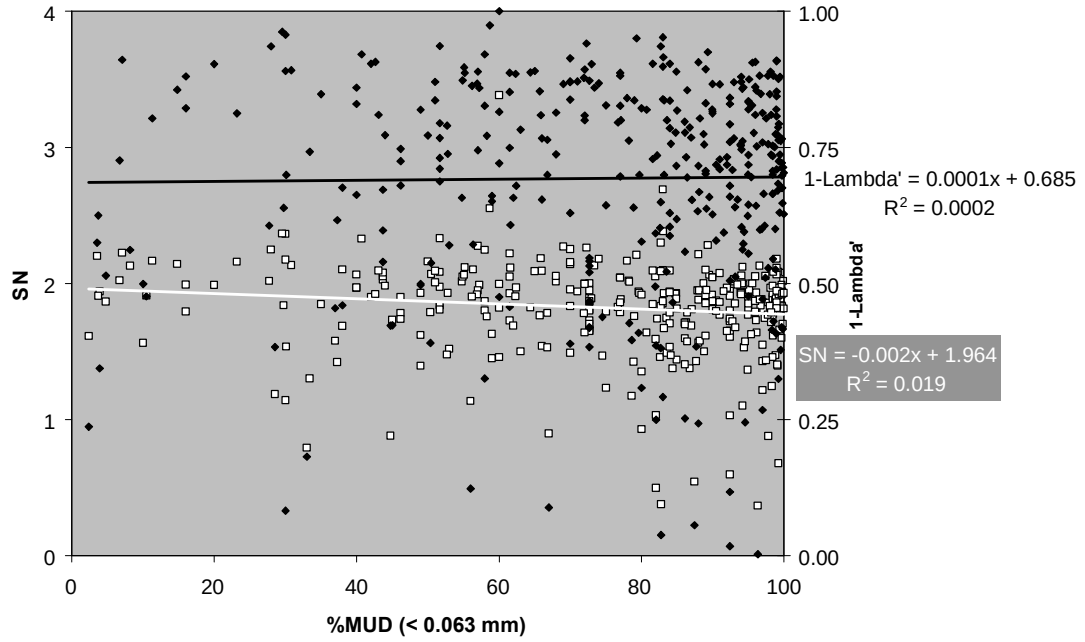

Figure 10. Correlations between SN (□) or 1-Lambda (♦) and % mud at polluted stations. The diversity index H' (not shown in the plot) also had a very weak correlation ($R^2 = 0.004$).

Since the 'x'-data (% mud) were the same for all indices, the different correlations implied that ranking of their values along the % mud values differed. A rank correlation test (Spearman) was therefore chosen for comparative analysis (**Table 8**).

Table 8. Rank correlation coefficients (rs-statistic) of different indices vs. % mud (< 0.063 mm) in the sediments of non-polluted and polluted stations. There were 591 samples from assumed non-polluted or non-disturbed stations, 348 samples from assumed polluted stations. The indices are sorted high-to-low in the table, according to their correlation value with % mud at the non-polluted stations.

Non-polluted		Polluted	
Index	Spearman	Index	Spearman
SN	-0.66	SN	-0.16
ES_{100}	-0.65	ES_{100}	-0.11
ES_{50}	-0.63	ES_{50}	-0.06
d	-0.60	*d*	-0.12
H'	-0.60	H'	-0.05
S_{04}	-0.56	S_{04}	-0.18
$\log_{10}(S+1)$	-0.56	$\log_{10}(S+1)$	-0.18
ES_{10}	-0.55	ES_{10}	-0.03
ES_5	-0.53	ES_5	-0.04
1-Lambda'	-0.51	1-Lambda'	-0.05
AMBI	0.45	AMBI	0.00
J'	-0.43	J'	-0.01
ISI	-0.14	ISI	0.07

S normalised to area=0.4 m^2 (S_{04}) had to be used instead of S when analysing the correlation with % mud. Rank of $\log_{10}(S+1)$ (Sweden) is equivalent to rank of S_{04}.

All indices (AMBI uses a reversed scale) showed negative correlations to % mud, but the strength of the correlations differed. The index showing the highest rank correlation with sediment grain size is probably the most sensitive index to differences caused by sediment grain size, and may be assumed to be a good descriptor of diversity changes caused also by other factors, e.g. pollution. The analyses indicate that 1-Lambda' may be a less good descriptor than SN, ES_{100}, ES50 or H'. The index H' is slightly less well correlated with sediment grain size than are SN, ES_{100} or ES_{50}. ES*n* seems to loose precision when choosing a low *n* (*n* = 5 or 10). ES_{50} or ES_{100} describe diversity almost as precisely as SN, but cannot be applied to samples having less than 50 (100) individuals. *SN is therefore the priority choice of diversity index for ecological status classification.*

Species number and evenness are the two basic parameters describing diversity in the fauna. The correlation coefficients in **Table 8** indicate that species number (S_{04}) is more dependent on sediment coarsness than evenness (J').

5. Correlation between SN and some other indices

Correlations between indices were calculated from all samples in the database. However, in order to avoid artefacts in the index values due to small number of animals, only samples with at least 6 individuals were included.

Figure 11 - **Figure 13** indicate the correlation between SN and some other indices. **Figure 14** indicates the correlation between the sensitive-species indices ISI and AMBI vs. SN.

