

Sub-catastrophic sewage and industrial waste contamination as revealed by marine nematode faunal analysis

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ABSTRACT: Studies in benthic biomonitoring have usually concentrated on the macrofauna. In theory the meiofauna possess characteristics which should facilitate their use in a biomonitoring role but unfortunately perceived difficulties in working with meiofaunal taxa have inhibited research into their application in this field. This paper reports an investigation into the effects of contamination at a sub-catastrophic level on some marine nematode assemblages of beaches in the Clyde inland sea area, Scotland. Overall abundance and analysis of feeding types revealed localized contamination effects in samples from Ayr Bay compared with reference sites. Stochastic model or simple diversity analyses were not effective. Neutral model and cladistic analyses detected effects in all assemblages exposed to sub-catastrophic contamination.

INTRODUCTION

The potential of marine nematodes for pollution monitoring has been discussed in detail by Ferris & Ferris 1979, Platt & Warwick 1980, Coull & Palmer 1984, and Platt 1984a. The case for nominating nematodes for this role rests on certain of their properties as a taxon. Nematodes are abundant with high species diversity, making them suitable for ecological and statistical analyses. They are ubiquitous and persistent as a taxon and are found in all environmental conditions that can support metazoan organisms. Their small size facilitates sampling and it is possible to take very precise spot samples from the environment using cores. Nematodes have conservative life cycles (i.e. no highly mobile pelagic life stages) so local contamination effects should not be hidden by immigration. They have a rapid turnover compared to the macrofauna and are in intimate contact with pore water. Thus they should demonstrate a fast response to contamination.

Ironically, the conceptual arguments against the use of these organisms for biomonitoring rest on this very property of diversity. This, coupled with chaotic taxonomy, has made the taxon difficult for the non-specialist. However, recent publications (Lorenzen 1981, Platt & Warwick 1983) have started to rectify this

situation bringing accurate nematode identification within the scope of extension workers.

Biomonitoring at the community level is still given a high priority as indicated at a recent interdisciplinary meeting on marine pollution (Platt 1984b). But most ecotoxicological studies have concentrated on the macrofauna, with a depressing lack of reproducible results. It is still impossible to predict the effects of contamination at levels lower than those causing catastrophic destruction. As Platt et al. (1984) have noted, 'Macrobenthic analysis has so far failed to provide the answer; it is time to turn the spotlight on the meiofauna'.

This study set out to investigate aspects of the practical potential of marine nematodes as pollution monitoring organisms. To this end nematode assemblages from an anthropogenically contaminated region were compared with assemblages from a relatively uncontaminated geographically close area similar in the major ecological parameters, sediment grain size and salinity (Ferris & Ferris 1979). A variety of ecological analytical techniques were used in an attempt to detect a pollution effect on the nematode assemblages. This report considers techniques based on overall abundance, feeding types, diversity, stochastic and neutral models, and multi-variate analysis and ecolo-

gical cladistics. The nematode-copepod ratio was calculated (Raffaelli & Mason 1981) but rejected as a reliable indicator of contamination, as reported elsewhere (Lamshead 1984). This paper will concentrate on the nematode assemblages.

MATERIALS AND METHODS

Study area. The inland sea area of the Firth of Clyde was chosen for the study. This estuary offered a number of advantages for the analysis of pollution effects on marine nematode assemblages. Highly contaminated regions were found in close proximity to relatively uncontaminated regions which were similar in important ecological parameters. Sediments in these regions consisted of fine sand rather than the muddy sediments more often associated with pollution. Nematode assemblages in sand have a higher diversity than mud assemblages (Ferris & Ferris 1979) facilitating the detection of pollution-induced changes. The contaminated Ayrshire coast had already been the subject of an extensive nematode survey by Jayasree (1976). Sediments in these regions were similar to those in relatively near, uncontaminated areas whose nematode assemblages have been previously reported on: Strangford Lough, Northern Ireland (Platt 1971, 1977a, 1977b) and Loch Ewe, west coast of Scotland (McIntyre et al. 1970, Zhang unpubl.).

An initial survey was used to screen likely reference stations to ensure that they had similar sediments to putative contaminated stations. The Clyde estuary is rather unusual in that reference and contaminated sites with similar sediment characteristics can be found in close proximity.

The main potential disadvantage of the Firth of Clyde for this study is that it is an estuary and so may be subject to salinity fluctuation. Such variations are undesirable as they could mask possible effects of contamination. However, Johnston et al. (1974) described the Clyde as 'not a typical estuary' which 'in many respects can be regarded as a shallow sea'. They reported that salinity varies only gradually and that the salinity at 3 m ranged from about 31.5 ‰ at the mouth of the River Clyde to 33.7 ‰ near the Mull of Kintyre. Much of the Firth had salinities in the range 33.2 ‰ to 33.5 ‰ and zonation as a result of salinity is only likely to occur in salinities ranging from 3 ‰ to 30 ‰ (Ferris & Ferris 1979).

The sources and scale of waste disposal in the Firth are extensively covered in the Clyde River Purification Board's (1976) internal report 'Water Quality, A Baseline Report'. There are 3 main sources of contamination in the inland sea area of the Clyde. Large quantities of domestic, industrial and agricultural

waste are washed into the area by the River Clyde. Activated sludge and industrial waste is dumped in the Firth south of Garroch Head, Isle of Bute, in enormous quantities of around 1,000,000 tons per year. Sewage and industrial waste is disposed of through effluent pipes off the Ayrshire coast, particularly Irvine Bay, and into the Garnock/Irvine estuary. In addition the Firth suffers from oil contamination from both normal shipping and tankers using the Loch Long terminal in the upper Firth.

Sampling stations. Six sampling stations (numbered 1 to 6) were chosen, 3 in heavily contaminated areas and 3 in areas thought to be free from direct contamination (Fig. 1). Stations were set at extreme low water mark, defined by sampling at the water line at low tide as given in Admiralty Tide Tables (1978). Samples were taken in this part of the beach to minimise natural disturbance (Platt 1977a) which might otherwise tend to mask a potential pollution effect. All the stations were sampled on 16 and 17 September 1978. A single set of samples were taken within this short period of time to avoid seasonal effects.

