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An Assessment of the Potential Use of the Nematode to Copepod Ratio in the Monitoring of Metals Pollution. The Chañaral Case

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We discuss the use of the nematode to copepod ratio in relation to determining the extent of metals impact using the Chañaral area of northern Chile, where the dumping of copper mine tailings has taken place for many years, as an example. Data were collected from 12 beaches in the area on eight occasions between January 1997 and October 1998. We find that the ratio is not a good predictor of pollution due to the generally low densities of meiofauna on impacted beaches and the absence of harpacticoid copepods from those beaches. We suggest that in the case of metal pollution the mean number of Harpacticoida per site may be a better indicator of impact stress. We rule out the use of the nematode to copepod ratio as an indicator in biomonitoring studies where metal enrichment is thought to occur. © 2001 Elsevier Science Ltd. All rights reserved.

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Introduction

Raffaelli and Mason (1981) first proposed the use of the nematode to copepod ratio as a tool for biomonitoring. The validity of this technique subsequently became the subject of discussion among meiobenthologists, with some (e.g. Coull *et al.*, 1981) arguing that it was an over generalization and others (e.g. Warwick, 1981) suggesting modifications to improve its utility. Lambshead (1984) cast doubt on the usefulness of the ratio by pointing out the difficulties in separating the effects of pollution on the ratio from the effects of other environmental variables. This nematode to copepod ratio has not been examined in relation to metals pollution or in the exposed oceanic beach situation which predominates along much of the Chilean coastline.

The meiofauna, unlike the macrofauna, spend their entire life cycle within the sedimentary environment. As a result of direct benthic recruitment and short generation times meiofauna are more responsive to the input of a pollutant to the sedimentary environment than the macrofauna (Coull and Chandler, 1992). Direct benthic recruitment implies that the effects of pollution on the community structure are not masked by recruitment of individuals from outside the impacted area. Short generation times and asynchronous reproduction of the majority of meiofaunal species have the advantage that all stages in the life cycle are exposed to the pollutant (Coull and Chandler, 1992), which results in a short response time by the community to a pollution event.

Copper mine tailings disposal has taken place in the Chañaral area of northern Chile since 1938. The tailings are from two mines, El Salvador and Potrerillos (now closed), located above 3000 m in the Andes mountains and approximately 150 km from the coast. Originally the untreated tailings were sent down the dry river bed of the Rio Salado, which empties onto the coast at Chañaral. Dumping took place at this location from 1938 to 1975 and resulted in the formation of a large tailings beach in the bay. As a result, the shore line is over one kilometer further out to sea than it was originally and the beach is estimated to be nine meters deep in tailings. In 1975 the tailings were redirected from the Rio Salado, via a canal, to a new dumping point at Caleta Palito, 200 m south of Playa Palito (Castilla, 1983). Dumping took place at Caleta Palito from 1975 to 1990, with an estimated discharge of 125–150 million tones of tailings over the entire period, with total copper concentrations of 6000–7000 μg l^{-1}. In 1990, an environmental court action ruled that a settlement dam should be constructed in the high desert between the mine at El Salvador and the coast, and that only ‘clear water’ tailings, containing no more than 2000 μg total

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Cu l^{-1}, should be dumped at Caleta Palito (Castilla, 1996).

The situation described for Chañaral area and surroundings represents a unique case to study the effect of copper mine tailings on the meiofaunal communities. Thus in this study we assessed the utility of the nematode to copepod ratio in conditions where metals are the dominant pollutants.

Methodology

Study sites

The beaches sampled for this study are centred around the city of Chañaral, Region III of northern Chile, and were selected to represent the full range of beach types present in this area. Surface seawater temperatures in this area range from 14°C (August) to 20°C (February), and mean annual salinity is 34.5‰ (SHOAC, 1996). The beaches sampled were as follows (Fig. 1), from north to south: Puerto Pan de Azucar (Pue), Frente Isla Pan de Azucar (Fre), Playa Blanca (Bla), Caleta La Lancha (Lan), Caleta Agua Hedionda (Hed), Palito 1000 m Norte (Mil), Playa Palito (Pal) 200 m north of the end of the tailings canal, Palito 2000 m Sur (Dos), Playa Chañaral (Cha), Las Piscinas (Pis), Torres del Inca (Tor), and Playa Zenteno (Zen) (see Table 1 for details). The type designation of the beaches follows the scheme of Short (1996). All beaches were composed principally of silicate sands except Tor which is shell sand. Lan, Hed and Cha are all beaches composed of 100% tailings.