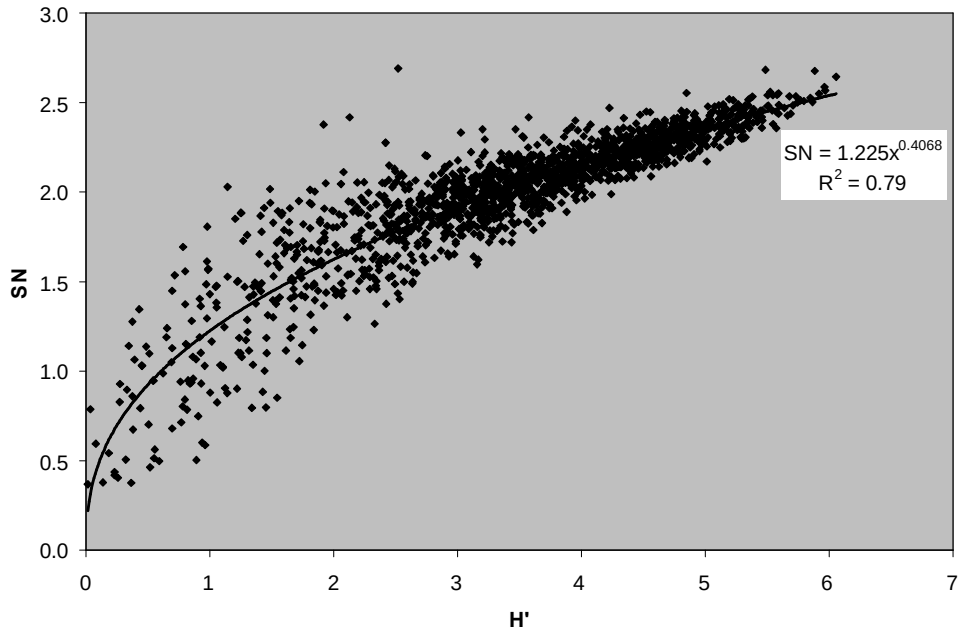

Figure 11. SN vs. H'. The plot shows a high correlation between SN and H' (Power regression, R^2=0.79; Spearman rank, rs statistic = 0.91).

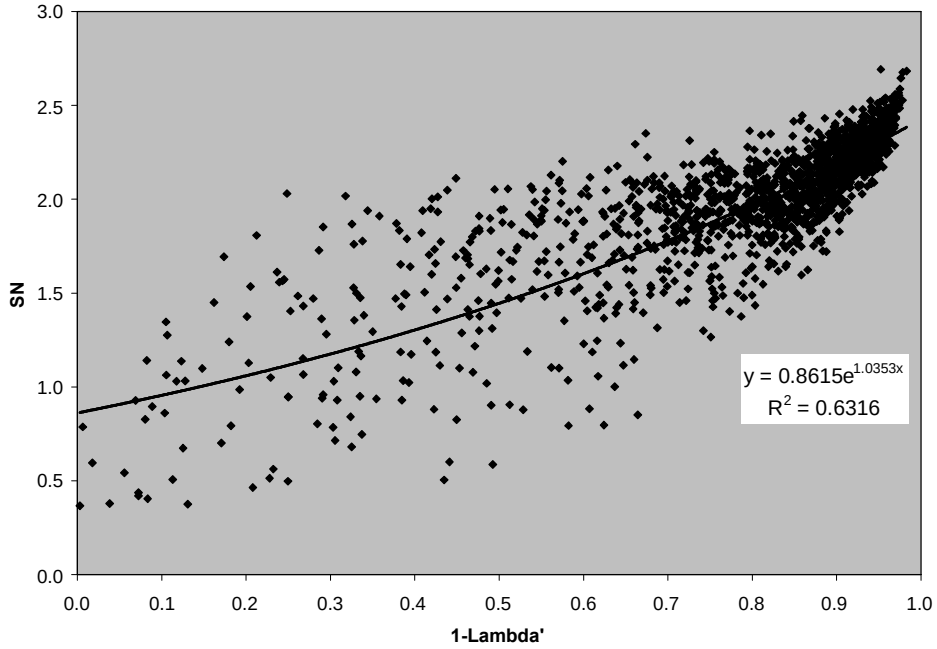

Figure 12. SN vs. 1-Lambda'. The plot shows a moderately high correlation between SN and 1-Lambda' (Exponential regression, R^2=0.63; Spearman rank, rs statistic = 0.84).

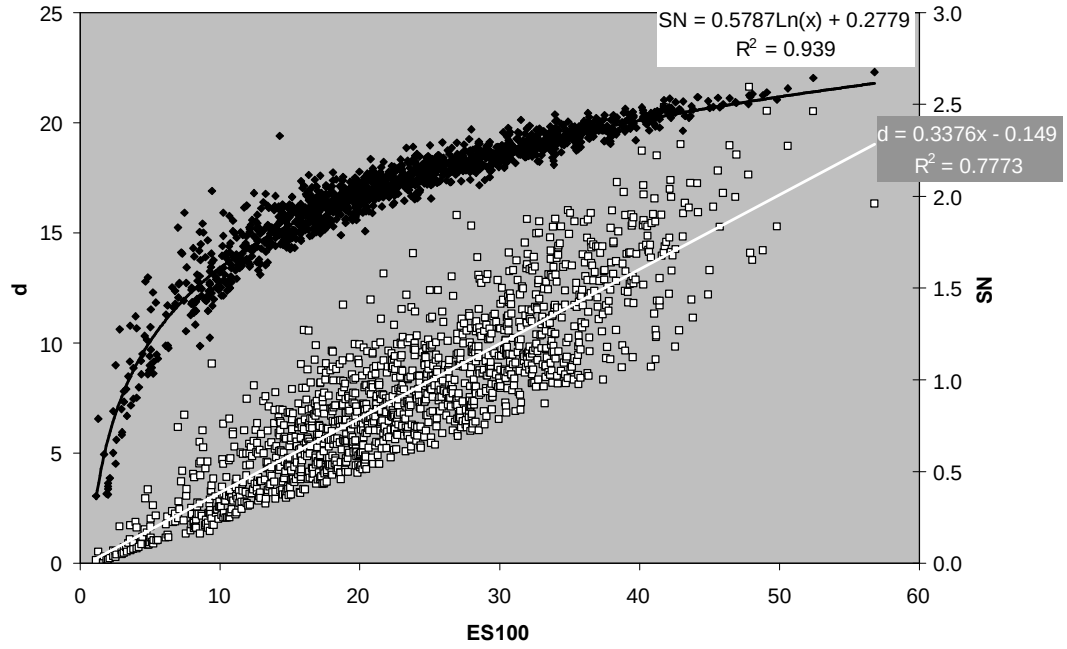

Figure 13. Species richness *d* (□) and SN (♦) vs. diversity ES_{100}. The correlation between SN and ES_{100} is high (logarithmic regression, R^2=0.939; Spearman rank, rs statistic = 0.97), tighter than between *d* and ES_{100} (linear regression, R^2=0.777; Spearman rank, rs statistic = 0.88).

