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Freeliving marine nematodes as a pollution indicator of the Bohai Sea

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Abstract: A hierarchical diversity index—taxonomic distinctness index Δ^+, which was first defined by Warwick and Clark in 1998, was employed to evaluate the pollution status of the Bohai Sea with freeliving marine nematodes. The result showed that the Bohai Bay and other coastal sampling sites might be affected by oil and gas production and other anthropogenic influences. In other words, anthropogenic disturbance was affecting this component of the benthos in these locations. And most offshore sampling sites in the middle of the Bohai Sea were clear and unpolluted.

Keywords: taxonomic distinctness index Δ^+; freeliving marine nematodes; the Bohai Sea

Introduction

The Bohai Sea is a marginal sea enclosed on three sides and connected to the Huanghai by the Bohai Sea Strait(Fig. 1). It is a shallow sea, with a maximum depth of 86m and an average depth of 18m(Geng 1981). The area is of great commercial importance, providing important spawning and feeding grounds for many commercially importance species of fish and shellfish and supporting extensive fisheries and aquaculture. In the early 1970s industrial expansion on the shores of the Bohai Sea created massive pollution and gave rise to massive impacts on aquatic life, particularly in the intertidal zone(Fan, 1989). In recent years the Bohai Sea has been subject to intensive offshore exploration for, and production of, natural gas and petroleum reserves (Fan, 1988). Partly as a result of these factors, general contamination has increased enormously and red tides have become a frequent occurrence. The major pollutants were inorganic materials including active phosphate, oil, and lead. Ecological studies of the biota inhabiting the sediments in the Bohai Sea have examined the composition of the macrofauna(Zhang, 1990a; 1990b; Sun, 1991) and foraminifera (Zheng, 1990). Few ecological examinations have dealt with meiofauna, other than preliminary studies of the abundance and biomass in the subaquatic delta of Huanghe(Yellow River) (Zhang, 1989; 1990), and some taxonomic works(Zhang 1990, 1992).

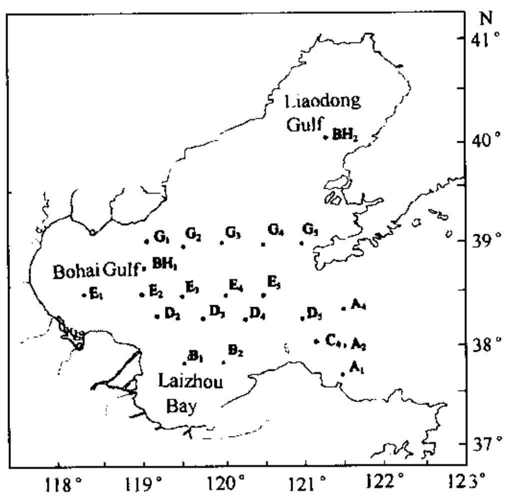

Fig.1 Map of the sampling stations in the Bohai Sea

Meifauna have evoked considerable interest as potential indicators of anthropogenic perturbation in aquatic ecosystems(Coull, 1992) as they have several potential advantages over macrofauna, which have traditionally been the component of the benthos examined in pollution monitoring surveys. Freeliving marine nematodes have been shown to be sensitive to a range of anthropogenic disturbances(Heip, 1988; Coull, 1992; Somerfield, 1995). The present study has one objective to assess the pollution status of the Bohai

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Sea using freeliving marine nematodes.

1 Materials and methods

1.1 Field sampling

Five stations B_1, E_1, E_5, BH_1 and BH_2 were selected in the first cruise in June 1997, and a grid of 20 stations besides stations BH_1 and BH_2 from the first cruise in second and third cruises in September 1998 and again in April/May 1999 (Fig. 1). Samples were collected from above mentioned stations. Undisturbed sediments were brought on-deck using a modified 0.1 m^2 Gray-O'hara box-corer. In order to reduce the influence of small-scale spatial variability within box-cores, 3 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. One replicate samples were collected for other sampling stations besides 6 replicates for station E_1 in September 1998.

1.2 Faunal analysis

Samples were stained with Rose-Bengal for 24 hrs and washed on a 48 μm sieve to remove formalin and some of the sediment fraction. Heavier sediment particles were extracted from the remaining sample using centrifugation in Ludox-TM with a specific gravity adjusted to 11.15 (Heip, 1985; Warwick, 1998a). The remaining light sample fraction was washed into a lined petri-dish, copepods were picked out, and major taxon were identified and counted. A 1/5 subsample of the remaining material was taken for the enumeration and identification of nematodes. 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 in a glycerol were mounted on slides using individual mounts for samples from the first and second cruises, and ecological bulk mounts(Somerfield, 1996) for samples from the third cruise. All nematodes in the subsample were identified to putative species using a compound microscope, bright-field illumination and a × 100 oil immersion len.

