

# Large-scale patterns in the community structure and biodiversity of freeliving nematodes in the Bohai Sea, China

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Freeliving marine nematodes were sampled on two occasions from an extensive grid of 20 stations in the Bohai Sea and its approaches. Differences within stations between sampling periods were small, resulting from small changes in abundance of dominant species. Differences between stations were significant, and were used to cluster stations into groups with similar species composition. These station groupings revealed a weak faunal gradient leading from the mouth of the Huanghe (Yellow River) to the Bohai Strait. Analyses relating faunal composition to environmental variables showed that there were significant differences in environmental variables between faunally-defined groups of stations. The variables most closely correlated with community structure were silt/clay and sand, depth, phaeopigment concentrations below the sediment surface, organic content and arsenic. These reflect natural processes within the Bohai Sea. A suite of univariate measures were related to distance from the river mouth, with a major discontinuity about 120 km into the Bohai Sea. Comparison of values of the biodiversity measures average taxonomic distinctness ( $\Delta^+$ ) and variation in taxonomic distinctness ( $\Lambda^+$ ) suggest that the meiobenthos of the Bohai Sea as a whole is not under major pollution stress.

## INTRODUCTION

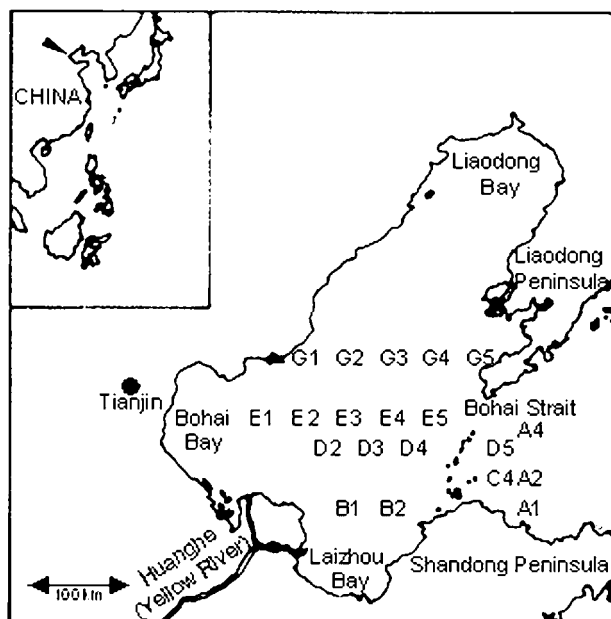
The Bohai Sea has an area of approximately 77,000 km<sup>2</sup>. It is a marginal sea with restricted water-exchange, enclosed by the Liaodong and Shandong peninsulas and connected to the Huanghai (Yellow Sea) by the Bohai Strait (Figure 1). It is shallow, with a maximum depth of 86 m (in the Bohai Strait) and an average depth of 18 m (Geng, 1981). The area is of great commercial importance, providing important spawning and feeding grounds for many species of fish and shellfish and supporting extensive fisheries and mariculture, particularly of prawns, bivalve molluscs, holothurians and nudibranchs. In recent years the Bohai Sea has also been subject to intensive offshore exploration for, and production of, natural gas and petroleum reserves (Fan & Zhang, 1988). The Bohai Sea is considered to be highly polluted and the state of the environment in the area is of great concern to the Chinese government and agencies. Although considerable effort is invested in measurement and monitoring of environmental variables (Xu & Zheng, 1991) little information is available to the wider scientific community.

Although overfishing was recognized as a problem as far back as 1955, when certain areas were closed to fishing, the Bohai Sea has no closed fishing seasons. In the early 1970s industrial and domestic expansion on the shores, and in the catchments of rivers entering the Bohai began to create massive pollution and gave rise to deleterious impacts on aquatic life, particularly in the intertidal

zone (Fan, 1989). Approximately 137 rivers flow into the Bohai Sea, bringing in some 2.8×10<sup>9</sup> tn of waste water and 7×10<sup>5</sup> tn of other pollutants each year. This approximates 50% of China's total maritime discharge of pollutants. According to the Chinese State Environmental Protection Administration the combination of overfishing and pollution, from excessive amounts of waste water (including industrial effluent) and untreated sewage, has severely depleted marine resources in the Bohai Sea. Red tides have become frequent, occurring more than 30 times in the Bohai Sea between 1991 and 1998. Although the blooms are not generally toxic they damage fishery resources by reducing oxygen levels, with losses since 1990 running into millions of dollars.

Meiofauna have evoked considerable interest as potential indicators of anthropogenic perturbation in aquatic ecosystems (see review by Coull & Chandler, 1992), free-living nematodes have been shown to be sensitive to a range of anthropogenic disturbances (Coull & Chandler 1992; Somerfield et al., 1995), and large-scale studies are useful precursors to applied monitoring programmes (Schratzberger et al., 2000). In order to assess the impacts of energy exploration and increased pollution on mariculture activities a national and international oceanographic and ecosystem study of the Bohai Sea has been launched which includes annual sampling for benthos, including meiofauna, from a large-scale grid of stations.

This study explores relationships between nematode community structure and environmental variables reflecting natural and anthropogenic influences in



**Figure 1.** Map of the Bohai Sea, showing major geographical features and sampling stations.

samples from this programme and has three objectives: (1) to document large-scale patterns in nematode community structure in the Bohai Sea; (2) to relate the observed patterns to measured environmental variables reflecting natural and anthropogenic influences; and (3) to evaluate whether nematode community structure is affected by the pollution status of the Bohai Sea.

## MATERIALS AND METHODS

### *Field sampling*

A grid of 20 stations, giving a broad geographic coverage of the Bohai Sea and the Bohai Strait (Figure 1) was selected. Two samples were collected from each station during September 1998 and one in April/May 1999. Undisturbed sediments were brought on-deck using a modified 0.1 m<sup>2</sup> Gray-O'Hara box-corer. In order to reduce the influence of small-scale spatial variability within box-cores, three subsamples from each box-core were taken using a sawn-off syringe (internal diameter 26 mm) to a depth of 50 mm, taking care to avoid core-compression in the process. These subsamples were pooled and preserved in 5% formalin pending further analysis. Additional samples for the later determination of environmental variables were also collected from each box-core and frozen at  $-20^{\circ}\text{C}$ .