Three reference stations (1 to 3) were located on the west coast of the Isle of Bute. This area is relatively sparsely inhabited with little industry and hence minimal contamination. However, the dumping ground off Garroch Head is only 3 km south of the island and

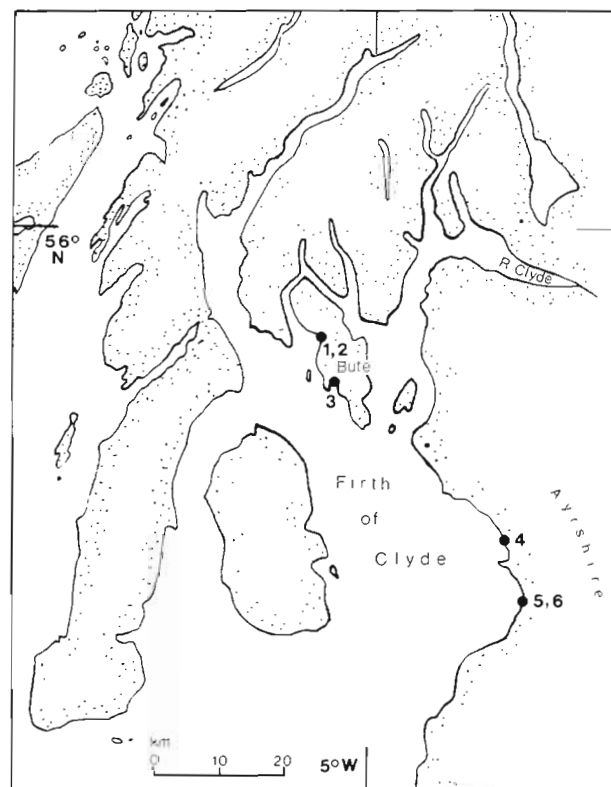


Fig. 1. Map of the Firth of Clyde inland sea area showing the locations of Stn 1 to 6

Mackay & Topping (1970) demonstrated with sea bed drifters that benthic currents could conceivably carry contaminants as far north as the island of North Bute (between Stn 2 and Stn 3). In the same paper the authors noted that although there was some build-up of metal waste in the dumping area, carbon analysis indicated that the sludge was either spread very thinly over the ground or the carbon content was quickly assimilated by organisms. Further work by Mackay et al. (1972), Topping & McIntyre (1972), and Halcrow et al. (1973, 1974) suggested that the effects of sludge dumping were localized. So there is no reason to believe that the reference stations might have been contaminated from this source.

Stn 1 and 2 were sited in the north and south part of Ettrick Bay respectively, about 1 km apart. Stn 3 was located on the western half of Scalpsie Bay.

Three contaminated stations (4 to 6) were located on the Ayrshire coast on the east side of the sea area. Stn 4 was sited in the southern part of Irvine Bay. This is a popular tourist area and 15 pipes release sewage and some industrial waste into the bay; in addition there are 3 pipes disposing of industrial effluent. Some of these pipes terminate above the low water mark. Much of the sewage is untreated and the industrial effluents are a complex cocktail including heavy metals, notably copper, and nutrients. The Clyde River Purification Board (1976) quoted the BOD (biological oxygen demand, i.e. the amount of oxygen required to degrade organic material and oxidise reduced substances in a water sample) of the various effluents as 28,660 tonnes yr^{-1} .

Stn 5 and 6 were sited in Ayr Bay. This has a lower effluent load than Irvine Bay with a BOD of about 3,800 tonnes yr^{-1} . Seven short pipes dispose of sewage and industrial waste into the bay, the sewage receiving only preliminary if any treatment before disposal. Stn 5 was positioned close to a domestic sewage/stormwater pipe. One core (5A) was taken from the terminus of the pipe. Since it seemed possible that this core, or to some extent all of Stn 5, might be unduly influenced by localized heavy contamination another station, Stn 6, was sited approximately 30 m to the south.

Four core-samples (A to D) were taken at each station using a 22 mm diameter hand-held corer. One (D) was used for grain size analysis, the remaining 3 being used for biological samples (3 represents an optimum balance between the time taken to process the samples and statistical accuracy; Platt 1977b). Samples A to C were sectioned at roughly 5 cm intervals and fixed in the field with generous applications of 4 % formalin in seawater.

All the biological samples (except Cores 3A and 4A) were subsampled using a sample-splitter. Nematodes were extracted by decantation through a 35 μm filter

and the efficiency checked as recommended by Platt & Warwick (1983). Sediment grain sizes were analysed according to the dry-sieve method devised by Morgans (1956), using 1,000, 710, 500, 355, 250, 180, 125, 90 & 63 μm sieves. Subsieve analysis was unnecessary as less than 1 % of the samples passed through the 63 μm sieve (Morgans 1956).

RESULTS AND DISCUSSION

Physical component

Grain size analysis

Cumulative grain size curves demonstrated that the sediments were well sorted with regular distributions, the parameters of which are shown in Table 1. It should be noted that the stations displayed closely similar sediment characteristics, with the exception of

Table 1. Sediment grain size analysis. QD (quartile deviation) and Skq (skewness) indicate the degree of sorting of the sediments (after Morgans 1956)

| Station | Median (μm) | QD (μm) | Skq (μm) |
|---------|--------------------------|----------------------|-----------------------|
| 1 | 157 | 20 | - 2 |
| 2 | 190 | 34 | - 1 |
| 3 | 265 | 79 | +28 |
| 4 | 139 | 22 | - 1 |
| 5 | 147 | 17 | 0 |
| 6 | 145 | 18 | + 2 |

Stn 3 which was coarser and less well sorted in contrast to the results suggested by the initial survey for this station. As Stn 3 was clearly anomalous only 1 core from this station was worked up.

Biological component

From the 16 cores, 9,775 specimens were recovered and all but 300 (3 %) were separated into 113 distinct species; of these 73 were identified down to the specific level (65 %), 10 appeared to be new to science (9 %), 28 were determined to generic level (25 %), and 2 to family level (2 %).

Nematode densities

Nematode densities at the 6 stations are shown in Table 2. The contaminated stations (4 to 6) do have a significantly lower mean density than the reference

Table 2. Density of nematodes in Clyde samples

| Station/core | Nos. of individuals | Density per 10 cm ² |
|--------------|---------------------|--------------------------------|
| 1A | 515 | 5,417 |
| 1B | 570 | 5,995 |
| 1C | 971 | 10,213 |
| 2A | 479 | 5,038 |
| 2B | 455 | 4,786 |
| 2C | 606 | 6,374 |
| 3 | 1,476* | 3,881 |
| 4A | 2,405* | 6,324 |
| 4B | 608 | 6,395 |
| 4C | 616 | 6,479 |
| 5A | 51 | 536 |
| 5B | 175 | 1,841 |
| 5C | 175 | 1,841 |
| 6A | 232 | 2,440 |
| 6B | 152 | 1,559 |
| 6C | 269 | 2,829 |

* Cores with their total number of individuals counted, rather than a 25 % subsample

stations (1 to 3), 5 % > p > 2 % (Student's *t* test assuming unequal variances). However, contaminated Stn 4 alone (Irvine Bay) is significantly different from Stn 5 and 6 (Ayr Bay), 0.1 % > p, but not from the reference stations, p > 25 % (*t* test). The Ayr Bay stations therefore exhibit a significantly lower nematode density than the other stations.

Core 5A was taken from within 1 m of the end of the effluent pipe in Ayr Bay. The nematode density of this core was 3.1 standard deviations lower than the mean of the other 5 cores from Ayr Bay. The results from contaminated Irvine Bay show that nematode densities in the assemblages examined were unaffected by the non-catastrophic levels of contamination. The reduced densities at Ayr Bay, especially the core taken from the terminus of the effluent pipe, would seem to be due to a localized contamination.