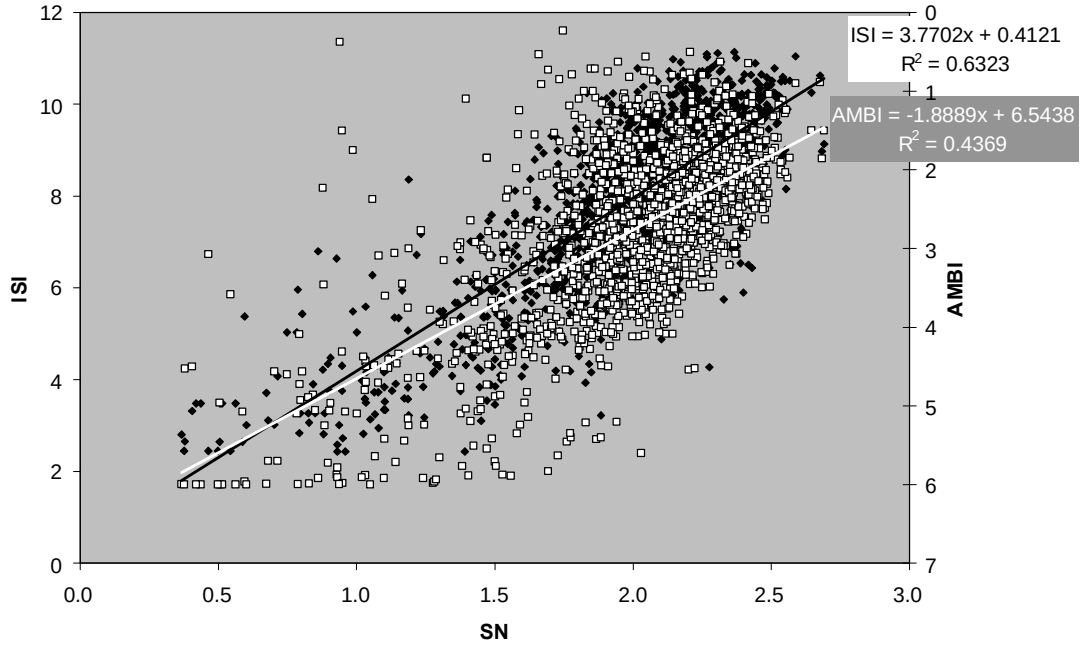

Figure 14. Correlations between ISI (♦) or AMBI (□) vs. SN at all stations (non-polluted and polluted). AMBI values in reversed order.

ISI was more highly correlated with SN than AMBI. This was expected, since ISI was derived using stations ES_{100}-values, and the SN and ES_{100} are closely correlated indices (**Figure 13**).

6. Normalisation of index values to sediment type and depth

As the results in **Figure 7**, **Figure 8** and **Figure 9** show, when comparing index values in samples from different sediment types, the values need to be normalised to common sediment type (sediment consisting of 100 % mud).

Mud = Sediment grains smaller than 0.063 mm.

6.1 Normalisation of species richness index (SN) to sediment type

SN_{63} = Observed SN normalised to 100% mud.

Expected SN at 100% mud = -0.0032*100 + 2.4191 = 2.0991 (regression equation in **Figure 7**)

Expected SN at observed % mud:

$$SN_{63} = SN_{obs} * \left[\frac{\text{expected SN at 100 \% mud}}{\text{expected SN at observed \% mud}} \right] = SN_{obs} * \left[\frac{2.0991}{-0.0032*\%mud_{obs}+2.4191} \right]$$

6.2 Normalisation of diversity index (H') to sediment type

H_{63} = Observed H' normalised to 100% mud.

Expected H' at 100% mud = -0.013*100 + 5.162 = 3.862 (regression equation in **Figure 8**)

Expected H' at observed % mud:

$$H_{63} = H_{obs} * \left[\frac{\text{expected H at 100 \% mud}}{\text{expected H at observed \% mud}} \right] = H_{obs} * \left[\frac{3.862}{-0.013*\%mud_{obs}+5.162} \right]$$

6.3 Normalisation of sensitive species index (AMBI) to sediment type

$AMBI_{63}$ = Observed AMBI normalised to 100% mud.

Expected AMBI at 100% mud = 0.0087*100 + 1.8319 = 2.7019 (regression equation in **Figure 9**)

Expected AMBI at observed % mud:

$$AMBI_{63} = AMBI_{obs} * \left[\frac{\text{expected AMBI at 100 \% mud}}{\text{expected AMBI at observed \% mud}} \right] = AMBI_{obs} * \left[\frac{2.7019}{0.0087*\%mud_{obs}+1.8319} \right]$$