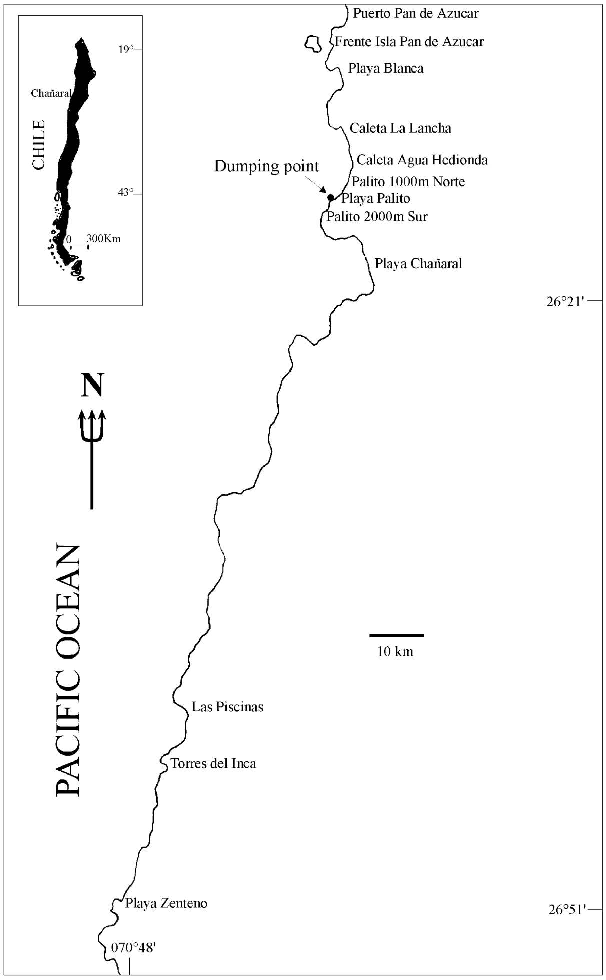

Fig. 1 Map of the Chañaral area and the beaches included in the study.

Samples

The data used in this paper come from a series of sampling occasions starting in January 1997. The sampling strategy and the number of beaches sampled varied slightly from one to the other and therefore the means used here were calculated using different numbers of samples for each beach (minimum 25 samples). During January 1997 samples were collected from Pue, Fre, Bla, Pal and Zen. Ten 125 ml samples were collected on each beach along two transects running between mean high water and mean low water. Subsequent sampling took place during April, May, July, September and December of 1997, January and October of 1998. All 12 beaches were sampled on all occasions with the exception of Mil and Tor which were not sampled in April and May of 1997. Five 50 cm^3 samples were collected from the swash zone of each beach using a modified plastic syringe. All samples were fixed in a 10% formalin solution and meiofauna were extracted using a simple decantation method through a 44 μm sieve (95% extraction efficiency, unpub. data).

Abiotic data

Sediment from the meiofauna samples was analysed by dry sieving through a graded series of sieves. The median diameter (Md) of the sediment of each site is included in Table 1. Md values are presented in units of phi (φ), where negative values indicate coarser sediment and positive values finer sediment, a value of zero is equivalent to 'coarse sand' on the Wentworth scale.

Labile copper concentrations (Fig. 2) were ascertained from both the porewater and overlying seawater using the diffusion gel technique (DGT) of Zhang and Davidson (1995). Porewater samples were collected using cores as the environment was too dynamic to leave the samplers *in situ*. Seawater samples were collected by placing samples in Ziploc bags with the sampler as again the environment was too dynamic to leave the samplers *in situ*. Samples were analysed by inductively coupled plasma-mass spectrometry (ICP-MS, Varian Ultramass) using a direct injection nebulizer (CETAC) at the University of Lancaster by Zhang, see Zhang *et al.* (1998) for the methodology. It was assumed that the labile copper concentration was equivalent to the bioavailable concentration of copper.

The relative amount of tailings deposited on each beach is presented in Table 2. The sites were ranked based on a visual observation of the tailings deposition

TABLE 1

Localities included in the study, type of beach and geographic location.