Table 3. Nematode densities, organic carbon levels and median sand grain size for 6 stations at low water locations in Irvine Bay (Jayasree 1976)

| Station | Nematodes per 10 cm ² | Organic carbon (µg C g ⁻¹ sand) | Median grain size (µm) |
|---------|----------------------------------|--|------------------------|
| I | 8,677 | 1,149 | 158 |
| II | 9,495 | 739 | 142 |
| III | 988 | 777 | 186 |
| IV | 14,053 | 1,032 | 160 |
| V | 48,827 | 1,175 | 140 |
| VI | 12,665 | 1,158 | 138 |

Previous work has noted changes in nematode densities with contamination ranging from increases (McIntyre 1977, McLachlan et al. 1977) to catastrophic declines (Wormald 1976, Giere 1979, Boucher 1980) so clearly dose-response effects on overall abundances are complicated.

From these results it might be tempting to suggest that nematode densities could be indicative of localized contamination but comparison with published data immediately reveals certain difficulties. For example, Table 3 shows nematode densities, collected using similar techniques to those employed here, from 6 stations in Irvine Bay in August 1974 (Jayasree 1976). The range of variation was rather large (50×) and cannot be satisfactorily associated with contamination; the correlation between nematode densities and organic carbon content was +0.54 compared to -0.55 for sediment grain size.

Feeding types

The concept of nematode feeding types was devised by Wieser (1953) who suggested 4 groups based on the morphology of the buccal cavity. Initially the groups were linked to suggested feeding strategies but it is probably safer to regard them solely as morphological types whose relative abundances reflect to some degree ecological parameters. This simple concept has stood the test of time and has been found a valuable tool by a number of authors (e.g. King 1962, Hopper & Meyers 1967, Tietjen 1969, Ferris & Ferris 1979, Platt & Warwick 1980, Kito 1984).

The 4 feeding types are 1A – buccal cavity absent or minute, no teeth; 1B – conical or cup-shaped buccal cavity, teeth absent or minute; 2A – medium sized buccal cavity, small to medium teeth; 2B – large buccal cavity, large teeth or other well-developed cuticularized structures. It should be noted that there is not complete agreement as to which species belongs to which feeding category. The system used here is recorded in the species list (see 'Appendix'). Generally it follows the same system used by Platt (1971) and Jayasree (1976). Platt (1971) is followed in including *Spirinia* in the 1A category rather than the 2A and Jayasree (1976) is followed in including *Metachromadora* in 2A rather than 2B. Where other authors are quoted their data have been revised to ensure comparability with the system used here.

The percentage abundances of the 4 feeding types in the cores are shown in Table 4. Stn 1 to 4 were dominated by 2A and 1A nematodes. However, while the 2A group was still an important component of Stn 5 and 6, the 1B group was the second major component, indeed the dominant group in the core (5A) closest to

the effluent pipe. In the specific sediments studied it would appear that the 2A group are a constant factor but that contamination is associated with a relative increase in the 1B group. This can be conveniently expressed as a 1B/2A ratio (Table 4). The mean of this ratio for the Ayr Bay stations is significantly different from the mean of the ratio of the other stations using Student's *t* test (1% > *p* > 0.2%).

Table 5 shows percentage abundances of feeding types for published data from projects which have used similar collecting techniques to those employed here. The first 4 samples are all from low water/sublittoral fine sandy sediments of the Scottish west coast and so are directly comparable with the Clyde samples studied here. The distribution of feeding types shows marked similarity to Table 4, with 1B/2A ratios of less

Table 4. The percentage abundances of the 4 nematode feeding types for each of the core samples and for the amalgamated data for each station

| Station/ core | Feeding types | | | | 1B/2A ratio |
|------------------|---------------|----|----|----|----------------|
| | 1A | 1B | 2A | 2B | |
| 1A-C | 28 | 13 | 57 | 2 | 0.23 |
| 1A | 28 | 17 | 52 | 2 | 0.33 |
| 1B | 20 | 12 | 65 | 3 | 0.18 |
| 1C | 36 | 9 | 54 | 2 | 0.17 |
| 2A-C | 49 | 7 | 41 | 3 | 0.17 |
| 2A | 51 | 4 | 43 | 2 | 0.09 |
| 2B | 49 | 10 | 36 | 6 | 0.28 |
| 2C | 46 | 7 | 45 | 2 | 0.16 |
| 3 | 9 | 15 | 73 | 3 | 0.20 |
| 4A-C | 36 | 8 | 52 | 3 | 0.15 |
| 4A | 24 | 10 | 61 | 5 | 0.16 |
| 4B | 33 | 4 | 59 | 4 | 0.07 |
| 4C | 52 | 9 | 37 | 1 | 0.24 |
| 5A-C | 10 | 50 | 36 | 4 | 1.39 |
| 5A | 6 | 62 | 26 | 6 | 2.38 |
| 5B | 6 | 41 | 49 | 4 | 0.84 |
| 5C | 18 | 48 | 33 | 1 | 1.45 |
| 6A-C | 19 | 38 | 40 | 3 | 0.95 |
| 6A | 17 | 39 | 41 | 3 | 0.95 |
| 6B | 12 | 46 | 39 | 3 | 1.18 |
| 6C | 28 | 28 | 41 | 3 | 0.68 |

than 0.5. The Lynher Estuary sample differs in 2 respects; it was taken from a midtide station on a mudbank. The 1B/2A ratio is distinctly higher at 0.7, closer to the Ayr Bay stations which have a mean of 1.17. It may be speculated that the contamination at Stn 5 and 6 had the effect of producing an assemblage more typical of muddy or more disturbed sediments.

There is some evidence in the literature to support this speculation. Heip & Decraemer (1974) identified 2 allegedly contaminant-disturbed stations by means of diversity analysis. Their nematode assemblages were dominated by *Sabatieria* and *Theristus*. The authors ascribed this domination to the fact that these genera may be 'generalists'; they are also 1B feeding types.

Tietjen (1980) used cluster analysis to divide New York Bight stations into 2 groups, conforming roughly to a sand group and a fine and silty sand group. However, there were 2 anomalous sand stations which clustered with the silty sand group. The anomalous stations showed higher levels of contamination (as measured by organic carbon and metal concentration) than the other sand stations. Tietjen noted that the sand group was characterized by Chromadoridae (2A), Desmodoridae (2A), *Pomponema tautraense* (2A), *Monoposthia duodecimalata* (2A), *Xyala striata* (1B) and *Theristus problematicus* (1B), with only 1 species of Comesomatidae (1B). In contrast the silty sand/contaminated group was characterized by Comesomatidae (1B), *Terschellingia longicaudata* (1A), *Tripyloides gracilis* (1B) and *Odontophora armata* (2A). In this group he noted an absence of Monoposthiidae (2A) and low abundances of Chromadoridae (2A) and Desmodoridae (2A). In other words the 2 anomalous stations shared 2 characteristics with the silty sand stations: (a) contamination and (b) a proportional reduction in the 2A feeding type compared to the 1B.