### *Faunal analysis*

Each sample was stained with rose-Bengal for 24 h and then washed on a 48- $\mu\text{m}$  sieve to remove formalin and some of the fine sediment fraction. Heavier sediment particles were removed using centrifugation in Ludox-TM with a specific gravity adjusted to 1.15 (Heip et al., 1985; Warwick et al., 1998). A 1/5 subsample of the remaining material was taken from which, after being

counted, nematodes were picked out and placed in a mixture of 5% glycerol, 5% ethanol and 90% water in a cavity block. This mixture was partially covered and placed in a desiccator for a few days, leaving the nematodes in pure anhydrous glycerol. Nematodes were mounted on slides using individual mounts for samples from the first cruise, and ecological bulk mounts (Sommerfield & Warwick, 1996) for samples from the second cruise. All nematodes in the subsample were identified to putative species using a compound microscope, bright-field illumination and a  $\times 100$  oil immersion lens.

### *Analyses of environmental variables*

Table 1 summarizes the environmental variables measured. Following extraction of dried sediment with C<sub>6</sub>H<sub>6</sub>, oil content was determined using fluorescent spectrophotometry (Welz & Sperling, 1999). As, Cu, Pb, Cr and Cd concentrations were determined by non-flame atomic absorption spectrophotometry following digestion with HClO<sub>4</sub>-HNO<sub>3</sub>. Hg concentrations were determined using cold atomic spectrofluorometry following digestion with HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub>. Organic content was determined by titrating, with FeSO<sub>4</sub>, the excess K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> not reduced after sediments were digested with a K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>-H<sub>2</sub>SO<sub>4</sub> mixture. For the determination of chlorophyll-*a* and phaeophorbide concentrations, samples were extracted using acetone in the dark and analysed spectrophotometrically. For the determination of granulometry of particles > 63  $\mu\text{m}$  in diameter sediment samples were wet-sieved, and the different fractions dried at 95°C and weighed. Pipette analysis was used to determine the distribution of particles < 63  $\mu\text{m}$  in diameter (Buchanan, 1984).

### *Data analyses*

For the majority of analyses non-parametric multivariate techniques were used. Most of these techniques are discussed Clarke & Warwick (1994), and are included in PRIMER (Plymouth Routines In Multivariate Ecological Research) version 5.

The significance of Spearman rank correlations, adjusted for ties, between corresponding elements in similarity/dissimilarity matrices was determined using

**Table 1.** Suite of environmental variables measured.

Total hydrocarbons	Total organic content	Sediment water content
Cu	Total Chlorophyll- <i>a</i>	Gravel fraction
Pb	Chlorophyll- <i>a</i> 0–2 cm in sediment	Sand fraction
Cr	Chlorophyll- <i>a</i> 2–5 cm in sediment	Silt fraction
As	Total phaeopigments	Clay fraction
Hg	Phaeopigments 0–2 cm in sediment	Silt/clay fraction
Cd	Phaeopigments 2–5 cm in sediment	Median particle diameter
		Quartile deviation
		Skewness
		Sorting
		Water depth

the non-parametric Mantel test RELATE (Clarke & Warwick, 1994; Somerfield et al., 1995). Relationships between multivariate community structure and environmental variables were examined using BVSTEP (Clarke & Warwick, 1998a), a stepwise algorithm which sets out to select subsets of variables from one matrix which maximize the correlation between their intersample distances (defined in terms of some similarity or dissimilarity measure), and another similarity/dissimilarity matrix. In this case the algorithm was used to select the subset of environmental variables from which a normalized Euclidean distance matrix provided the 'best match' with a Bray–Curtis similarity matrix derived from  $\sqrt{\cdot}$ -transformed nematode abundances.

Univariate measures calculated included: total abundance ( $A$ ), number of species ( $S$ ), Shannon–Wiener diversity index ( $H'$ , calculated using natural logarithms), Pielou's evenness measure ( $J'$ ) and Simpson's dominance index ( $\lambda$ ). In addition to these more traditional measures, two biodiversity indices based on the taxonomic relatedness of species, average taxonomic distinctness  $\Delta^+$  (Clarke & Warwick, 1998b) and variation in taxonomic distinctness  $\Lambda^+$  (Clarke & Warwick, 2001), were calculated. Both indices are sample-size independent and may therefore be compared across studies with differing and uncontrolled degrees of sampling effort. Being based on the presence/absence of species, they can be calculated using simple species lists.  $\Delta^+$  is the mean path-length through the taxonomic tree connecting every pair of species recorded at a location.  $\Lambda^+$  is the variance of these pairwise path-lengths, and reflects the evenness of the taxonomic tree. Both indices can be tested for departure from expectation by constructing simulation distributions using random subsets from a master list of species. The joint distribution of both indices can be summarized as probability contours in a 2-d ( $\Delta^+$ ,  $\Lambda^+$ ) plot, against which real values can be compared.

## RESULTS

A total of 168 putative species of nematodes were identified, making up 79–95% of the total meiofauna at the various sampling stations. This study is concerned with patterns of small animals (requiring small samples) over a large area. In order to be assured that conclusions drawn from the dataset are robust, two facts need to be confirmed. The first is that the spatial patterns observed are consistent, and not a chance consequence of sampling. The second is that these patterns are consistent through time. The minimal replication within the sampling design is adequate for two-way ANOSIM tests addressing these issues. A two-way ANOSIM test for differences in community structure between cruises and stations, based on Bray–Curtis similarities calculated using untransformed nematode abundances, shows that variability within stations is very much less than variability between stations (Global  $R=0.91$ ,  $P<0.001$ ), but also that there is a significant difference between samples collected in the two cruises (Global  $R=0.40$ ,  $P=0.02$ ). Repeating the analysis following a mild ( $\sqrt{\cdot}$ ) transformation of the data again shows the importance of the differences between stations ( $R=0.89$ ,  $P<0.001$ ) but the difference between the cruises is no longer significant ( $R=0.2$ ,  $P=0.17$ ). The difference in

results from analyses based on raw and mildly transformed abundances shows that changes in abundance of dominant species cause the small differences in nematode community structure between the two cruises.

