Do Meio- and Macrobenthic Nematodes Differ in Community Composition and Body Weight Trends with Depth?

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Abstract

Nematodes occur regularly in macrobenthic samples but are rarely identified from them and are thus considered exclusively a part of the meiofauna. Our study compares the generic composition of nematode communities and their individual body weight trends with water depth in macrobenthic (>250/300 μm) samples from the deep Arctic (Canada Basin), Gulf of Mexico (GOM) and the Bermuda slope with meio-benthic samples (<45 μm) from GOM. The dry weight per individual (μg) of all macrobenthic nematodes combined showed an increasing trend with increasing water depth, while the dry weight per individual of the meio-benthic GOM nematodes showed a trend to decrease with increasing depth. Multivariate analyses showed that the macrobenthic nematode community in the GOM was more similar to the macrobenthic nematodes of the Canada Basin than to the GOM meio-benthic nematodes. In particular, the genera Enoploides, Crenopharynx, Micoletzkyia, Phanodermella were dominant in the macrobenthos and accounted for most of the difference. Relative abundance of non-selective deposit feeders (1B) significantly decreased with depth in macrobenthos but remained dominant in the meio-benthic community. The occurrence of a distinct assemblage of bigger nematodes of high dry weight per individual in the macrobenthos suggests the need to include nematodes in macrobenthic studies.

Introduction

Free-living nematodes are an abundant and diverse phylum that is usually considered in studies of meio-benthos. While several macrobenthic studies note their presence, they are identified only to phylum level and not considered in macrobenthic assemblages [1]. A comparison of body weight in individuals of different taxa showed that nematodes had the highest percent carbon dry weight suggesting an important role in the carbon cycle [2]. The main reason for the omission of large nematodes in macrobenthos studies is that the distinction between macro- and meio-benthos was originally based purely on sieve size [3]. Meiobenthos are defined as fauna retained on 42/63 μm sieves [4] and macro-benthos are defined as, depending on habitat, fauna retained on 0.25–1 mm sieve apertures [5]. A more flexible definition of these groups is now widespread and is based on taxonomic composition [6]. Macro-benthos sensu stricto excludes nematodes, harpacticoids and ostracods [4]. Large nematodes are, therefore, hardly ever taxonomically identified in any study from a given region.

The following trends on biomass and nematode size variation with water depth have emerged from numerous studies. Both total macrobenthic and meio-benthic biomass generally decreases with water depth though the rate of decline is greater for larger organisms [7]. This decline is partially explained by the decrease in average metazoan size with water depth [8]. The size of individual nematodes has also been noted to decrease with depth in the deep sea [9,10] and has been attributed to the typically decreasing availability of food and decreasing sediment grain size. Studies on the functional diversity of nematodes as determined by buccal morphology show that with increasing water depth deposit feeders predominate while predators were less dominant [10]. However, macrobenthic nematodes have not been included in these studies and our current study emerged from an interest in evaluating the role of these larger nematodes in benthic communities and food webs.

Here we test the null hypothesis that meio- and macrobenthic nematode communities do not differ in structure and function. Specifically, the structure of the nematode community was examined by (1) measuring average weights of individual nematodes in macrobenthic samples from the Gulf of Mexico, high Arctic Canada Basin and Bermuda transect and in meio-benthic samples from the Gulf of Mexico in relation to water depth, and (2) comparing the community composition of macro-benthic (GOM, Canada Basin, Bermuda transect) and meio-benthic (GOM) nematodes using a multivariate approach. The functional role of meio- and macrobenthic nematodes was...
determined by comparing the feeding group distribution based on
their buccal morphology. Taxonomic and abundance data for the
Canada Basin can be found in Sharma and Bluhm [11] and the
GOM and Bermuda transect data are not yet published.

Methods

Meio- and macrobenthic samples were collected from the Gulf
of Mexico with a GOMEX boxcorer (0.2 m²) during May and
June 2000 (Figure 1) [12]. 28 samples were collected from 14
stations in water depths of 212–3000 m. A set of five subsamples
were set within the box thus reducing the total area sampled for
macrobenthos to 0.17 m². Sediments were removed, along with
overlying water, down to a depth of 15 cm within the box and
sieved on a 300 µm sieve. The meiobenthic samples were two of
the seven subsamples with the box, each with a 5.5 cm inner
diameter and a circular surface area of 22.9 cm². The meiofauna
were extracted by sieving through a 45 µm mesh sieve and
centrifugation with Ludox [9].