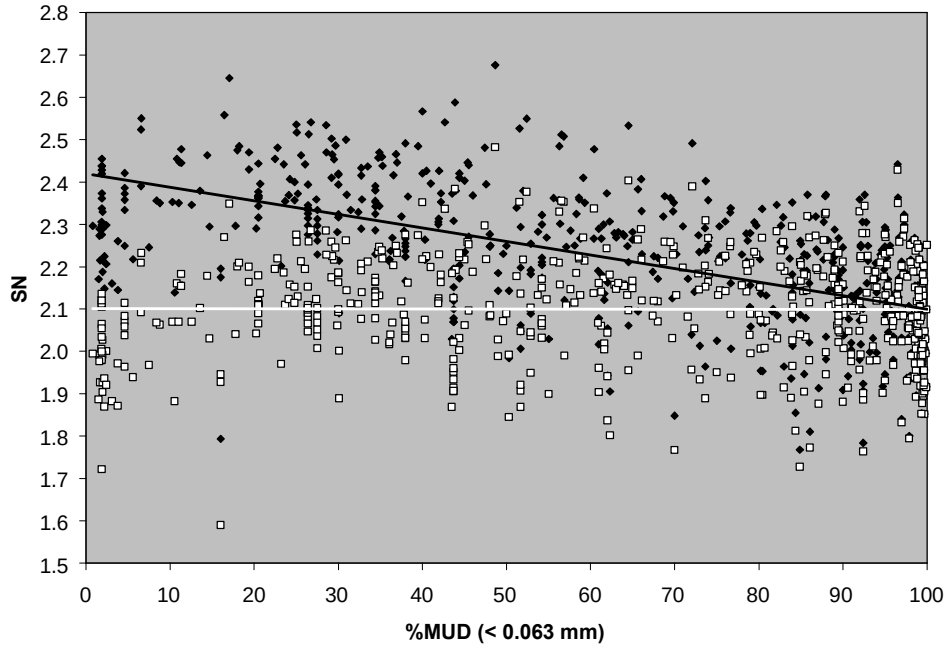

Figure 15. Plot of SN vs. % mud before (♦) and after (□) normalisation to 100% mud.

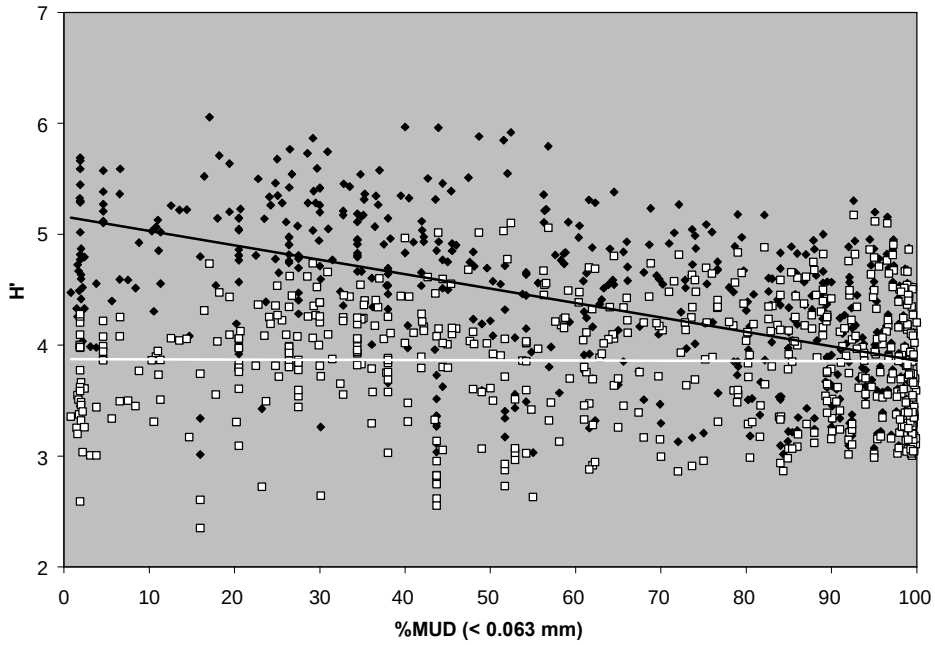

Figure 16. Plot of H vs. % mud before (♦) and after (□) normalisation to 100% mud.

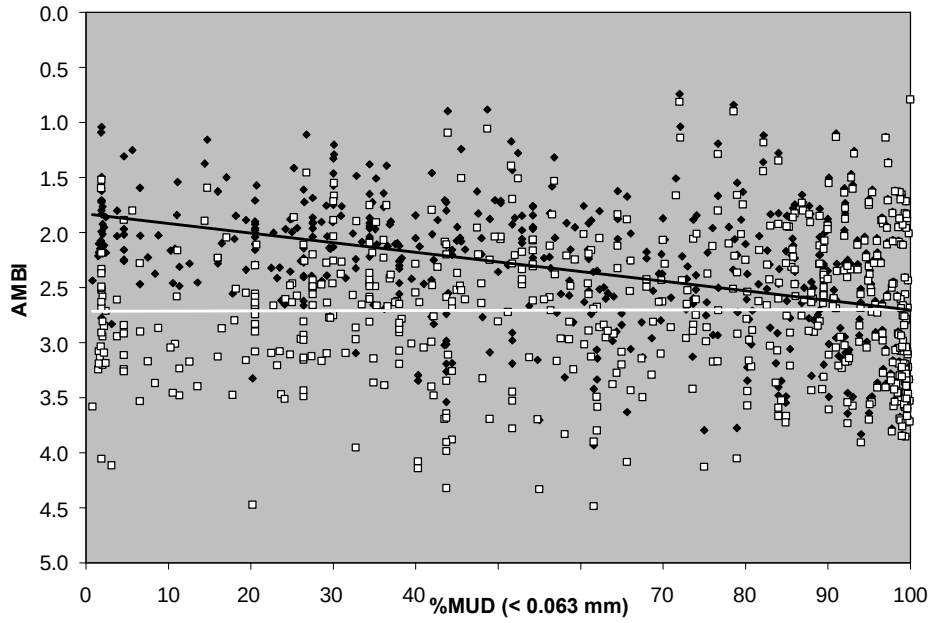

Figure 17. Plot of AMBI vs. % mud before (♦) and after (□) normalisation to 100% mud. AMBI values in reversed order.