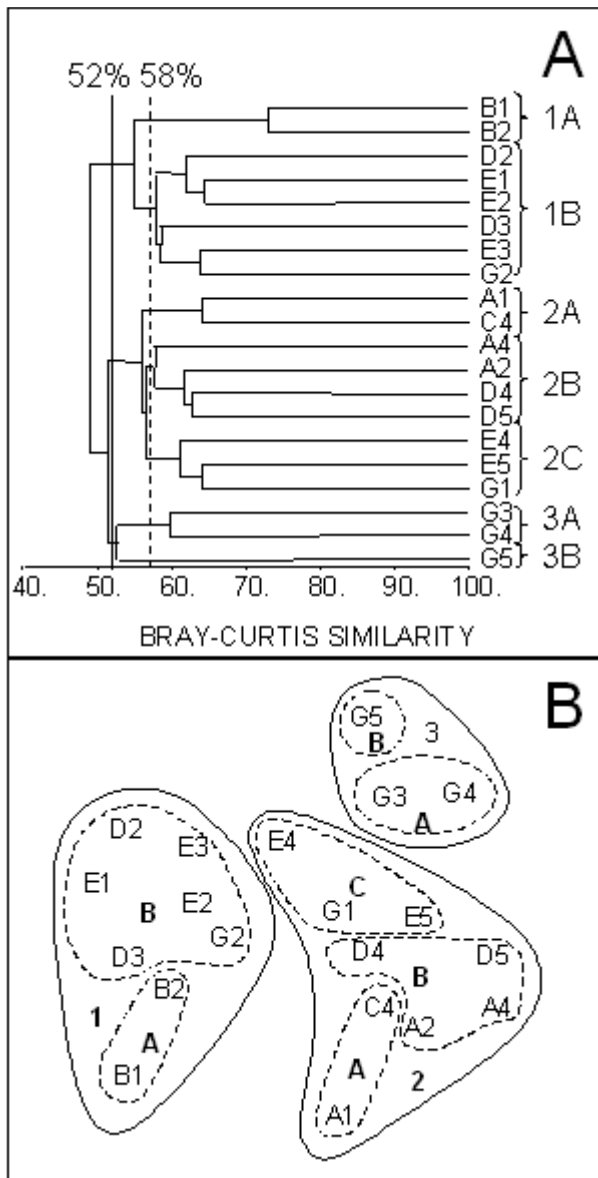
Results from RELATE tests (Table 2) between similarity matrices derived from  $\sqrt{\cdot}$ -transformed abundances from the two cruises show that the patterns of changes in community structure at the different stations are significantly correlated. Furthermore, correlations between interstation distances, nematode community structure in the two cruises, and variation in the measured environmental variables, are all significant. These results confirm that the spatial patterns observed are consistent, not a chance consequence of sampling, and that differences in community structure between the two cruises are relatively minor in comparison to the differences between different stations. Additional replicate samples would be required if pairwise tests of differences between stations or times were required, but in the context of examining large-scale patterns in a correlative framework the data are adequate.

In order to examine variation in community structure across stations, a Bray–Curtis similarity matrix was constructed using the weighted-average abundances of nematodes from the two cruises, and a  $\sqrt{\cdot}$  transformation. The correlation of this matrix with interstation distances ( $\rho=0.520$ ,  $P<0.001$ ) indicates that the differences between stations in this matrix are similar (as expected) to those in the original matrices from which it is derived, and that there is a significant relationship ( $\rho=0.299$ ,  $P=0.003$ ) with the measured environmental variables. The dendrogram derived from this matrix (Figure 2A) is remarkably flat, with the majority of divisions occurring at similarities between 50 and 65%. Thus the overall pattern of variation in community structure between stations is not marked, and it is unlikely that strong gradients in community structure exist. Arbitrary division of the samples at similarity levels of 52 and 58% (Figure 2A) was used to produce two sets of sample groupings for further analysis. At the 52% level, three groups of samples (labelled 1, 2 and 3) are defined, whereas at the 58% level these three groups are further subdivided into a total of seven groups (labelled 1A and B, 2A–C, and 3A

**Table 2.** Nonparametric Mantel (RELATE) tests of relationships between Bray–Curtis similarity matrices derived from  $\sqrt{\cdot}$ -transformed nematode abundances, and normalized Euclidean distance matrices derived from interstation distances and  $\ln$ -transformed environmental variables.

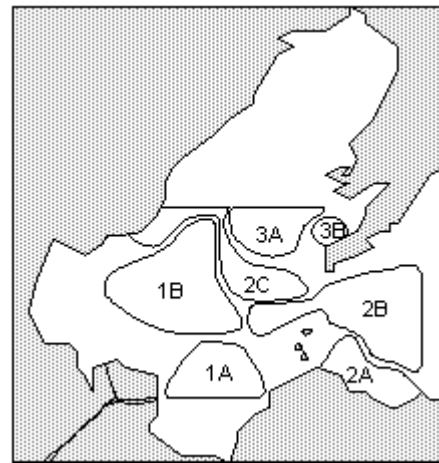
	$\rho$	$P$	$\rho$	$P$	$\rho$	$P$
Nematodes, 1999	0.319	0.001				
Station location	0.535	<0.001	0.164	0.037		
Environmental variables	0.228	0.017	0.323	0.005	0.269	0.003
			Nematodes, 1998	Nematodes, 1999	Station location	

$\rho$ , Spearman's rank correlation (adjusted for ties), calculated between corresponding elements in each matrix;  $P$ , significance estimated by permutation of one of the matrices and recalculation of the statistic.