It may therefore be concluded that *in this type of habitat* contamination has the effect of altering the proportional abundance of feeding types in the assemblage from a structure characteristic of a sandy community to one characteristic of a mud or silty sand community. This may be generally true but it would be naïve to assume that the ratios obtained in this study

Table 5. The percentage abundances of the 4 nematode feeding types for various British shallow sublittoral, low and mid-water stations

| Station | Feeding types | | | | 1B/2A ratio | Source |
|---|---------------|----|----|----|----------------|----------------------|
| | 1A | 1B | 2A | 2B | | |
| Oct sample from low water stn, N. Irvine Bay, Clyde | 11 | 22 | 62 | 5 | 0.35 | Jayasree 1976 |
| Jul sample from low water stn, Firemore Bay, Loch Ewe | 11 | 20 | 56 | 3 | 0.36 | McIntyre et al. 1970 |
| Oct sample from sublittoral stn, Firemore Bay, Loch Ewe | 28 | 10 | 50 | 12 | 0.20 | Zhang unpubl. |
| Sep sample from low water stn, Strangford Lough | 12 | 26 | 55 | 7 | 0.47 | Platt 1971 |
| Oct sample from mid-water stn, Lynher Estuary | 26 | 30 | 43 | 1 | 0.70 | Warwick & Price 1979 |

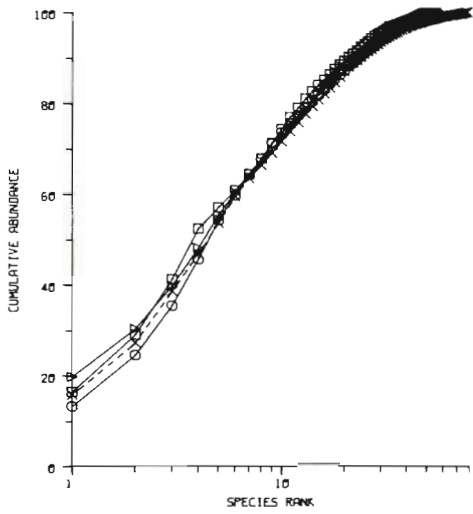


Fig. 2. *k*-dominance curves for nematode assemblages from Stn 1. (□-□) Core A; (○-○) Core B; (Δ-Δ) Core C; (×---×) combined cores

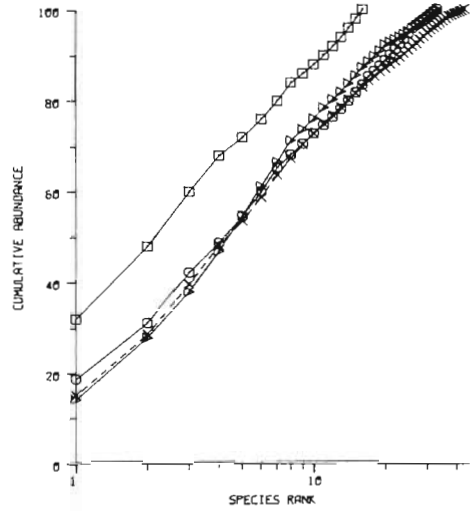


Fig. 5. Stn 5, legend as for Fig. 2

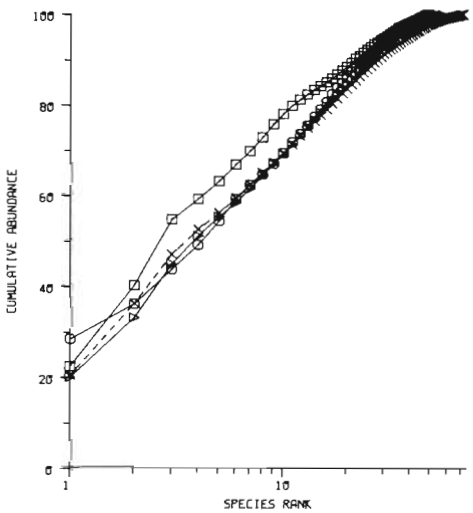


Fig. 3. Stn 2, legend as for Fig. 2

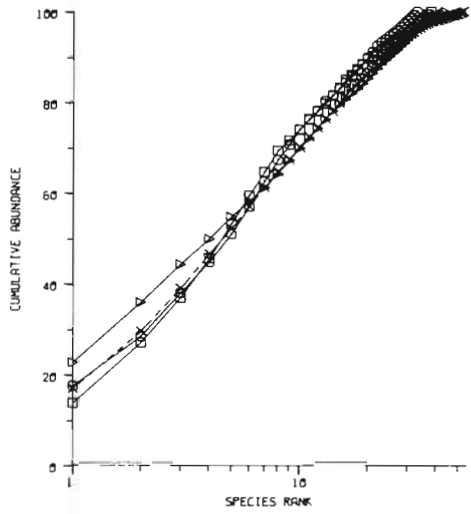


Fig. 6. Stn 6, legend as for Fig. 2

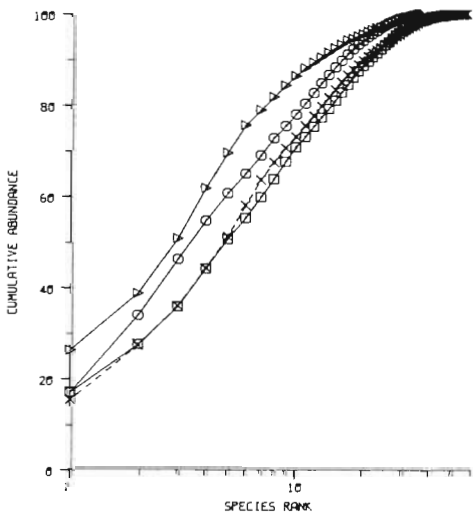


Fig. 4. Stn 4, legend as for Fig. 2

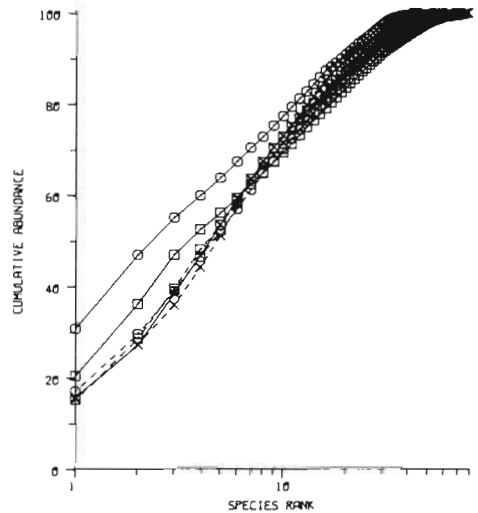


Fig. 7. *k*-dominance curves for amalgamated data from Stn 1, 2, 4, 5 and 6 and the single core from Stn 3. Reference stations (solid line): (×) Stn 1; (□) Stn 2; (○) Stn 3. Contaminated stations (dashed line): (×) Stn 4; (□) Stn 5; (○) Stn 6

Table 6. Percentage abundances of the 4 nematode feeding types for the Exe Estuary (Warwick 1971)

| Sediment type | Feeding types | | | | 1B/2A ratio |
|--------------------------------|---------------|----|----|----|-------------|
| | 1A | 1B | 2A | 2B | |
| Mud | 20 | 35 | 25 | 20 | 1.40 |
| Muddy sand | 5 | 48 | 24 | 24 | 2.00 |
| Fine sand | 10 | 29 | 14 | 48 | 2.07 |
| Well-drained sand | 10 | 14 | 48 | 29 | 0.29 |
| Coarse sand (with water table) | 5 | 26 | 35 | 35 | 0.74 |

are of any value except for comparing contaminated and reference stations from within the same habitat. For example, Table 6 shows feeding type abundances for various uncontaminated sediments in the Exe Estuary (Warwick 1971). As predicted, the 1B/2A ratio decreases from muddy to coarse sand. However, in this location, unlike the Clyde, fine sand was similar to silt sand and in general the ratios were larger.