6.4 Normalisation to depth

The relationships of the indices SN, H' and AMBI to depth were found to be weak and unsystematic (**Figure 18 - Figure 20**).

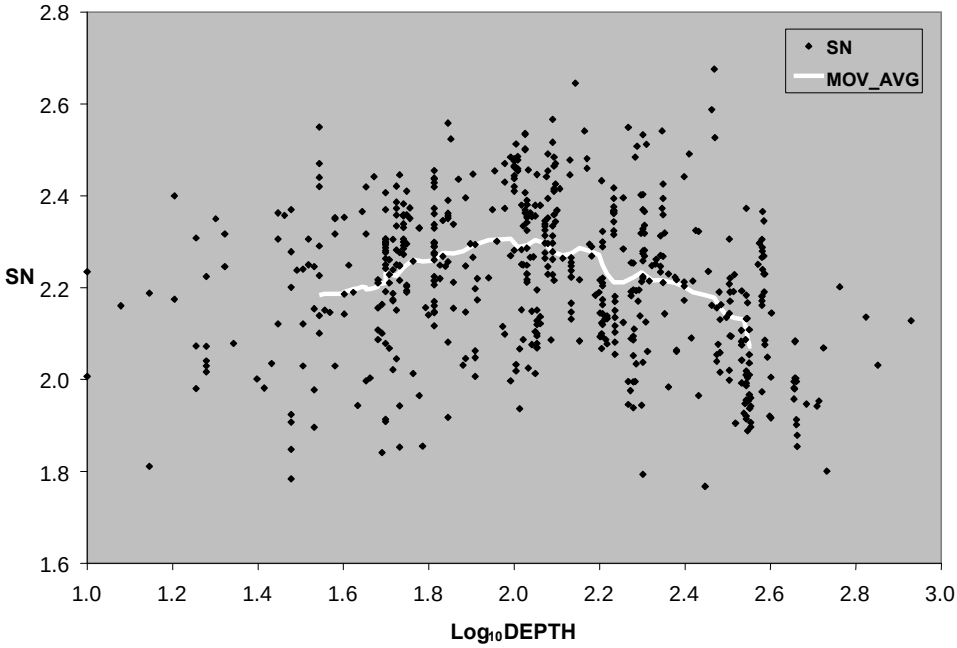

Figure 18. Plot of SN vs. $\log_{10}$ of depth. Moving average of SN (series=100) is shown.

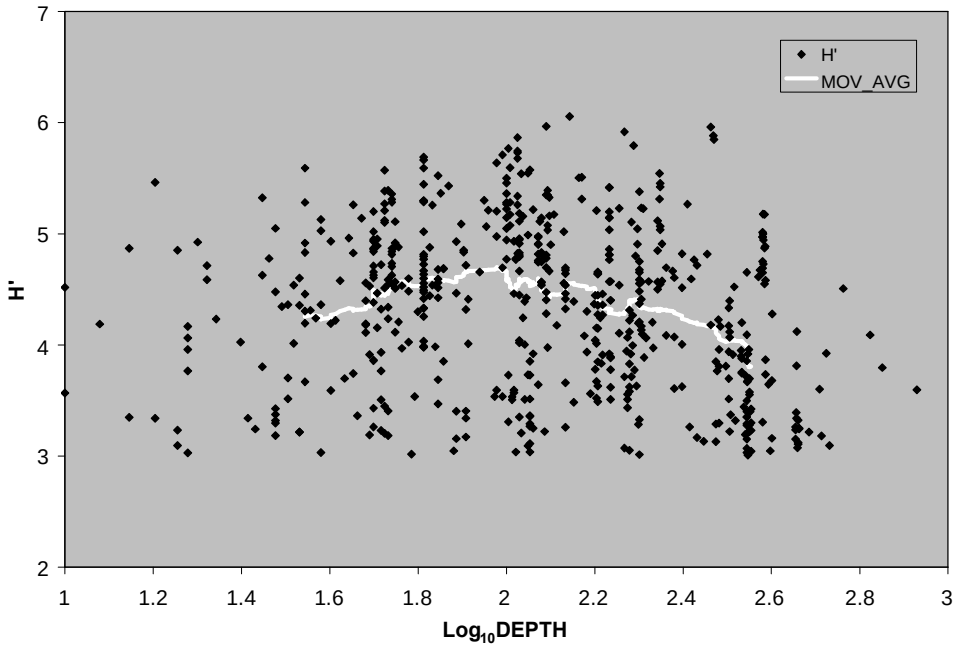

Figure 19. Plot of H' vs. $\log_{10}$ of depth. Moving average of H' (series=100) is shown.

Maximum values of SN and H' were observed at depths around 100 m (log = 2). This is in accordance with the relationship found between diversity (H' and ES_{100}) and depth by Rygg (1996). Considering the large variation in SN or H' at similar depths and lack of linear relationship, normalisation of SN or H' to depth is not recommended.