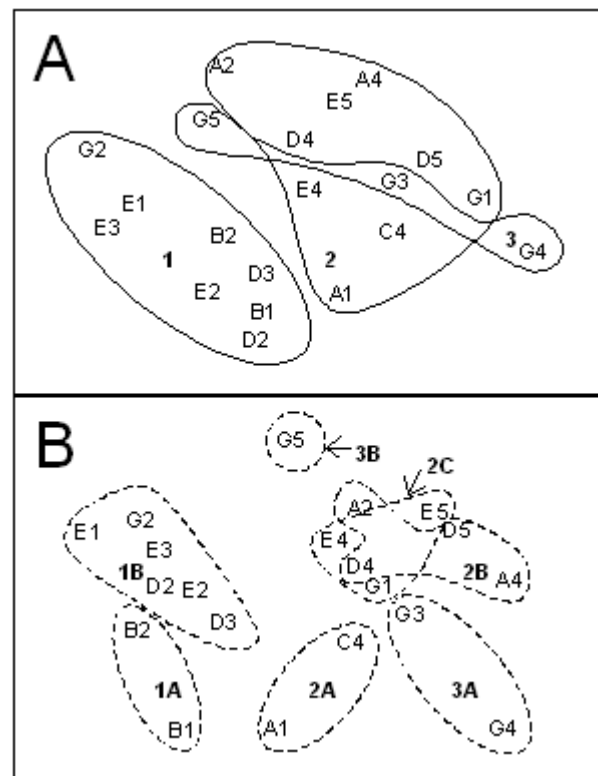


**Figure 2.** Analyses of Bray–Curtis similarities between  $\sqrt{}$ -transformed weighted average abundances from each station: (A) dendrogram derived by hierarchical agglomerative clustering using group average linkage, showing arbitrary division into three and seven groups at 52 (solid lines) and 58 (dashed lines) % similarity; (B) MDS ordination (stress=0.13). Superimposed clusters are divisions into three and seven groups based on cluster analysis (A).

and B). Overlaying these groupings on the multidimensional scaling (MDS) plot derived from the same similarity matrix (Figure 2B) clarifies the inter-relationships between them. Stations in group 1 are from the inner Bohai Sea (Figure 3), group 1A in Laizhou Bay, and group 1B in the western part of the central Bohai. Stations in group 2 are generally from the eastern part of the Bohai. The three groups 2A, 2B and 2C are clearly geographically separated, 2A representing stations from the north-west and central parts of the study area, 2B stations from the eastern central Bohai, the Bohai Strait and the eastern parts of the study area, and 2C coastal stations to the south of the Bohai Strait. Stations in group 3 are from the southern part of Liaodong Bay, 3B

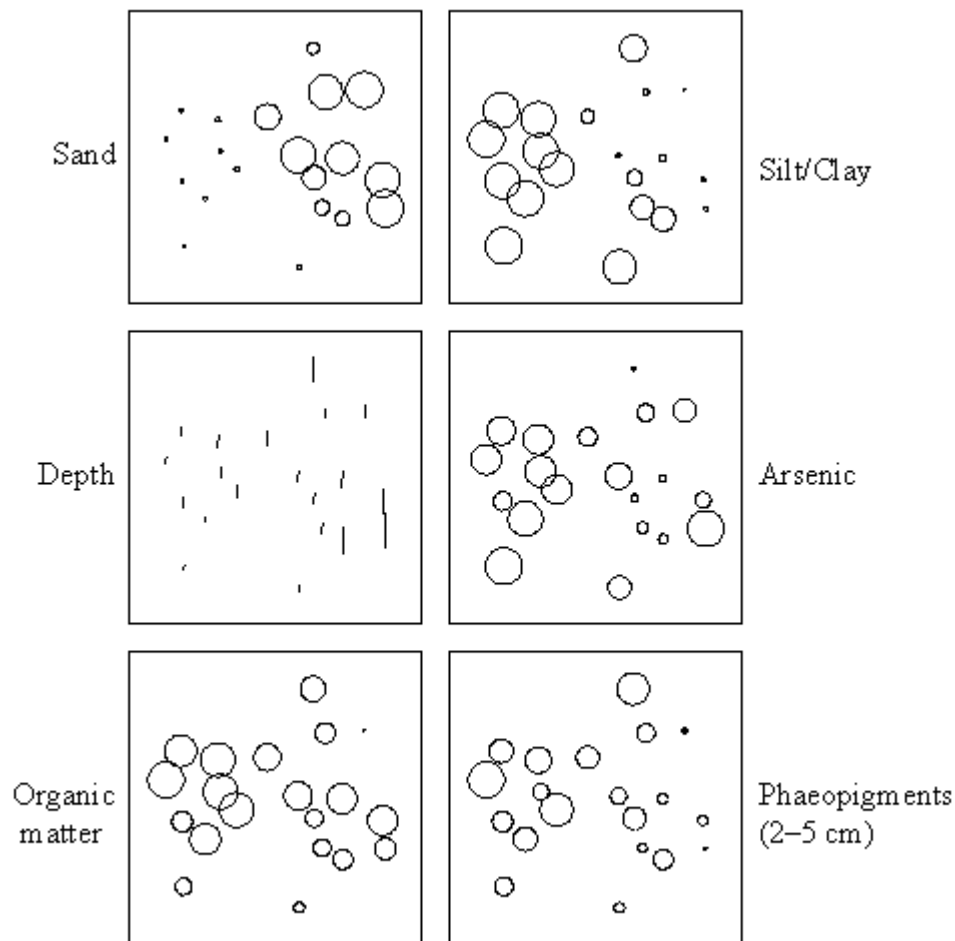


**Figure 3.** Schematic map of the Bohai Sea, showing the areas occupied by each of the faunally defined station groups.



**Figure 4.** Principal component analysis ordinations of sites based on environmental variables: (A) full suite of measured environmental variables (total variance explained by the first two principal components=64%). Superimposed clusters are station groups with a faunal similarity of 52%; (B) six variables (ln-As, ln-organic matter, silt/clay, sand, depth, phaeopigments at 2–5 cm) best explaining variation in nematode community structure (total variance explained by the first two principal components=71%). Superimposed clusters indicate station groups with a faunal similarity of 58%.