Diversity

The diversity of the nematode assemblages was examined using *k*-dominance curves (Lambshhead et al. 1983). Fig. 2 to 6 show the *k*-dominance curves for Stn 1, 2, 4, 5 and 6, respectively. The solid lines show the diversity of each core while the dotted lines show the diversity of the amalgamated data from the 3 cores taken at each station.

Stn 1, 2 and 6 showed close similarity in the diversity of the replicates. There was some variation in the diversity of the contaminated Irvine Bay cores, Stn 4. Stn 5 exhibited a sharp discontinuity between Core 5A and Cores 5B and 5C. There was a marked reduction in the diversity of the 5A sample which was taken from the terminus of the effluent pipe.

Fig. 7 shows the comparison between *k*-dominance curves for the single core from Stn 3 and the amalgamated data from the other 5 stations. Clearly there is no distinction between the diversities of the contaminated and the uncontaminated stations. Stn 3 shows a slightly lower diversity than the others. The amalgamated data from Stn 1 is therefore used as representative of these Clyde stations to compare diversities with relevant published work.

Fig. 8 shows *k*-dominance curves of nematode assemblages from an uncontaminated low water station in Strangford Lough (Platt 1971), median grain size 190 to 200 μm , compared with Stn 1. The diversities are near identical. Fig. 9 shows a similar comparison with assemblages in uncontaminated Firemore Bay, a low water station of median grain size 178 to

200 μm (McIntyre et al. 1970) and a shallow sublittoral station of median grain size 190 to 200 μm . The Firemore Bay stations show a range of diversity. Stn 1 lies midway between the sublittoral and low water July samples. These diversity comparisons with uncontaminated samples show no evidence that the Clyde assemblages sampled were affected by contamination.

Stochastic models

Stochastic models, such as the log-normal, are not utilized here for the detection of the effects of contami-

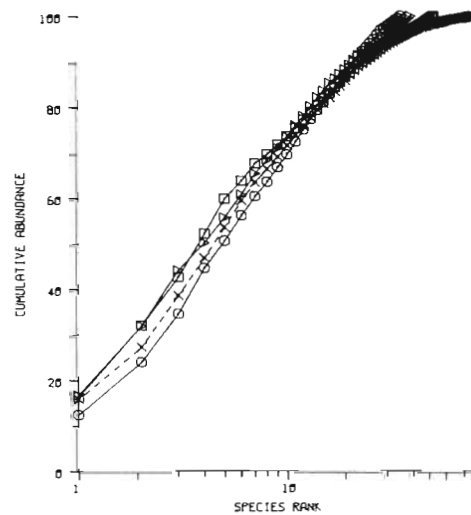


Fig. 8. *k*-dominance curves for nematode assemblages from Stn 1, Clyde inland sea area and Stn C (low water), Strangford Lough, Northern Ireland (after Platt 1971). (X --- X) Stn 1, Cores A to C. Stn C: (□) Jul; (○) Sep; (Δ) Nov

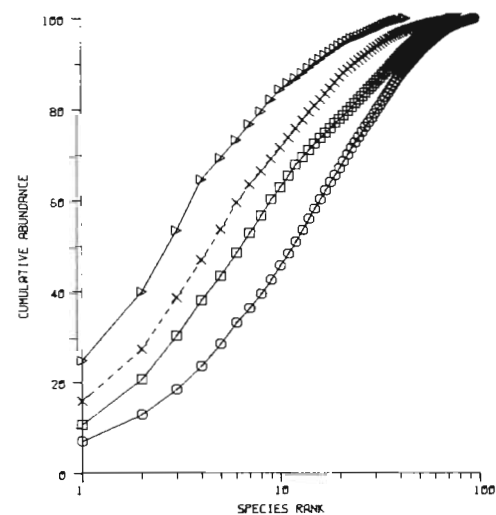


Fig. 9. *k*-dominance curves for nematode assemblages from Stn 1, Clyde inland sea area, a sublittoral station (after Zhang unpubl.) and a low water station (after McIntyre et al. 1970) in Firemore Bay, Scotland. (X --- X) Stn 1, Cores A to C. Sublittoral station: (□) Jul; (○) Oct. (Δ) Low water station, Jul

nation on the Clyde nematode assemblages for reasons which are discussed in detail elsewhere (Lamshead & Platt 1985, Platt & Lamshead 1985), including an examination of the data from this study.

Neutral model analysis

The diversity curves appeared to demonstrate that Core 5A had a reduced diversity, presumably due to a localized contamination effect from the effluent pipe. All diversity analyses are to some degree sample-size dependent. This is because diversity includes some measure of species richness and species richness is to some degree proportional to the number of individuals. This effect is most pronounced with small samples. Because a species can never be represented by less than 1 individual, as sample size decreases some rare species will be lost. Core 5A had a significantly lower number of individuals than the other Ayr Bay cores. It also had a low number of species (Table 7). It may be

Table 7. Number of identified individuals (N), species (S), simple dominance (d) and neutral model V statistic for each core

| Station/core | N | S | d | V |
|--------------|------|----|----|-------|
| 1A | 508 | 49 | 17 | -1.05 |
| 1B | 549 | 56 | 13 | -1.28 |
| 1C | 950 | 56 | 20 | -0.65 |
| 2A | 464 | 49 | 22 | -2.45 |
| 2B | 425 | 47 | 28 | -1.00 |
| 2C | 578 | 57 | 20 | -1.42 |
| 3* | 1415 | 44 | 31 | -0.52 |
| 4A* | 2325 | 50 | 17 | +1.30 |
| 4B | 600 | 35 | 17 | +0.59 |
| 4C | 609 | 36 | 26 | -1.41 |
| 5A | 50 | 16 | 32 | -1.15 |
| 5B | 170 | 33 | 19 | -0.08 |
| 5C | 168 | 33 | 14 | -0.23 |
| 6A | 231 | 38 | 15 | -0.38 |
| 6B | 147 | 32 | 18 | -0.08 |
| 6C | 266 | 43 | 23 | -1.22 |

* Cores with their total number of individuals determined, rather than a 25% subsample

therefore that the lower diversity exhibited by this core is an artifact of a smaller sample. It would still be an artifact even if the low density was a genuine reflection of a contamination effect.

One way to avoid the sample size problem is to concentrate on the evenness component of diversity which is far less susceptible to sample size effects. The simplest way to measure evenness is by calculating simple dominance, i.e. the percentage of the sample dominated by the commonest species (Shaw et al.