Macrobenthos from the Arctic deep-sea Canada Basin were
collected from a total of 22 quantitative box corer casts at a total
of 8 stations at depths of 640–3961 m (Figure 1). The samples
were collected with three replicates at each station in 2005 with 0.06 m²
surface area for replicates 1 and 2 and 0.03 m² for replicate three
[13], and mostly without replication in 2002 (0.04 m² surface area)
[1]. The top 10 cm of sediment and the overlying water was sieved
through a 250 µm sieve. Details about the study area and
additional benthic collections can be found in Bluhm et al. [1] and
MacDonald et al. [13].

The Bermuda Slope samples were collected with an epibenthic
sled at 1535–2200 m depth with details on sampling locations and
methods in studies of Sanders and Hessler [14]. The samples
examined are deposited at the Museum of Comparative Zoology,
Harvard University, Cambridge, MA. The GOM and Canada
Basin samples will be archived at the National Museum of Natural
History, Washington, D.C.

Samples from all areas were preserved in 4% buffered formalin
and later transferred to 70% ethanol. Nematodes were sorted by
hand under WILD M3 and Leica M12 stereo-microscopes and
processed to glycerin [15] for identification. All nematodes were
identified to genus rather than species as many deep-sea species
are not described. The total length and width of ethanol-preserved
nematodes were measured on a Zeiss Axioscop to the nearest
20 µm. Five representative individuals (juveniles and adults) of
each genus were measured to obtain average measurements.
Biomass was calculated by the formula: Wet Weight = Length ×
Width²/16000000 [16]. Dry Weight was calculated as 25% of wet
weight [17].

Multivariate community analysis was carried out using
PRIMER® version 6 [18]. Bray-Curtis similarity was calculated
on the abundance matrix after presence-absence transformation.
Similarities between station groups were tested using analysis of
similarities (ANOSIM) in which global R = 1 indicates complete
separation of groups and global R = 0 indicates no separation [19].
A similarity profile test (SIMPROF) was performed on group
average cluster analysis to test the null hypothesis that the macro-
and meiofaunal samples do not differ from each other.

The functional diversity of the nematode community was
analyzed by classification into one of four feeding groups: Selective
deposit feeders (1A), non-selective deposit feeders (1B), epigrowth
feeders (2A), predators and omnivores (2B). Though there have
been further divisions of these feeding categories [20] the original
scheme introduced by Wieser [21] is used here.

Results

In all study areas combined, 177 nematode genera were found
representing 38 families. Detailed lists and abundances in for the
Canada Basin are included in Sharma and Bluhm [11], 128
nematode genera occurred in the GOM meiofauna while 60
genera occurred in the GOM macrobenthos (Sharma and
Baguley, unpublished). There were 75 genera from 25 families
in the Canada Basin samples (ibid.). The Bermuda slope transect
had 15 nematode genera from 8 families (Sharma and Baguley,
unpublished).

Macrobenthic nematodes measured 400–8600 µm in body
length and 14–130 µm in width, translating into a weight range
of 0.04–7.34 µg per individual (2.11±2.70 µg; mean ± SD).
Meiobenthic nematodes measured 300–3500 µm in body length
and 25–110 µm in width translating into a weight range of 0.60–
3.03 µg (1.38±0.78) per individual. The mean dry weights of
individual macrobenthic nematodes showed a non-significant
increase with water depth while the biomass of meiobenthic
nematodes decreased non-significantly with increasing depth
(Figure 2).

Cluster analysis of nematode genera indicates that the
macrofauna nematodes of the GOM were more similar to those
of the Canada Basin and Bermuda slope than to the meiofaunal
nematodes at the same GOM stations (Figures 3). High R values in
the ANOSIM support the separation of GOM meio- and
meiobenthic nematode communities while no significant separa-
tion was seen between the GOM and Canada Basin macrobenthic
nematode communities (Table 1).

A SIMPER analysis indicated 73% dissimilarity between the
meio- and macrofauna nematode genera in the GOM with genera
such as Enoploides, Crenopharynx, Micoletzkyia and Phanodermella
present in macrobenthic samples and accounting for part of the
difference (combined 7%). These larger nematodes that were
predominant in the GOM, Canada Basin and Bermuda slope
occurred rarely in the meiofauna samples. The highest within-
area similarity was found in the GOM meiofauna nematodes
(60%) with genera such as Halalanus, Desmoscolex, Microcamus
and Anomotheristus contributing most to this similarity (combined 17%).
The pattern did not change greatly when the analysis was run at
the family level (Figure 4).