represented by a single station (G5) close to the Liaodong Peninsula, and 3A a pair of stations further offshore. The conclusion from these analyses is that there are consistent, if small, differences in nematode community structure



**Figure 5.** Multidimensional scaling plots derived from nematode abundances (see Figure 2B) overlain with symbols proportional in size to values of individual environmental variables in order to visualize the relationship between variation in community structure and variation in the individual variable.

between groups of stations related to their position in different parts of the Bohai Sea.

Within the suite of measured environmental variables (Table 1) some, like oil and heavy metals, reflect possible anthropogenic inputs, some such as organic content and pigment concentrations reflect productivity, and others like sediment parameters and depth reflect natural variability. Surprisingly, no intervariable correlations (Pearson or Spearman) are high ( $r > 0.95$ ) with the exception of two with obvious mechanistic links, namely median particle diameter with clay content, and percentage of sand with percentage of silt/clay. This suggests that gradients over the area as a whole are not strong.

Principal component analysis (PCA) of the measured environmental variables (Figure 4A) shows that the primary division in environmental variables between the three biotically defined groups of stations is between biotic group 1 and the other two. ANOSIM confirms the significance of these differences ( $R=0.487$ ,  $P < 0.001$ ). BVSTEP was used to explore the relationship between groups of environmental variables and the biotic similarity matrix, and it consistently defined a group of six variables: As; organic content; phaeopigment concentrations 2–5 cm below the sediment surface; % sand; % silt/clay; and depth, as providing the best match ( $\rho=0.52$ ) with variations in nematode community structure. The PCA of this

subset of variables (Figure 4B) shows that there is a close match between the majority of the seven biotically groups of stations and their measured environmental variables. Analysis of similarities (ANOSIM) again confirms the significance of these differences ( $R=0.401$ ,  $P=0.002$ ). Taking each variable in turn, BIOENV identified sand and silt/clay as providing the best match ( $\rho_w=0.353$ ), followed by depth (0.237), As (0.180), Cu (0.157), phaeopigments at 2–5 cm depth (0.150), gravel content (0.129) and organic matter (0.098). When silt and clay were entered into the analysis separately, clay had a slightly higher correlation (0.245) than silt (0.196). Most of these correlations are low, representing fairly weak relationships, but the relationships between those variables selected by both BVSTEP and BIOENV and nematode community variation can be visualized by the use of MDS overlay plots (Figure 5). It is readily apparent that the primary factors influencing the differences in community structure between groups are those related to sediment composition, with less clear relationships being demonstrated with other physical (e.g. depth, As) and biological (e.g. organic matter and phaeopigments) factors.

Similarity percentages analysis (SIMPER) of  $\sqrt{}$ -transformed nematode abundances was used to determine the contribution from individual species to the Bray–Curtis dissimilarity between groups (Table 3). Group 1A is



**Table 3.** Summary of similarities terms (SIMPER) analysis. Differences (< and >) in average abundances of species contributing to Bray–Curtis dissimilarities between selected pairs of groups of stations identified in the cluster analysis based on  $\sqrt{\text{transformed weighted average abundances}}$ . A cut-off at a cumulative per cent dissimilarity of 40% was applied.