Beach	Code	Type	Md (φ)	Latitude	Longitude
Puerto Pan de Azucar	Pue	Reflective	−0.65	26°08.3′S	070°39.3′W
Frente Isla Pan de Azucar	Fre	Intermediate	1.52	26°08.4′S	070°40.0′W
Playa Blanca	Bla	Intermediate/dissipative	2.44	26°11.1′S	070°39.2′W
Caleta La Lancha	Lan	Intermediate	1.67	26°13.4′S	070°39.2′W
Caleta Agua Hedionda	Hed	Intermediate	2.09	26°15.3′S	070°38.5′W
Palito 1000m Norte	Mil	Reflective	−0.15	26°16.1′S	070°39.2′W
Playa Palito	Pal	Reflective	−0.98	26°16.3′S	070°39.3′W
Palito 2000m Sur	Dos	Intermediate	−0.06	26°17.0′S	070°39.6′W
Playa Chañaral	Cha	dissipative	2.71	26°20.5′S	070°37.4′W
Las Piscinas	Pis	Intermediate/dissipative	2.58	26°33.0′S	070°41.1′W
Torres del Inca	Tor	Reflective	−0.09	26°36.2′S	070°44.5′W
Caleta Zenteno	Zen	Intermediate	1.71	26°51.1′S	070°48.3′W

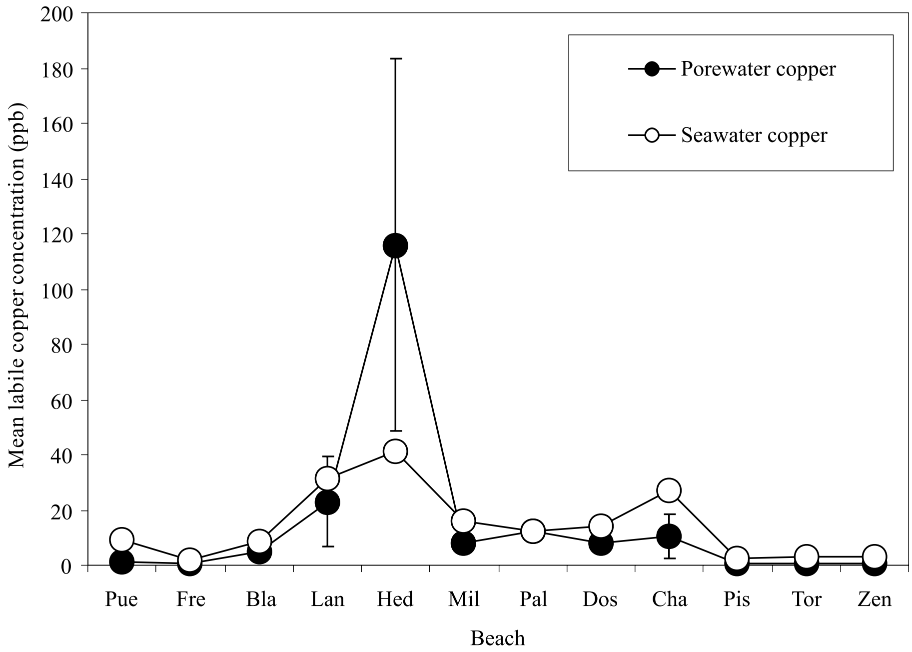

Fig. 2 The mean labile copper concentrations (ppb) in both the porewater and the overlying seawater at each of the beaches. The data were collected using the diffusion gel technique (DGT).

TABLE 2

A ranking of the beaches based on the amount of tailings present at each.[a]

Beach	Rank
Pue	6
Fre	4
Bla	5
Lan	11
Hed	11
Mil	8.5
Pal	7
Dos	8.5
Cha	11
Pis	2
Tor	2
Zen	2

[a] The lower the rank, the lower the amount of tailings.

at each beach. Physical sediment analysis was unable to clearly differentiate the amount of tailings at each beach as the Md of the tailings was very similar to some of the natural sediments at other sites. However, it was possible to distinguish the tailings from natural sediment on the basis of colour. Therefore, the rankings are based on personal observation and are subjective.

Statistical analysis

The nematode to copepod ratio was calculated by dividing the number of nematodes in a sample by the number of copepods. Where there were no copepods present in a sample the number of nematodes present was used to represent the ratio. Where nematodes were absent but copepods present the ratio was zero, and zero was also used where both groups were absent.

Data were interpreted using simple graphical representations, GLM repeated measures analysis, and Spearman's ρ correlation analysis using the SPSS software (SPSS Inc.). Data for January, April and May of 1997 were eliminated from the GLM analysis due to problems with the balance of the design.