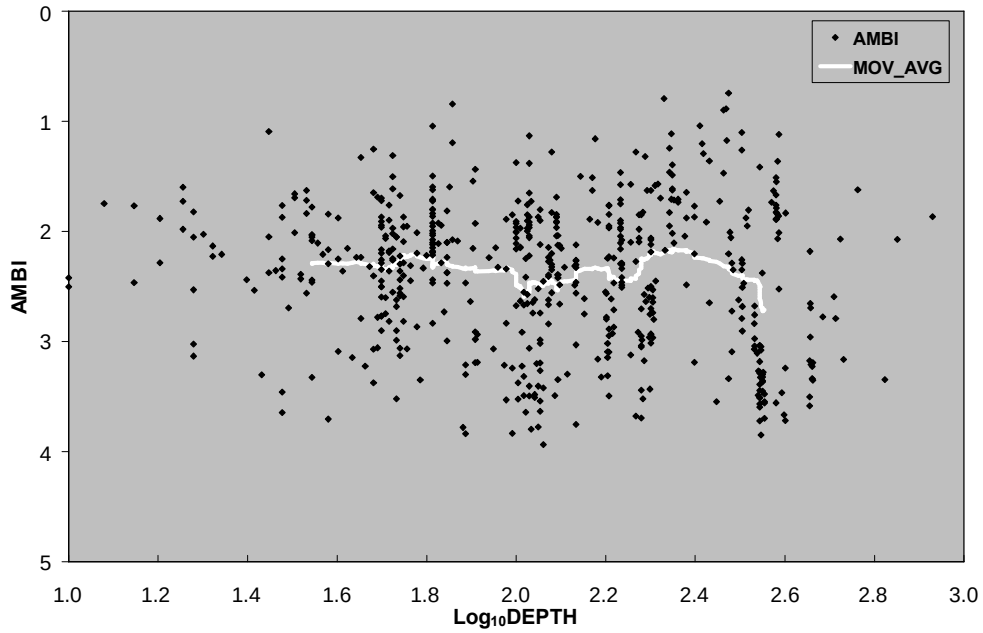

Figure 20. Plot of AMBI vs. $\log_{10}$ of depth. Moving average of AMBI (series=100) is shown. AMBI values in reversed order.

Considering the large variation in AMBI at similar depths and lack of linear relationship, normalisation of AMBI to depth is not recommended.

7. Suggested multimetric indices for classification of ecological status

7.1 Index alternative 1

Several countries have proposed multimetric indices consisting of three elements. In accordance with this, a Norwegian multimetric index is here suggested encompassing the following three elements:

1. Sensitivity (AMBI)
2. Diversity (SN)
3. A factor for downweighting artificially high index values of small samples (few individuals)

The index may take the form (Norway multimetric index 1) =

$$[\text{Weight1}*(1-\text{AMBI}_{63}/\text{AMBI}_{63}\text{max}) + \text{Weight2}*(\text{SN}_{63}/\ \text{SN}_{63}\text{max})*(N/(N+5))]$$

where $\text{AMBI}_{63}\text{max} = 7$, $\text{SN}_{63}\text{max} = 2.7$, and Weight1 + Weight2 = 1.0 (ensures an index value between 0 and 1; suggested values are Weight1 = Weight2 = 0.5

The correction factor (N/(N+5)) is adopted from the Swedish index (Blomqvist et al. 2006).

The maximum value of AMBI is 7 (no animals present). If one or more animals are present, the maximum value of AMBI is 6. The minimum value is 0, indicating the highest possible status (Borja et al. 2000). The choice of (1-AMBI/7) instead of AMBI/7 in the formula is because the AMBI values are reversed in relation to quality status (higher AMBI value = lower status).

To prevent artefact values of SN, the index should only be applied on samples with at least 6 individuals. If a sample contains at least 6 individuals, the highest possible value of SN is 3.07 (6 species each having 1 individual). Among the 1665 samples, the highest observed value of SN was 2.69. The maximum value of SN in the formula is set to 2.7. The minimum value of SN is 0 (only 1 species present). In cases where number of individuals are less than 6, specialist judgement is required, eventually the sample may be considered useless for classification purposes.

7.2 Index alternative 2

A correction factor to downweight artificially high index values at small sample sizes (few individuals) was proposed in the alternative-1 multimetric index (**7.1.**). Similar correction

factors were also included in the multimetric indices suggested by UK, Denmark and Sweden (Borja et al. 2006, Blomqvist et al. 2006).

However, choosing indices in the multimetric which show consistent values also at small sample sizes might make a downweighting correction factor superfluous. Therefore, a further comparison of the performance of different indices at small sample sizes (few individuals) was carried out.

Values of the diversity indices SN, H' and 1-Lambda' and the sensitivity indices AMBI and ISI were calculated on 122 small samples (40 or less individuals), and their behaviour along the span of N plotted (**Figure 21**). At each N (when N>1) different number of species and different evenness values were involved, causing variance in the diversity values. In particular, the occurrence of species with different sensitivity values caused high variability in the sensitivity indices (AMBI and ISI).