1.3 Data analyses

In grossly perturbed situations, communities are kept in an early successional stage with a low species diversity, and often comprise guilds of closely related species or single species with a high genetic diversity. Meiobenthic examples include the copepod genus *Tisbe* (Bergmans, 1979) and the nematode family Oncholaimidea(Bett, 1988) all of which characterize organically polluted habitats and have sympatric sibling species. Unperturbed benthic communities in a late successional stage tend to comprise a range of more distinct species belonging to many phyla. In less severely perturbed situations, change in community composition in response to anthropogenic disturbances of various kinds, which are unaccompanied by changes in species diversity, have repeatedly been revealed by multivariate analysis of species abundance (Warwick, 1991; Somerfield, 1995). Species diversity seems in some way to be homeostatic, at least to relatively low levels of disturbance. However, assemblages with the same species diversity may comprise species which are all closely related to each other taxonomically(belonging to the same genus or family) or be taxonomically more distant(belonging to different phyla), i.e. the hierarchical level of diversity may shift even though species diversity is maintained. For biological community data (species-by-sample abundance matrices), Warwick & Clarke(Warwick, 1995) defined two biodiversity indices, capturing the structure not only of the distribution of abundance amongst species but also the taxonomic relatedness of the species in each sample. The first index, taxonomic diversity (Δ), can be thought of as the average taxonomic "distance" between any two organisms, chosen at random from the sample: this distance can be visualized simply as the length of the path connecting these two organisms, traced through(say) a Linnean or phylogenetic classification of the full set of species involved. The second index, taxonomic distinctness (Δ^*), is the average path length between any two randomly chosen individuals, conditional on them being from different species. The special case was where the data consist only of presence/absence information. Clarke and Warwick(Clarke, 1998)defined it as the taxonomic distinctness index Δ^+:

$$\Delta^+ = \left[\sum\sum_{i<j} W_{ij}\right] / \left[S(S-1)/2\right].$$

Where the summation is taken over all species, i, j, the number of species is s, and ω_{ij} is the taxonomic path length through a hierarchical tree between species i and j. The index is sample-size independent and therefore is compared across studies with differing and uncontrolled degrees of sampling effect. Being based on the presence/absence of species, it can be calculated using simple species lists. Further, a statistical framework exists for detecting departures of the index from expectation. This uses Δ^+ values calculated from random subsets of species selected from a master species list in order to calculate a 95% confidence "funnel". These properties give the index theoretical and logistical advantages over other more traditional univariate measures used in environmental assessment studies (Warwick, 1998). In present paper the taxonomic distinctness index Δ^+ was applied to data on free-living marine nematodes from the Bohai Sea in order to evaluate the pollution status of the Bohai Sea.

2 Results and discussion

A total of 168 putative species of nematodes were identified, and nematode made up 79%—95% of the total meifaunal abundance at the various sampling stations. The taxonomic distinctness index Δ^+ for each of the stations were calculated. There is no relevant "master" species list of the marine nematodes from temperate NW Pacific Seas. That being said, it has been shown that the nematode communities in the Bohai Sea are broadly similar to those in similar habitats in temperate seas elsewhere in the world. The values from the Bohai Sea are therefore compared with the confidence funnel derived from the complete UK freeliving marine nematode species list(Platt, 1983; 1988; Warwick, 1998b).

Table 1 The measured value of Δ^+ for sampling stations in the Bohai Sea in two crusies

Stations	A_1	A_2	A_4	B_1	B_2	C_4	D_2	D_3	D_4	D_5
1999	4.614	4.787	4.756	4.415	4.474	4.671	4.533	4.619	4.75	4.631
1998	4.467	4.753	4.714	4.633	4.62	4.634	4.668	4.567	4.73	4.662
	E_1	E_2	E_3	E_4	E_5	G_1	G_2	G_3	G_4	G_5
1999	4.385*	4.559	4.444	4.654	4.647	4.679	4.573	4.587	4.62	4.685
1998	4.341	4.494	4.592	4.578	4.543	4.542	4.587	4.591	4.561	4.585

Note: * A average value from six replicate samples

All the measured values Δ^+ from 1998 and 1999 of two cruises in the Bohai Sea are shown in Table 1. The values Δ^+ from station E_1, which was located in the Bohai Bay, were always the lowest among sampling stations in two cruises. The values for stations A_1, B_1 and B_2, which were nearer to the shore, were relatively low compared with other sampling stations. The values for A_2, A_4, D_4, E_4, E_5 and G_5, which were in the middle of the Bohai Sea, were relatively high.