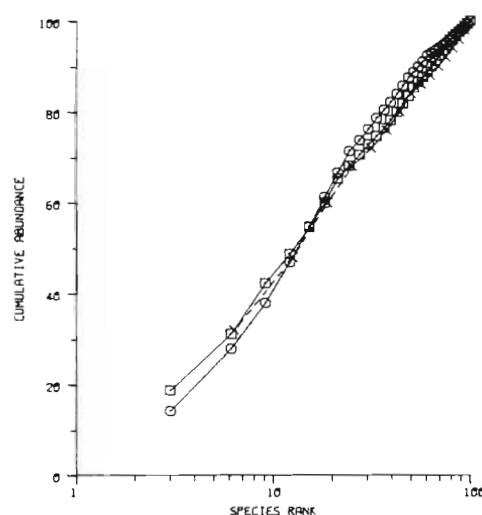


Fig. 10. Lorenz equitability curves for nematode assemblages from Stn 5. (X --- X) Core A; (□) Core B; (○) Core C

1983). Core 5A had a simple dominance index which, although higher than the other Ayr Bay cores (Table 7), was no higher than uncontaminated Stn 3. A more sophisticated method for measuring evenness is the Lorenz curve derived from a k -dominance curve (Lamshead et al. 1983). The Lorenz curves for Stn 5 (Fig. 10) indicate that Core 5A has an equitability curve indistinguishable from 5B or 5C. These results suggest that the low diversity measured for Core 5A is an artifact.

An extremely sensitive, and relatively sample-size independent, method for analysing diversity was developed by Caswell (1976) from a neutral model for genetic studies devised by Ewens (1972). This model generates a species distribution for a sample of given number of individuals and species assuming no inter-specific biotic interactions or differential responses to the environment (i.e. no species has any competitive advantage over other species and species do not interact). A theoretical diversity (EH') is produced from this distribution. A deviation statistic V is calculated by subtracting EH' from the actual diversity of the sample (H') and dividing by the standard deviation of EH' . The use of the neutral model to analyse patterns of marine benthic species diversity and its implications for the relationship between disturbance and diversity is discussed by Platt & Lamshead (1985).

Table 7 shows the V statistics for the Clyde samples. V was negative in all but 2 cases. This is not unusual for benthic data, positive V 's being characteristic of post-catastrophe situations (Lamshead & Platt 1985, Platt & Lamshead 1985). The reference cores had a mean V of -1.20 compared to a mean of -0.30 for the contaminated cores. The means were significantly different ($0.05 > p$ using a t test) indicating that the

contaminated cores had a significantly higher diversity than the reference cores. The V statistic for Core 5A was not particularly low; 5 of the other 15 cores had lower diversities.

Ordination

Five principal coordinates analyses were made of the data: Gower's index with joint absences included; joint absences excluded; reruns of these with data rescaled into 5 logarithmic abundance classes; and the Bray-Curtis index of similarity.

Fig. 11 shows a plot of Vectors I and II of the principal coordination analysis using Gower's index with joint absences included. As all the principal coordination runs using Gower's index produced similar results this plot will be quoted as representative of them all.

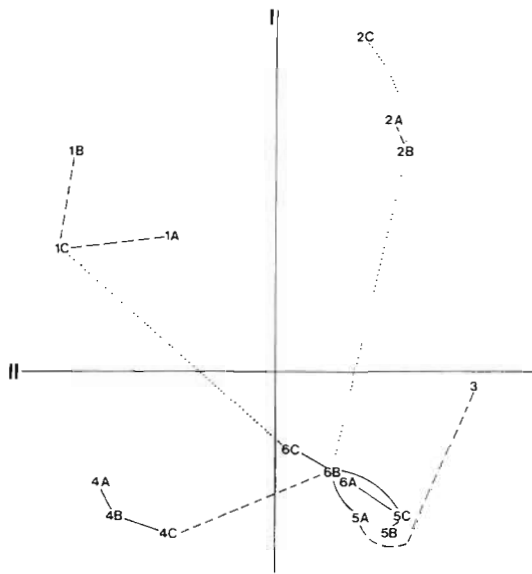


Fig. 11. Principal coordinate analysis of cores using Gower's similarity coefficient with joint absences included; y axis = Vector I, x axis = Vector II. The minimum spanning tree (MST) is overlaid, dotted line indicates similarity of 74 to 76 %, dashed line 76 to 84 % and unbroken line 85 to 89 %

The minimum spanning tree was imposed over the plot. Examination of the eigenvalues, which give the proportion of the total variance accounted for by each axis, indicated that only 20 % of the variation was incorporated in the first vector. Five groups can be distinguished splitting the stations up geographically. Stn 5 and 6 which were only 30 m apart form a single group. Vector III conveyed no useful information so it is not plotted. It is tempting to ascribe the alignment of the stations along Vector I to contamination because the contaminated stations were plotted negative to the reference stations, but uncontaminated Stn 3 lies closer to the contaminated stations than the other refer-

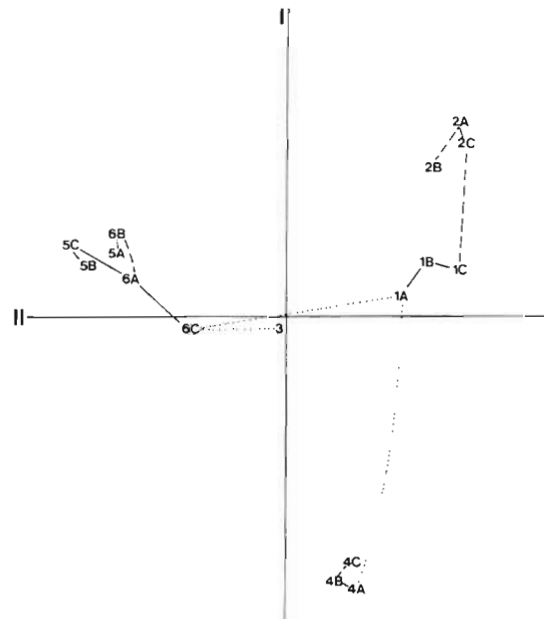


Fig. 12. Principal coordinate analysis of the cores using the Bray-Curtis index of similarity; y axis = Vector I, x axis = Vector II. The MST is overlaid, dotted line indicates similarity of 35 to 50 %, dashed line 51 to 63 % and unbroken line 64 to 73 %

ence stations so the results are equivocal. Also the minimum spanning tree does not suggest a split into 2 groups, contaminated and uncontaminated.

The plot for Vectors I and II for the 5th ordination carried out using the Bray-Curtis index of similarity is shown in Fig. 12. The results are similar to Gower's index, except there is even less suggestion of a contaminated and uncontaminated split. Vector III, which is not plotted, split off Core 5A as an outlier. This core has only a low percentage connection (37 %) with the other Ayr Bay cores according to the minimum spanning tree.

Fig. 13 shows the results for a reciprocal-averaging analysis of the data. The figure shows the sites plotted on Vectors I and II. The square roots of the eigenvalues gave a correlation between sites and species of 0.69 for Vector I, 0.67 for Vector II and 0.56 for Vector III, which is reasonable. The grouping produced for the cores was similar to the principal coordinates analyses except that the stations in north Etrick Bay and south Etrick Bay were grouped together by reciprocal averaging. Stn 3 is separated as an outlier and there is no suggestion of a fundamental split into contaminated and reference stations.