Results

The nematode to copepod ratios fluctuated significantly from beach to beach though to a lesser extent in the unimpacted beaches. The impacted beaches are characterized by the absence or near absence of copepods (Fig. 3).

The results of the GLM repeated measures test (Table 3), included only data from July 1997 onwards (five sampling occasions) in order to keep the design balanced. As the assumption of sphericity was violated (Mauchly's $W = 0.004$, $df = 9$, $p < 0.001$) the degrees of freedom were multiplied by an epsilon value of 0.370 (Huynh–Feldt) and the probabilities for F recalculated. There are significant within-subject effects, with the nematode to copepod ratio changing with time ($F = 5.381$, $p < 0.05$). There was also a significant time-beach interaction indicating that the ratio changes both with time and over the range of beaches ($F = 5.909$, $p < 0.05$). Finally, there was also a significant between-subject effect that indicated that the ratio changed significantly over the range of beaches ($F = 12.371$, $p < 0.025$). *Post hoc*, Student–Newman–Keuls test ($p < 0.05$) indicated that only Bla can be consistently separated from the rest of the beaches.

Spearman's ρ correlations were carried out to examine the relationship between the ratio, the labile porewater copper concentration and the median sediment grain size (Md) (Table 4). Correlations were also calculated between the mean number of harpacticoid copepods, the labile porewater copper concentration and

TABLE 3

GLM repeated measures analysis, as the assumption of sphericity was violated the degrees of freedom were multiplied by an Epsilon of 0.370 and the probabilities for the *F* statistic recalculated.

Within-subject effects	*df*	*F*	*p*
TIME	1	5.381	0.05
TIME X BEACH	16	5.909	0.05
Between-subject effects			
BEACH	4	12.371	0.025

TABLE 4

Spearman's ρ correlations between the nematode to copepod ratio, the mean number of copepods, a measure of the interstitial bioavailable copper, and the mean sediment grain size.

	Nematode to Copepod ratio	Mean number of Copepods
Labile porewater copper	−0.014	−0.895**
Md (φ)	−0.350	0.322

** $p < 0.001$

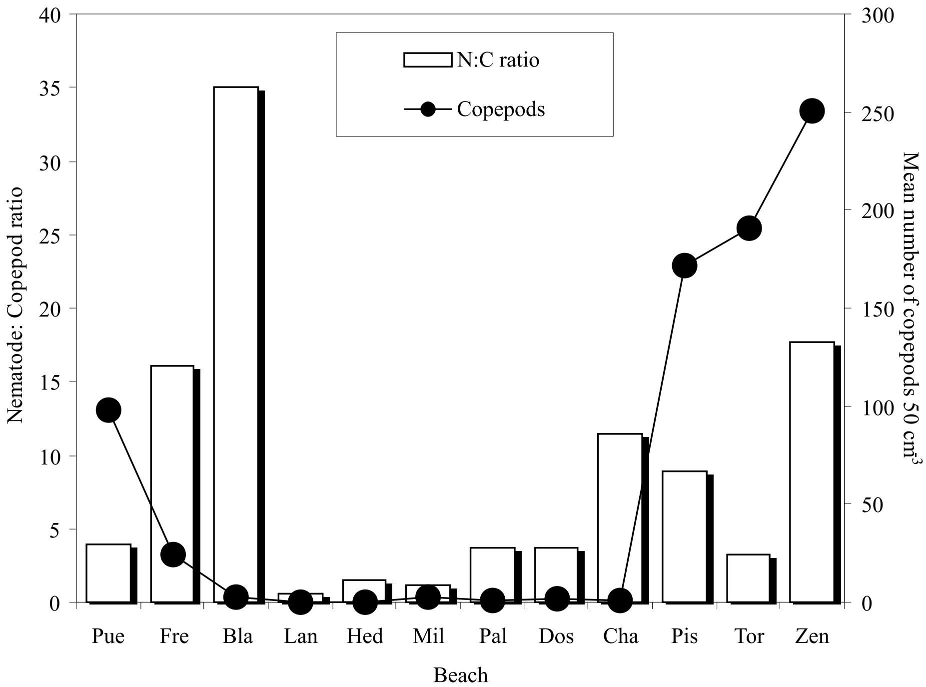

Fig. 3 Nematode to copepod ratio (bars) and the mean number of harpacticoid copepods (line) over the range of beaches sampled. The three divisions of the graph were proposed by Lee *et al.* (2000).

the median sediment grain size (Md) (Table 4). There was a significant correlation (-0.895, $p < 0.001$) between the mean number of harpacticoid copepods and the labile porewater copper concentration only.