All the measured values Δ^+ from three cruises in the Bohai Sea were presented as points on the 95% confidence funnel. E_1 station in 1997 cruise had Δ^+ values outside these 95% confidence intervals(Fig. 2). 6 replicate samples from E_1 station in 1998 cruise had all Δ^+ values outside these 95% confidence intervals(Fig. 2). Two other stations A_1 and E_2 in 1998 cruise had Δ^+ values outside these 95% confidence intervals(Fig. 3). 2 stations E_1 and B_1 in 1999 cruise have Δ^+ values outside these 95% confidence intervals (Fig. 4). Warwick and Clarke (Warwick, 1998) demonstrated that the taxonomic distinctness of nematode from environmentally degraded locations is reduced in comparison with that of more pristine locations, often significantly so. When the values of Δ^+ from unpolluted offshore shallow soft sediments were plotted against the number of species as points on the simulated 95% confidence funnel, they fell within the lower part of the confidence funnel. When the values of Δ^+ from polluted offshore shallow soft sediments were plotted against the number of species as points on the simulated 95% confidence funnel, they fell outside the lower part of the confidence funnel.

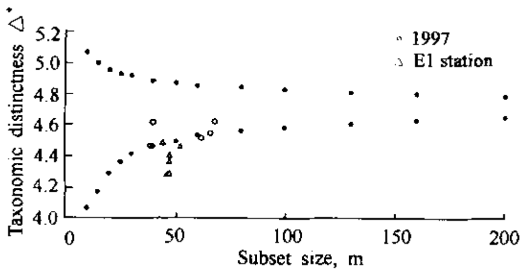

Fig.2 Measured taxonomic distinctness values of the Bohai Sea in 1997 cruise and at E_1 station in 1998 cruise, plotted against the number of species (m) as points on the simulated 95% confidence funnel

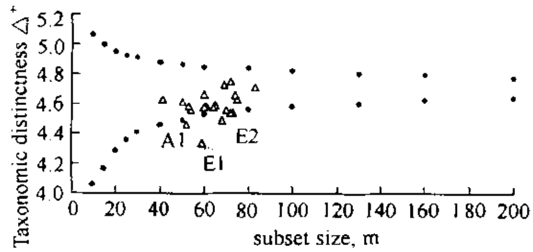

Fig.3 Measured taxonomic distinctness values of the Bohai Sea in 1998 cruise, plotted against the number of species(m) as points on the simulated 95% confidence funnel

The samples from the station E_1, therefore was from an impacted offshore area, with the value for the station E_1 outside the 95% confidence limits. This, coupled with the results of other analyses showing that natural processes determine variation in nematode community structure, can be taken as clear evidence that anthropogenic disturbance was affecting this component of the benthos in the Bohai Sea, or in other words, analyses of nematode community structure showed the Bohai Bay to be polluted. The samples from stations A_1, B_1 and B_2—which were nearer to the shore—with the values for them mostly outside the 95% confidence limits. This seemed to show that these sampling sites might be polluted. And the values for A_2, A_4, D_4, E_4, E_5 and G_5, which were in the middle of the Bohai Sea, were relatively high and within the 95% confidence limits. These locations were clear and unimpacted. By the way, the samples for this particular study were collected from an extensive grid of stations appropriated to the study of large-scale processes within the area. In order to determine the effects of anthropogenic resulting from oil exploration, pollution, aquaculture, and so on at the Bohai Bay and coastal stations, survey plans appropriate in scale for the effects being studied should be designed.

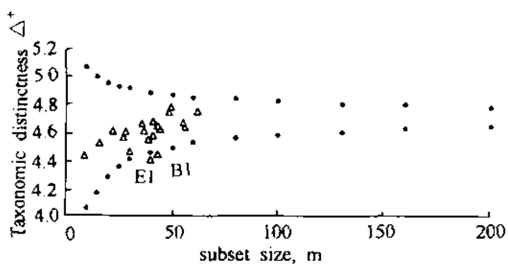

Fig.4 Measured taxonomic distinctness values of the Bohai Sea in 1999 cruise, plotted against the number of species (m) as points on the simulated 95% confidence funnel

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