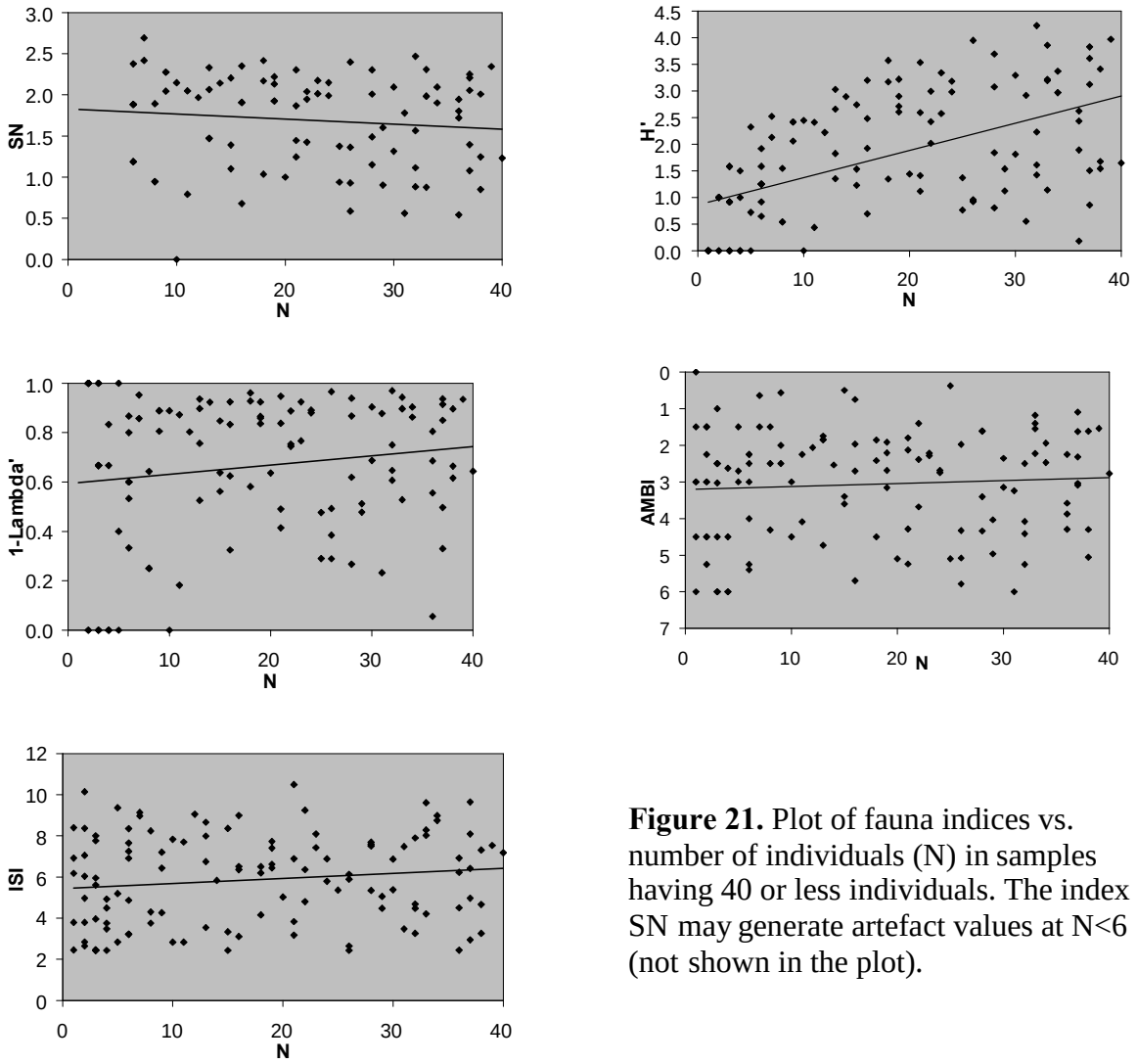

Figure 21. Plot of fauna indices vs. number of individuals (N) in samples having 40 or less individuals. The index SN may generate artefact values at N<6 (not shown in the plot).

The results indicate that the diversity index H' maintains low values at low N. The reason for this is that H' is dependent on species number (S), which necessarily must be low when N is low. The other indices sometimes show very high values also at low N, at other times, very low values. In other words, their values are very variable at low N. In some cases therefore, when index values are incidentally low, the downweighting "correction" could in fact be an "incorrection".

As an alternative-2, a multimetric index may be suggested incorporating H' as the diversity element and omitting the correction factor:

This index may take the form (Norway multimetric index 2) =

$$[\text{Weight1}*(1-\text{AMBI}_{63}/\text{AMBI}_{63}\text{max}) + \text{Weight2}*(\text{H}_{63}/\text{H}_{63}\text{max})]$$

The maximum value of AMBI is 7. Values of H' practically never exceed 6 (maximum value of H' in the NIVA database is 6.06). In the multimetric index, maximum value of H' is set to

6. The suggested values of the weights are Weight1 = Weight2 = 0.5 ensuring an index value between 0 and 1.

Index 2 = [0.5*(1-$AMBI_{63}$/7) + 0.5*(H_{63}/ 6)]

7.3 Comparison of index 1 and index 2

Correlation plot of index 1 (containing diversity element SN) and index 2 (containing diversity element H') is shown in **Figure 22**. The correlation is very high. The similarity with the plot of SN vs. H' (**Figure 11**) is evident.

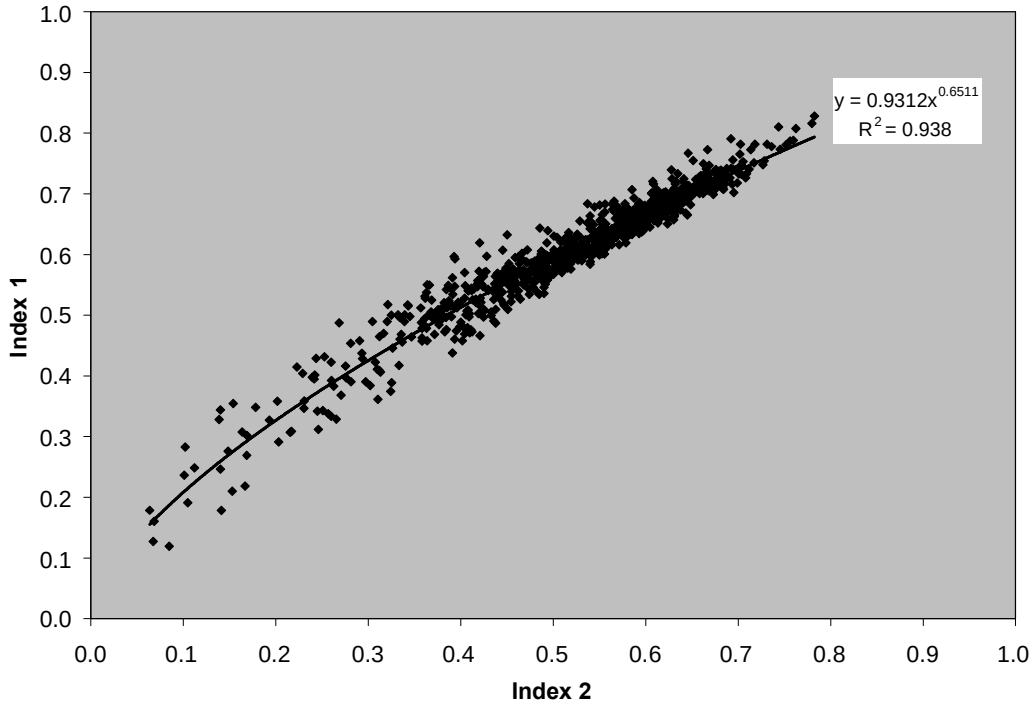

Figure 22. Correlation plot of index 1 and index 2, with regression curve (power).