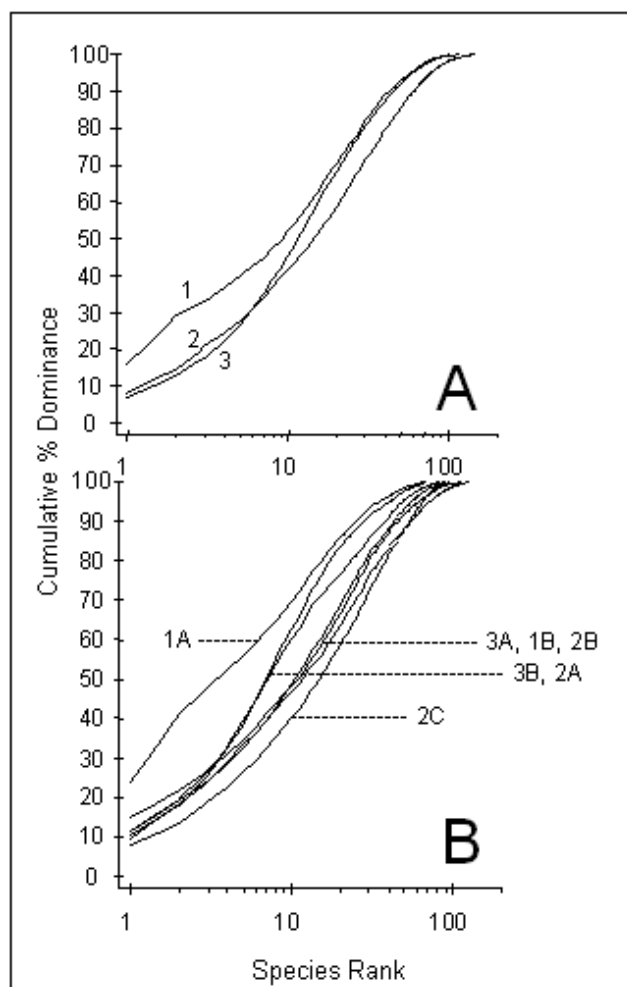
Station group:	1A	1B	2C	2B	2A
<i>Parodontophora</i> sp.	60.25	>9.83	<13.17		
<i>Eleutherolaimus</i> sp.	16.63	>0.04			
<i>Dorylaimopsis</i> sp.	43.13	>20.75	>4.83		
<i>Sabatieria</i> sp.	9.75	>2.25	>0.25	<6	<22
<i>Halalaimus</i> sp.	0.63	<6.71		10.31	>4.63
<i>Hopperia</i> sp.	4.13	>0.17	<2.25		
<i>Monhysteroides</i> sp. 2	7.5	>1.38			
<i>Thalassironus</i> sp.	0	<2.83			
<i>Retrotheristus</i> sp. 1	7.5	>1.83			
<i>Terschellingia</i> sp. 1	6.75	>2	<8		
<i>Daptonema</i> sp. 5	6.25	>1.88		0.56	<3.63
<i>Daptonema</i> sp. 6	11.38	>5.17			
<i>Terschellingia</i> sp. 2	3.13	>0.88	<4.17	>3.81	>1.63
<i>Actinonema</i> sp.	0.42	<7.25	>4.13	>0.75	
<i>Pseudochromadora</i> sp.	0.04	<3.33	<13.56	>0	
<i>Maryllynnia</i> sp.	0.29	<4.58			
<i>Paranticoma</i> sp.	0	<3.08	<8.06	>1.75	
<i>Microilaimus</i> sp. 1	1.38	<8	<20.19	>12.5	
<i>Chromadorina</i> sp.	0.17	<4.33			
<i>Leptolaimus</i> sp. 2	2.75	<7.42	<26.88	>13.5	
<i>Parasphaerolaimus</i> sp.	1.71	<3.67			
<i>Paramesonchium</i> sp.	0.25	<2.75	>0.13		
<i>Paracyatholaimus</i> sp.	0.63	<2.83			
<i>Viscosia</i> sp.	0.04	<1.42			
<i>Daptonema</i> sp. 7	2.25	<2.58			
<i>Daptonema</i> sp. 1	2.92	>2			
<i>Rhabdocoma</i> sp. 1	0.21	<2.08	>0		
<i>Desmodora</i> sp.	0.04	<1.58			
<i>Desmolaimus</i> sp.		0.33	<8.06	>1	
<i>Amphimonhystera</i> sp.		0.75	<3.88	>0.63	
<i>Quadricoma</i> sp.		5	<4		
<i>Daptonema</i> sp. 4		1.5	<5.13		
<i>Thalassironus</i> sp.		4.63	>1		
<i>Anticoma</i> sp.		0	<2.13		
<i>Metacylicolaimus</i> sp.		0	<2.88		
<i>Odontophora</i> sp.		1.58	<1.94		
<i>Neochromadora</i> sp.		1.42	<3.5	<16.38	
<i>Sphaerolaimus</i> sp. 1		1.92	>0.13		
<i>Daptonema</i> sp. 8		3.08	>1.06		
<i>Thalassomonhystera</i> sp.		3	>1.25		
<i>Paracyatholaimus</i> sp.		2.83	>1		
<i>Spilophorella</i> sp.			0.81	<10.63	
<i>Metalinhomoeus</i> sp. 3			2.25	<6.5	
<i>Chromadora</i> sp.			0.88	>0.69	
<i>Sphaerolaimus</i> sp. 2			2.63	>0	
<i>Dichromadora</i> sp.			2.88	>0.13	

separated from group 1B by the general occurrence of relatively high abundances of species common to both groups, including *Parodontophora* sp. (*P. marina*), *Eleutherolaimus* sp., *Dorylaimopsis* sp. (*D. rabalaisi*) and *Sabatieria* sp., suggesting that the primary differences between these groups results from changes in dominance, rather than diversity. The majority of species contributing to dissimilarities between samples in group 2 and samples in group 1 occur in low abundance in group 1 but are more abundant in group 2, such as

*Terschellingia* spp., *Actinonema* sp., *Pseudochromadora* sp., *Maryllynnia* sp., *Microilaimus* sp. 1 and *Chromadorina* sp. Species contributing to dissimilarities within group 2 have a tendency to be more abundant in group 2B, such as *Pseudochromadora* sp., *Paranticoma* sp., *Microilaimus* sp. 1, *Leptolaimus* sp. 2, *Desmolaimus* sp. and *Amphimonhystera* sp. Some species, including *Sabatieria* sp., *Neochromadora* sp., *Spilophorella* sp. and *Metalinhomoeus* sp. 3, are relatively abundant in group 2A. Group 3 stations (not shown in Table 3) are characterized by relatively high abundances of *Rhabdocoma* sp. 1, *Sphaerolaimus* sp. 2, *Daptonema* sp. 2 and *Odontophora* sp., and low abundances of a variety of species common to other groups.

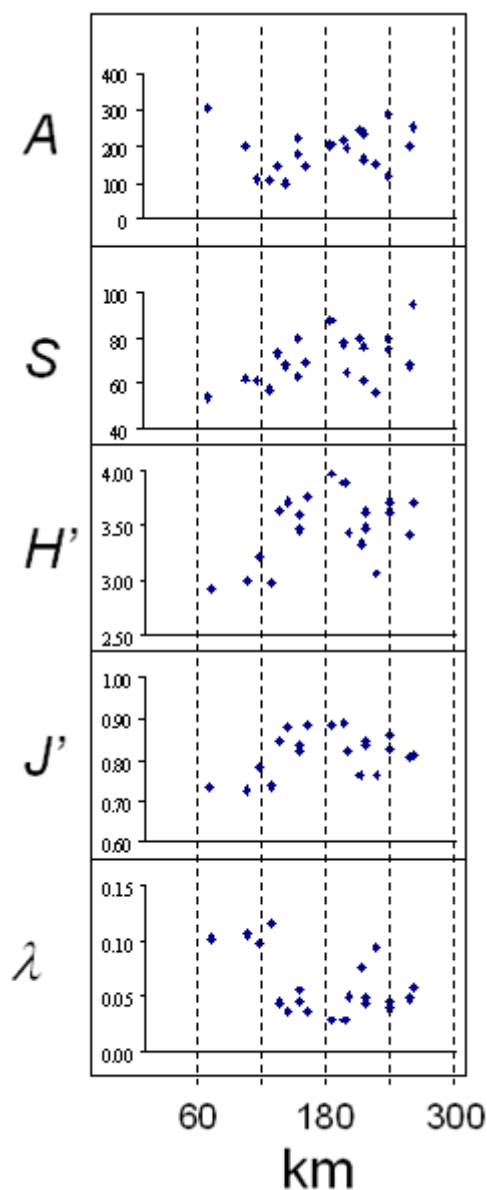
The curves of *k*-dominance for the three biotically defined groups of stations (Figure 6A) show that in terms of dominance and diversity groups 2 and 3 are similar, whereas group 1 is more highly dominated. Comparison of the curves for the seven biotic groups (Figure 6B) show that group 1A is the most highly dominated, whereas group 2C is the most diverse. These findings support the results of SIMPER analysis. The differences between the other groups are less amenable to interpretation as the curves cross.