The feeding group composition of the macrofauna nematodes
from the GOM, Canada Basin and Bermuda transect combined
shows a non-significant increase of predators and omnivores (2B)
and selective deposit feeders (1A) with increasing water depth.
Group 1B decreased significantly with depth in macrofauna
(p = 0.002, Figure 5) but remained dominant in the meiofauna
community (Figure 6). In the meiofauna samples, feeding group
composition did not change significantly with depth nor were
there any obvious trends, though as noted above, the non-selective
deposit feeders remained dominant at all depths. The dominant
feeding group in both the meiofauna and macrofauna commu-
nities was 1A. Group 2A was the least represented feeding group in
the macrofauna sample. The slight increase of epigrowth feeders
with increasing water depth in the meiofauna samples is due to
increased presence of Desmodoridae genera. Group 1B was
abundant at all stations and represented by larger genera of the
Comesomatidae (Sabatieria) and Phanodermatidae (Micoletzkyia,
and Phanodermella). Group 2B was only present at some deeper
stations of the Canada Basin.

The cluster analysis of nematode feeding groups from meio- and
macrofauna defined three significant clusters with meiofauna all
grouping in one cluster but including three Canada Basin stations
(Figure 7). High R values in the ANOSIM again supported the
separation of GOM meio- and macrobenthic nematode communities while no significant separation was seen between the GOM and Canada Basin macrobenthic nematode communities (Table 1). Again, the highest average within group similarity (81%) was in the feeding group composition of the GOM meiofauna (SIMPER analysis). The group 1A contributed 53% to this similarity. The highest dissimilarity between groups was between the GOM meio- and macrofauna.
A frequency distribution of individual body weight suggests a bimodal distribution with one mode including the meiobenthic nematodes and the second comprised of macrobenthic nematodes (Figure 8). There are fewer genera among the larger weight classes that comprises nematodes of 4 μg to 15 μg dry wt per individual than among the 1–3 μg dry wt per individual weight classes.

Discussion

1. Size and biomass
Meiobenthic nematodes are defined as such by size and are logically smaller than macrobenthic nematodes. The plot of nematode sizes shows a predominance of smaller nematodes that

Figure 2. Station mean dry weight per individual of nematodes across genera from meio- and macrobenthos in the Gulf of Mexico, Canada Basin and the Bermuda transect.
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Figure 3. Hierachical cluster analysis with SIMPROF test on similarity of nematode genera from meio- and macrobenthos in the Canada Basin and the Bermuda transect based on presence/absence of genera.
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are part of the meiofauna (Figure 8). The meio- and macrobenthic faunas of shallow subtidal regions are characterized by a defined bimodal distribution of body sizes [22]. In the deep sea, however, this distinction between the meio- and macrobenthos body sizes is not well defined [23]. Nevertheless, the depth trend in our data, although non-significant, combined with the depth frequency distribution, may suggest a size gap between meio- and macrobenthic nematodes in the deep sea areas examined.

Larger-bodied nematode genera such as *Crenopharynx*, *Micoletzkyia* and *Phanodermella* and families such as Leptosomatidae, Thoracostomopsidae and Phanodermatidae dominated our macrobenthos samples at greater depths and are seldom recorded in meiofaunal studies. The relationship of our GOM meiofaunal nematodes to water depth, although not significant, combined with the depth frequency distribution, may suggest a size gap between meio- and macrobenthic nematodes in the deep sea areas examined.

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The upper limit to the size of meiofauna has been suggested to be 0.5–1.0 mm [27] as the organisms would shift from an interstitial to burrowing lifestyle. Although not tested in this study, environmental factors such as sediment properties, trophic interactions, and sampling factors such as fixation method and sieve size influence body size [11] and the environmental factors are related to depth [24]. The finer sediments found at deeper water depths may limit interstitial organism size. Thus long thin nematodes such as *Halalaimus* that can move through finer sediments are well represented in deeper waters in both meio- and macrobenthic fauna. These thin nematodes are also well represented in meiobenthic studies as they pass through the 1.0 mm sieve [16]. Warwick [22] suggests that meiofaunal traits such as feeding and resource partitioning are optimized at 45 μg dry weight body size. The larger size may facilitate burrowing and overcome movement barriers in the finer sediments in deeper waters [27]. The smaller flocculents generally reported at greater water depths may also allow for less restricted movement at greater water depths. The only published record of macrobenthic nematodes apart from ours is from a polluted river