Figure 23 shows index values at low number of individuals in the samples. N below 6 may generate artificially high values of SN (the diversity element in index 1). The use of H' (diversity element in index 2) prevents high index values at low N.

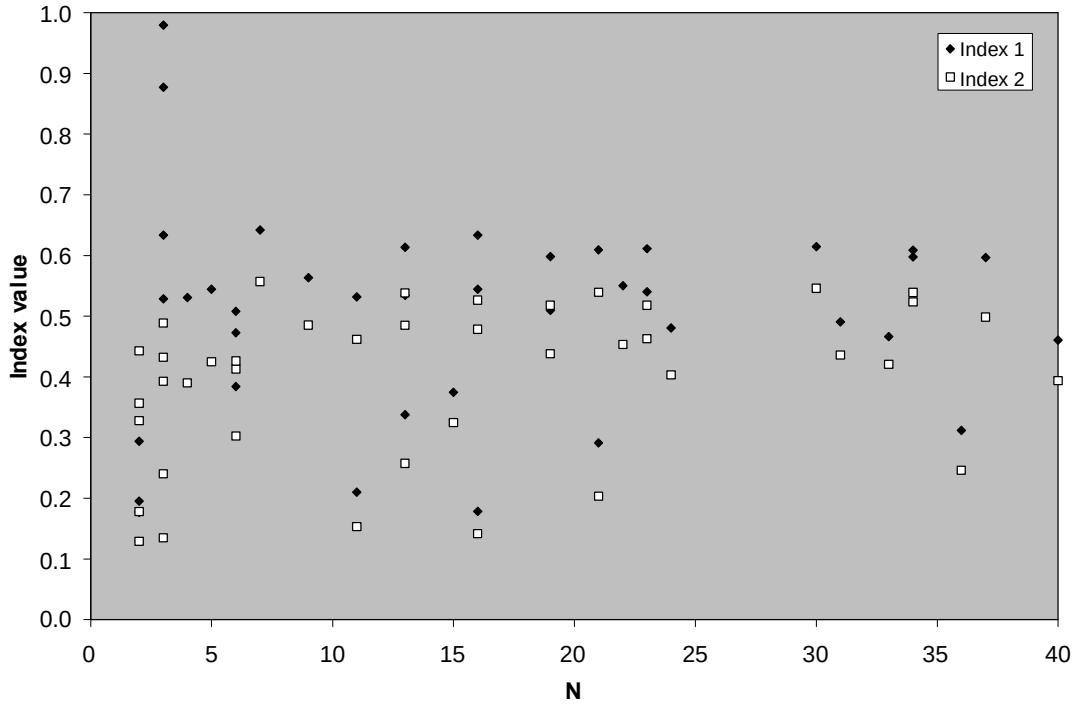

Figure 23. Index values at low N.

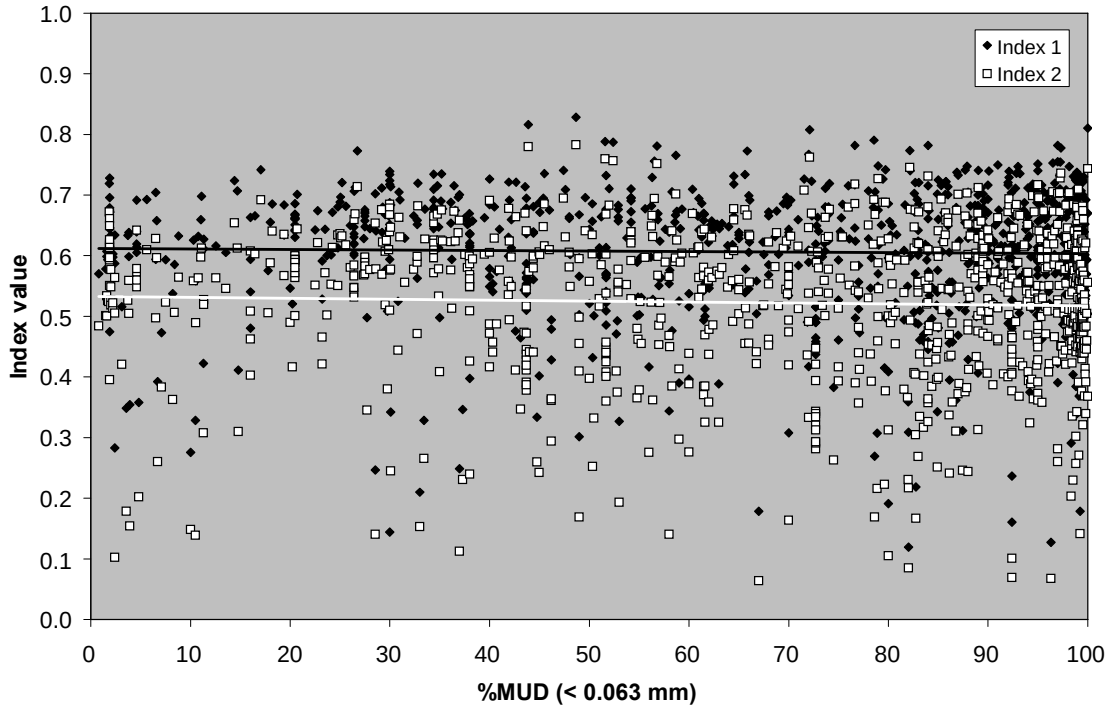

Figure 24. Linear regression of multimetric indices vs. % mud.

Since the elements in the multimetric indices were normalised to 100% mud in the sediment, the index values were expected to be independent of sediment coarseness. This assumption seems to hold good (**Figure 24**).

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