The overall pattern, therefore, appears to be one of small but consistent differences between different parts of the Bohai Sea, which are primarily linked to variations in



**Figure 6.** Average *k*-dominance curves derived from nematode abundances in: (A) station groups 1, 2 and 3; (B) the seven station groups identified in multivariate analyses.

natural environmental variables. The primary input of silt/clay to the Bohai Sea is the Huanghe (Yellow River). Variation in univariate measures with distance from the river mouth (Figure 7) illustrates the importance of the river in determining aspects of nematode community structure. There appears to be a major discontinuity approximately 120 km from the river mouth. At lesser distances communities are highly dominated with low diversity and evenness. At greater distances dominance is low, evenness and diversity are relatively high. The number of species increases with distance from the river mouth, whereas abundance is high near the mouth of the river, decreasing to a distance of 120 km, and then rising again. If one considers the input of sediment to the Bohai Sea to be an example of disturbance, it is interesting to note that diversity increases to a distance of 180 km from the mouth of the river, and then decreases again,

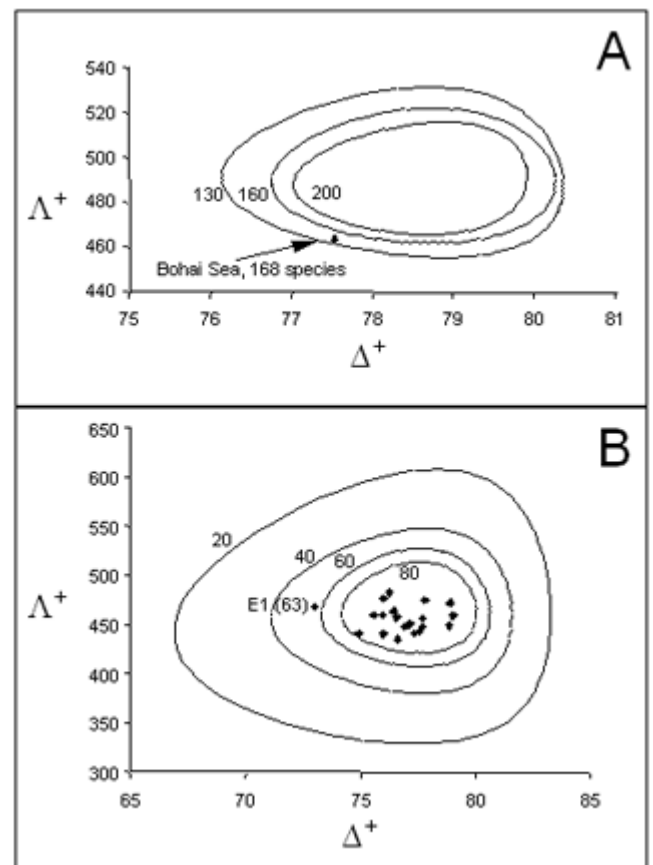


**Figure 7.** Variation in univariate measures abundance (A), numbers of species (S), Shannon–Wiener diversity ( $H'$ ), Pielou's evenness measure ( $J'$ ) and Simpson's dominance index ( $\lambda$ ) with distance from the present mouth of the Huanghe (Yellow River).

providing an example of intermediate disturbance leading to high diversity.

Average taxonomic distinctness ( $\Delta^+$ ) and variation in taxonomic distinctness ( $\Lambda^+$ ) were calculated for the total 168 species from all stations. There is no relevant 'master' species list of the marine nematodes from temperate north-west Pacific seas, but it is of interest to know whether a community of similar composition from a better-known part of the world would be regarded as unimpacted. The values from the Bohai Sea are therefore compared with 95% confidence ellipses derived from the complete UK freeliving marine nematode species list (Platt & Warwick, 1983, 1988; Warwick et al., 1998). The value for the Bohai Sea as a whole lies outside the 95% confidence limits because  $\Lambda^+$  is lower than expected (Figure 8A).

Ninety-five per cent confidence intervals were recalculated for subsets of the 168 species found in this study (Figure 8B). All stations lie within them, except for one station (E1) for which  $\Delta^+$  is lower than expected. This station lies deep within the inner Bohai, an area possibly affected by oil and gas production and other anthropogenic influences. It can be calculated, however, that there is a 64% probability of this result occurring by chance,



**Figure 8.** Measured values of  $\Lambda^+$  and  $\Delta^+$  from; (A) the Bohai Sea species list (168 species) plotted against fitted 95% probability contours for joint  $\Lambda^+$ ,  $\Delta^+$  distributions from 1000 simulations of 130, 160 and 200 species drawn randomly from a full list of 395 UK nematode species; (B) individual stations in the Bohai Sea plotted against fitted 95% probability contours for joint  $\Lambda^+$ ,  $\Delta^+$  distributions from 1000 simulations of 20, 40, 60 and 80 species drawn randomly from the full Bohai Sea species list (168 species).

and therefore to interpret it as clear evidence of an impact at this particular station is not justified.