Discussion

The information gathered in this study strongly suggests that the nematode to copepod ratio is not a reliable indicator of the metal enrichment of coastal waters. GLM repeated measures analysis indicates that there is a significant variation in the nematode to copepod ratio from beach to beach. However, this variation is not ordered from impacted to unimpacted beaches (Fig. 3) and the correlation between the nematode to copepod ratio and the labile porewater copper concentration is poor (-0.014). On the other hand, the changes in the density of harpacticoid copepods are associated with the change from impacted to unimpacted beaches and there is a highly significant correlation ($-0.895, p < 0.001$) between the mean number of harpacticoid copepods and the labile porewater copper concentration. This further supports the hypothesis that harpacticoid copepods are particularly sensitive to metals (Van Damme *et al.*, 1984; Hicks and Coull, 1984), and suggests that in metal enriched environments their densities are themselves indicators of pollution stress.

There is a significant change in the ratio over time (GLM repeated measures, within-subject comparison) indicating that either the ratio is sensitive to seasonal changes or it is responding quickly to changes in the metal concentrations in the interstitial water. At present, however, data do not exist to support either hypothesis, although, it is more likely to be the former bearing in mind the poor correlation (-0.014) between the ratio and the labile porewater copper concentration.

Neither the ratio nor the mean number of harpacticoid copepods correlates with the median sediment grain size (Md) (-0.350 and 0.322, respectively). This is an important observation as in unimpacted environments the sediment structure is regarded as the 'super factor' in determining meiofaunal community structure (Platt *et al.*, 1984). The absence of a correlation of either measure with the sediment structure indicates that both parameters are under the influence of another variable, and in the case of the mean number of harpacticoid copepods there is good evidence that this is the metal enrichment resulting from the dumping of the mine tailings. There is, however, one confounding factor with regard to the sediment structure and that is that the deposition of the tailings themselves changes the sediment structure by reducing the amount of interstitial space. Irrespective of the metal loadings this reduction in interstitial space will change the meiofaunal community structure. Fortunately for this study, not all the beaches considered impacted by the tailings are affected by a reduction in interstitial space. Neither, Mil, Pal nor Dos are subject to the complete smothering of the site by tailings deposition but the values for the mean number of harpacticoid copepods are as low as on other impacted beaches.

As indicated previously there has been a great deal of discussion as to the use of the nematode to copepod ratio in assessing pollution impact on native meiofaunal assemblages. Unlike many of previous studies, we did not discuss the ratio in relation to sewage pollution but to metal enrichment, where the reaction of the harpacticoid copepod fauna is more predictable. Copepods are generally sensitive to metal pollution and decline rapidly with increasing metal concentrations (Van Damme *et al.*, 1984; Hicks and Coull, 1984). The beaches under discussion are all high-energy oceanic beaches and the epifaunal copepods, which Shiells and Anderson (1985) recommended be excluded from the calculation, are not present in the swash zone from where the samples were taken. Lee *et al.* (2000) determined that metal enrichment generally drives down both diversity and density of meiofaunal assemblages. Thus, the nematode to copepod ratios in impacted areas are based on very low numbers of organisms and, therefore, small changes in the density of either taxa could have a significant effect on the ratio. Outside impacted areas, on the moderately impacted beaches (Pue, Fre, Bla), we found a decline in the ratio associated with distance from the source the metals and a concomitant increase in the mean number of harpacticoid copepods (Fig. 3). However, the three beaches considered in this study are insufficient to provide conclusive support of this trend. For the reference sites to the south (Pis, Tor and Zen) which are considered to be unimpacted by the tailings dumping there is no observable trend in the ratio.

The evidence presented here does not support the use of the nematode to copepod ratio as a stand-alone method for biomonitoring purposes on high energy open ocean beaches. However, it may prove a useful addition to the range of univariate measures that can be used in interpreting pollution impact where the dominant pollutant is not obvious but could be copper. On the other hand, harpacticoid copepod density seems to provide a useful indicator for broad-based surveys, where the dominant pollutant is expected to be copper, by drawing attention to those beaches that should be studied in greater detail.

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