Ecological cladistics

Ordination and other phenetic techniques based on 'overall' or 'raw' similarity have been largely replaced

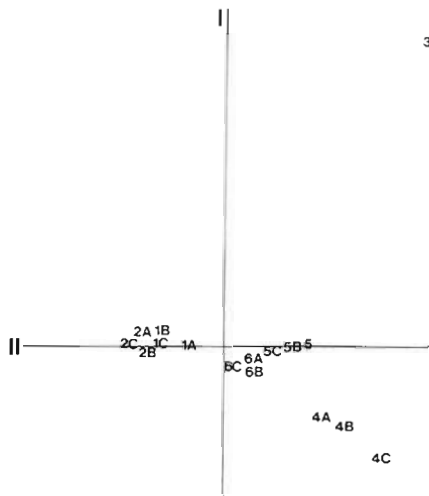


Fig. 13. Reciprocal averaging analysis of the cores; y axis = Vector I, x axis = Vector II

in systematics by cladistics. Cladistic analysis differs fundamentally from phenetics in that it involves the identification of monophyletic groups defined by the possession of one or more shared derived characters unique to that group. Lamshead & Paterson (in press) argue the case for the application of cladistics to ecological analysis and demonstrate that the method has some significant advantages over phenetic analysis.

Sites can be grouped cladistically using species as characters in exactly the same manner as phenetics. The presence of a species is coded as a derived or homologous character while absence is coded as primitive. The outgroup is defined as a string of primitive characters, i.e. a sample with no species present. The implication of ecological cladistics is that species presence can be used as a homologous character. That is, if a species is present in 2 samples then its presence is assumed to be due the same key environmental factors influencing both samples. There exists the possibility of species being excluded from 2 or more samples for the same reasons, e.g. a homologous absence. Unfortunately, this cannot logically be used to construct the groupings since it could never be ascertained whether an absence is homologous or analogous except by inference from the groupings themselves.

A key point when using cladistics to group sites is that the species used as characters must have the potential for dispersion into all the sites were it not for the influence of the ecological parameters under test. This is really another example of the classic ecological criterion that like should be compared with like. This criterion is fulfilled in these samples since there is no reason to doubt that any of the nematode species have the capability for dispersion into any of the stations.

Cladistics offers 2 important advantages over phenetics. The first is greater explanatory power. The second is the ability to select between competing explanations by a logical criterion, parsimony, which involves no assumptions by the investigator about the results. Different phenetic algorithms commonly give different results when applied to the same data and there is no accepted biological way of choosing between them that does not involve circular reasoning.

The numerical cladistic program used in this analysis was a Camin-Sokal program called PHYLIP. This is not the most effective program but it has the merit of running on a modest minicomputer and appears to give adequately parsimonious cladograms when applied to ecological data (Lamshead & Paterson in press).

Fig. 14 shows the most parsimonious cladogram for the relations between the cores. Dichotomy 1 divided the cores into 2 groups, a contaminated group and a reference group. Dichotomy 2 separated the reference cores spatially into Stn 1 and 2 (Ettrick Bay) and Stn 3 (Scalpsie Bay). Dichotomy 2' separated the contaminated cores spatially into Stn 4 (Irvine Bay) and Stn 5 and 6 (Ayr Bay) with the exception of Core 5A which is an outlier being included with Stn 4.

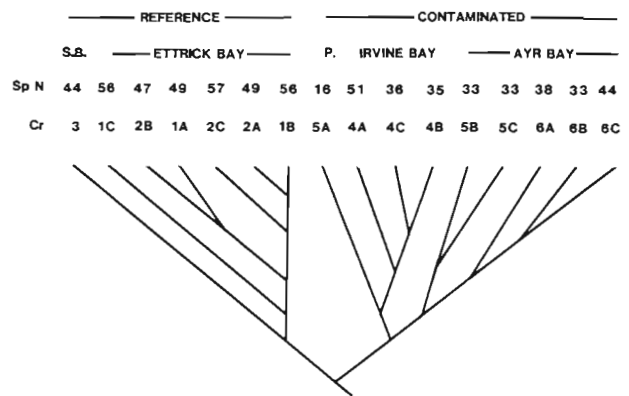


Fig. 14. Most parsimonious cladogram showing the relation between the cores. Cr = core, Sp N = number of species in sample, S.B. = Scalpsie Bay, P. = pipe (i.e. the core taken from the mouth of the effluent pipe)

CONCLUSIONS

The diversities, densities, feeding type ratios and V statistics for the nematode assemblages from the reference stations are consistent with the assumption that these assemblages are uncontaminated. In contrast, the V statistics, cladistic and, to some extent, multivariate analyses demonstrate that the nematode assemblages from the contaminated stations were detectably affected by non-catastrophic contamina-