## DISCUSSION

Whilst meiobenthic communities are sensitive to anthropogenic inputs to the marine environment (Coull & Chandler, 1992) it has long been recognized that the structure of the sediment is a major factor influencing meiobenthic community structure, and changes in sediment structure within an area may be expected to perturb that community structure (Heip et al., 1985; Somerfield et al., 1995; Warwick & Buchanan, 1970). The modern Huanghe (Yellow River) estuary is situated in Laizhou Bay. The Huanghe is the second largest river in the world (after the Ganges/Brahmaputra River) in terms of sediment load. Approximately  $1.1 \times 10^9$  tn of sediment predominantly derived from erosion of loess sediments in the river catchment are carried to the delta each year, representing approximately 10–15% of the total world riverine sediment load (Zhang et al., 1990). The majority of the material carried by the river is medium to coarse silt (Shanming, 1986), but as the river water discharge is relatively small and the tidal current is strong and approximately perpendicular to the river course, riverine suspended sediment is either deposited near the river mouth or transported by the tidal current along the coast rather than down the delta slope, reducing offshore sediment transport (Zhang et al., 1990). The long axis of the Bohai Sea, approximately 400 km in length, lines up with north winds that prevail during the winter months, which induce waves that are a major factor in reworking the Huanghe delta front and bottom sediments in the southern part of the Bohai Sea (Keller et al., 1990). Because of the shallow nature of the Bohai Sea and the high winter-induced waves, the upper intervals of the bottom sediments throughout are annually reworked, resulting in a degree of uniformity in time and a bottom topography which is relatively featureless (Keller et al., 1990). This explains the fact that variations in nematode community structure throughout the study area are not marked, and that differences between groups of stations from different parts of the Bohai, although significant, are relatively small. Patterns of sediment transport are entirely consistent with the observed variations in nematode community structure in this study, with a more highly dominated and less diverse community in siltier sediments near the river mouth, and a more diverse community further away. Species characteristic of communities closer to the delta front belong to the families Axonolaimidae, Linhomocidae, and Comesomatidae, all families commonly found in muddy sediments.

Although sediments derived from the Huanghe are the primary factor influencing variation in nematode community structure, other variables, namely depth, organic content of the sediment, phaeopigment concentrations at 2–5 cm depth within it, and arsenic concentrations, are also potentially important determinands of nematode distributions. Depth is a common correlate of community structure, most likely because it determines other factors, such as the amount and quality of phytoplankton-derived carbon reaching the benthos, which are not commonly measured in benthic investigations but which directly

affect the communities being investigated. The correlation with organic content of the sediment, and more interestingly the relationship with phaeopigments deeper in the sediment, also suggest that the supply and nature of food are important factors influencing nematode distributions. Phaeopigments are breakdown products of chlorophyll, and although both chlorophyll and phaeopigments were measured, at both 0–2 and 2–5 cm depth in the sediment, it is only the deeper phaeopigments that appear to be of importance in determining nematode community structure in the Bohai. The important species contributing to the major division between station groups 1 and 2 tend to be those that derive their carbon from diatoms or phytoplankton debris directly, such as *Actinonema* sp. and *Chromadorina* sp. (Chromadoridae), *Pseudochromadora* sp. (Desmodoridae), *Marylynnia* sp. (Cyatholaimidae) and *Microilaimus* sp. 1 (Microilaimidae), or from bacteria, such as *Terschellingia* spp. (Linhomocidae).

Marine nematodes are known to be sensitive to sediment metal concentrations (Somerfield et al., 1994). Comparisons with other pristine or less disturbed rivers elsewhere in the world reveal that levels of both dissolved and particulate arsenic are high in the Huanghe (Zhang, 1996). However, anthropogenic activities have not altered arsenic concentrations and the high levels of arsenic in the Huanghe appear to be controlled by natural weathering and lithology (Zhang, 1996; Zhang & Huang, 1993). Arsenic concentrations in the Bohai Sea are relatively stable and intimately associated with the sedimentary regime related to discharges from the Huanghe, implying that arsenic, which is not considered to be particularly harmful to aquatic organisms (Bryan & Langston, 1992), is not determining the observed distributions through some toxic mechanism, but is a factor reflecting natural processes related to fluxes from the Huanghe.

Warwick & Clarke (1998) demonstrated that nematode assemblages from offshore shallow soft sediments tend to have an average taxonomic distinctness slightly lower than the mean value derived from equivalent numbers of species selected at random from a full list from a variety of habitats. For  $\Delta^+$ , therefore, the samples from the Bohai are exactly as expected from an unimpacted offshore area, with the value for the Bohai Sea as a whole lying within the 95% confidence limits but below the mean. Clarke & Warwick (2001) show that in polluted areas where  $\Delta^+$  is less than expected, there is also a tendency for  $\Lambda^+$  to be lower than expected. The lower than expected value of  $\Lambda^+$  for the Bohai list indicates that the higher taxa which are present in the UK list, but missing from the assemblage in the Bohai Sea, are those which contain few lower taxa. These could be specialist taxa which cannot find a niche in the range of environments within the Bohai, or taxa that are restricted to north-west Europe for biogeographical reasons. In either case the reduced value of  $\Lambda^+$  in the absence of a significant departure from expectation for  $\Delta^+$  is not indicative of an assemblage under a severe pollution stress. This, coupled with the results of other analyses showing that natural processes determine variation in nematode community structure, can be taken as evidence that anthropogenic disturbance is not affecting this component of the benthos in the Bohai Sea.



The samples for this particular study were collected from an extensive grid of stations appropriate to the study of large-scale processes within the area. In order to determine the effects of anthropogenic disturbance, from oil exploration, pollution and aquaculture, it is likely that survey designs appropriate in scale for the effects being studied will show that individual parts of the Bohai Sea are being impacted by the activities of man.

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