Appendix. Continued

| Species list | Feeding type | 1A | 1B | 1C | 2A | 2B | 2C | 3 | 4A | 4B | 4C | 5A | 5B | 5C | 6A | 6B | 6C |
|---|--------------|-----|-----|-----|-----|-----|-----|------|------|-----|-----|----|-----|-----|-----|-----|-----|
| 56. <i>Microlaimus monstrosus</i> | 2A | 1 | 3 | - | 1 | - | 1 | - | 21 | 8 | 2 | 1 | 1 | - | 1 | 1 | 2 |
| 57. <i>Microlaimus ostracion</i> | 2A | 1 | - | 1 | 4 | 2 | 9 | 11 | 3 | - | 1 | - | 3 | 4 | 3 | 2 | 8 |
| 58. <i>Microlaimus A</i> | 2A | - | 1 | - | 3 | - | 1 | - | - | - | - | - | - | - | - | - | - |
| 59. <i>Monhystera venusta</i> | 1A | - | - | - | 1 | - | 2 | - | 94 | 50 | 161 | - | 1 | - | - | - | 1 |
| 60. <i>Monoposthia mirabilis</i> | 2A | 19 | 11 | 21 | 14 | 5 | 8 | 2 | 46 | 13 | 18 | - | 4 | 1 | 3 | 8 | 6 |
| 61. <i>Nannolaimoides A</i> | 2A | 3 | 9 | 13 | 1 | - | 3 | - | 192 | 26 | 20 | - | 1 | 2 | 6 | 7 | 9 |
| 62. <i>Nannolaimus fusus</i> | 1A | - | 1 | 1 | 2 | - | - | - | - | - | - | - | - | 2 | 1 | - | - |
| 63. <i>Neochromadora paractea</i> | 2A | 6 | 13 | 25 | 18 | 4 | 7 | 13 | - | - | 1 | - | 2 | 1 | - | 1 | 2 |
| 64. <i>Neochromadora tecta</i> | 2A | - | 1 | - | 2 | - | 5 | 438 | - | - | - | - | - | - | - | - | - |
| 65. <i>Neochromadora trichophora</i> | 2A | 84 | 56 | 90 | 14 | 22 | 12 | 230 | 397 | 102 | 47 | 4 | 32 | 23 | 35 | 14 | 61 |
| 66. <i>Neochromadora A</i> | 2A | - | - | - | - | - | - | - | 108 | 23 | 7 | - | - | - | - | - | - |
| 67. <i>Neotonchus boucheri</i> | 2A | - | - | - | - | - | 2 | 2 | 13 | 1 | 1 | - | - | - | - | 1 | - |
| 68. <i>Odontophora exharena</i> | 2A | 18 | 30 | 32 | 2 | - | - | - | - | - | - | - | - | - | - | - | - |
| 69. <i>Odontophora rectangula</i> | 2A | 3 | - | 5 | - | - | - | - | 51 | 15 | 3 | - | - | - | - | - | 1 |
| 70. <i>Odontophoroides paramonhystera</i> | 2A | 3 | 1 | 2 | 2 | 2 | 1 | 68 | - | 7 | 6 | - | 3 | 4 | 2 | 2 | 2 |
| 71. <i>Oxystomina elongata</i> | 1A | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - |
| 72. <i>Paracanthochus thaumasius</i> | 2A | - | 2 | 1 | - | - | - | - | 44 | 16 | 13 | - | 2 | - | 1 | 1 | 1 |
| 73. <i>Paracyatholaimus occultus</i> | 2A | - | - | - | - | - | - | 12 | - | - | - | - | - | - | - | - | - |
| 74. <i>Paracyatholaimus pentodon</i> | 2A | 13 | 60 | 47 | - | - | 1 | - | 9 | 9 | 2 | - | 2 | - | 1 | - | - |
| 75. <i>Polysigma fuscum</i> | 2A | - | - | 2 | 1 | 8 | 3 | - | - | - | - | - | - | - | - | - | - |
| 76. <i>Pomponema debile</i> | 2A | 1 | 3 | 11 | 1 | 5 | 5 | 9 | - | 1 | - | - | - | - | 2 | - | 1 |
| 77. <i>Pomponema effilatum</i> | 2A | - | - | - | - | - | 3 | 2 | - | - | - | - | - | - | - | - | - |
| 78. <i>Pomponema sedecima</i> | 2A | - | 1 | - | - | - | 1 | - | 4 | - | - | - | - | - | - | - | - |
| 79. <i>Prochromadorella ditlevseni</i> | 2A | 64 | 62 | 100 | 21 | 8 | 24 | 8 | 193 | 36 | 14 | 1 | 11 | 8 | 24 | 9 | 15 |
| 80. <i>Pselionema A</i> | 1A | - | - | 1 | 1 | - | 4 | - | - | - | - | - | - | - | - | - | - |
| 81. <i>Pseudonchus A</i> | 1B | - | - | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - | - |
| 82. <i>Rhabdocoma riemanni</i> | 1A | - | - | - | - | - | - | 4 | - | - | - | - | - | - | - | - | - |
| 83. <i>Rhabdodemania imer</i> | 1B | - | - | - | - | - | - | 9 | - | - | - | - | - | - | - | - | - |
| 84. <i>Rhynconema A</i> | 1A | 6 | 5 | 12 | - | - | - | 9 | - | - | - | - | 3 | 3 | 6 | 1 | 8 |
| 85. <i>Richtersia inaequalis</i> | 1B | - | - | - | 1 | - | - | - | 19 | - | - | - | - | - | - | - | 1 |
| 86. <i>Sabatieria longiseta</i> | 1B | - | - | 4 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 87. <i>Sabatieria longispinosa</i> | 1B | 24 | 18 | 15 | 4 | 10 | 6 | - | 12 | 2 | 1 | 1 | - | 11 | 9 | 5 | 13 |
| 88. <i>Sigmophoranema litorale</i> | 2A | - | - | - | - | - | - | 6 | - | - | - | - | - | - | - | - | - |
| 89. <i>Siphonolaimus ewensis</i> | 2B | - | - | - | - | - | - | 1 | 1 | - | - | - | - | - | - | - | - |
| 90. <i>Spiliphora hirsuta</i> | 2A | 18 | 47 | 69 | 3 | 3 | 11 | 43 | - | - | - | - | - | - | - | - | - |
| 91. <i>Spilophorella candida</i> | 2A | - | - | - | - | - | - | 30 | - | 5 | 5 | - | 3 | 3 | - | - | - |
| 92. <i>Spilophorella paradoxa</i> | 2A | 1 | - | - | - | - | - | 22 | - | - | - | - | - | - | - | - | - |
| 93. <i>Spirinia laevis</i> | 1A | - | 1 | 1 | - | 1 | - | - | - | 2 | - | - | - | - | 1 | - | - |
| 94. <i>Stephanolaimus elegans</i> | 1A | - | - | - | 6 | 6 | 5 | 2 | - | - | - | - | - | - | - | - | - |
| 95. <i>Stephanolaimus A</i> | 1A | - | - | - | 3 | - | 1 | - | - | - | - | - | - | - | - | - | - |
| 96. <i>Tarvaia angusta</i> | 1A | 1 | 1 | 1 | 1 | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 97. <i>Terschellingia communis</i> | 1A | - | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 98. <i>Terschellingia A</i> | 1A | - | 1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 99. <i>Thalassoalaimus A</i> | 1A | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - |
| 100. <i>Theristus bastiani</i> | 1A | - | 3 | 2 | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 101. <i>Theristus curvatus</i> | 1B | 4 | 1 | 6 | - | - | - | - | - | - | - | - | - | 3 | - | - | 2 |
| 102. <i>Theristus fleveniis</i> | 1B | 1 | - | - | 3 | 2 | - | - | - | - | - | - | - | - | - | - | - |
| 103. <i>Theristus interstitialis</i> | 1B | - | - | - | - | - | - | - | - | - | - | - | 3 | - | - | - | - |
| 104. <i>Theristus munnui</i> | 1B | 62 | 26 | 79 | 104 | 121 | 76 | 34 | 104 | 24 | 73 | 2 | 5 | 15 | 17 | 9 | 35 |
| 105. <i>Theristus pertenuis</i> | 1B | - | - | - | - | - | - | - | 3 | - | - | - | - | - | - | - | - |
| 106. <i>Theristus A</i> | 1B | 1 | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - | - |
| 107. <i>Theristus B</i> | 1B | - | - | - | - | - | - | - | - | - | - | - | 1 | - | - | 1 | 3 |
| 108. <i>Trefusia A</i> | 1A | 2 | - | - | 4 | 1 | 16 | - | - | - | - | - | - | - | - | - | - |
| 109. <i>Trichotheristus mirabilis</i> | 1B | 17 | 1 | 8 | 7 | 4 | 8 | 13 | 40 | 11 | 37 | 1 | 9 | 9 | 13 | 16 | 7 |
| 110. <i>Trileptium parisetum</i> | 2B | - | - | - | - | - | - | 1 | - | - | - | - | - | - | - | - | - |
| 111. <i>Viscosia cobbi</i> | 2B | 3 | 7 | 5 | 4 | 21 | 7 | 9 | 71 | 14 | 2 | - | - | - | - | 2 | 2 |
| 112. <i>Viscosia A</i> | 2B | 1 | 4 | 2 | - | 1 | 4 | - | 1 | - | - | - | - | - | - | - | - |
| 113. <i>Xyala riemanni</i> | 1B | - | - | - | - | - | - | 117 | - | - | - | 2 | - | - | - | - | - |
| Unidentified specimens | | 7 | 21 | 21 | 15 | 30 | 28 | 61 | 80 | 8 | 7 | 1 | 5 | 7 | 1 | 5 | 3 |
| Totals | | 515 | 570 | 971 | 479 | 455 | 606 | 1476 | 2405 | 608 | 616 | 51 | 175 | 175 | 232 | 152 | 269 |

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