| Pairwise comparison | R statistic | P-value |
|--------------------|------------|---------|
| Genus Global R = 0.687, p = 0.001 |
| GOM meio vs macrofauna | 0.995 | 0.003 |
| GOM macrofauna vs CB macrofauna | 0.261 | NS |
| Family Global R = 0.513 p = .001 |
| GOM meio vs macrofauna | 0.677 | 0.001 |
| GOM macrofauna vs CB macrofauna | 0.171 | NS |
| Feeding Group Global R = 0.603, p = .001 |
| GOM meio vs macrofauna | 0.908 | 0.001 |
| GOM macrofauna vs CB macrofauna | 0.351 | NS |

Footnote: GOM = Gulf of Mexico, CB = Canada Basin.

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Figure 4. Cluster analysis with SIMPROF test on similarity of nematode families from meio- and macrobenthos in GOM and from macrobenthos in Canada Basin and Bermuda transect.

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where they were associated with fine sediments [28] but were absent in coarse sediments.

The choice of sorting methods to extract nematodes may also affect their observed size in benthic studies that do not use an upper size limit to extract meiofauna. A study in the Gulf of Mexico found that manual sorting produced more taxonomic groups and higher abundance of nematodes than the traditional extraction method by Ludox centrifugation which removed the larger nematodes of the macrobenthos [29]. The biomass of hand-sorted nematodes was also significantly higher in samples from the abyssal sites. Further analysis of the size ranges of the nematodes could determine if this reflects higher biomass of abyssal nematodes that may constitute macrobenthos.

2. Community structure

The meiobenthic nematode community in the deep GOM was found to be significantly different than macrobenthic nematodes from the same and two other deep sea regions, while the macrobenthic nematodes from these three regions did not significantly differ from each other (Table 1). These families of macrobenthic nematodes, namely, Phanodermatidae and Leptosomatidae are seldom recorded in meiobenthic nematode studies. The generic composition of the meiobenthic samples is similar to that of other studies with a dominance of Comesomatidae and Xyalidae [10]. The large dissimilarity between nematode genera contributing to meiobenthic and macrobenthic communities observed here is a significant finding and supports the theory that meiofauna and macrofauna are functionally separate communities [27]. Our study is the first record of several genera from the GOM and western Atlantic, namely, Thoracostomopsis, Micoletzkyia, Phanodermella and Synonchus as they have not been considered in previous meiobenthic studies in these regions [30]. Similarly, at least seven genera found in the Canada Basin samples were new records for the Arctic deep sea [11].

3. Functional groups

Among the macrobenthic nematodes, the observed trend of increasing proportion of predators and omnivores, such as Oncholai-
midae and Enchelidiidae at increasing depth may be related to reduced competition by other macrobenthic predators. Omnivory is also a useful trait in the deep sea where food is scarce. These families are almost absent from deeper waters in studies of meiobenthic nematodes [31,32]. The selective deposit feeding families Phanodermatidae and Leptosomatidae apparently predominate at greater depths and displace the non-selective deposit feeders, Monhysteridae and Comesomatidae that are prevalent at the shallower water depths. While the classification of marine nematodes into feeding groups is based solely on stoma structure, observations in the lab have shown that nematodes are flexible in feeding preferences [33]. However, given the significant reduction of selective deposit feeders in the macrobenthic community but dominance of this feeding group in the meiofauna community, we interpret these data as further support of the conclusion that the meiobenthic and macrobenthic nematodes constitute structurally and functionally different communities.

4. Conclusions
Data presented here support the idea that meio- and macrofauna represent two unique communities, rather than one continuous community within a taxonomic group (e.g. Nematoda) as evidenced by: 1) different community structure (meio- vs. macrobenthic nematodes), 2) different body sizes, regardless of depth trends, and 3) the different functional response with depth as evidenced by the loss of the dominance of non-selective deposit feeders (1B) among macrobenthos.

Figure 7. Cluster analysis with SIMPROF test on similarity of nematode feeding groups from meio- and macrobenthos in the Gulf of Mexico (GOM) and from macrobenthos in Canada Basin and Bermuda transect.
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Figure 8. Numbers of nematode genera in each weight class.
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References

1. Bluhm BA, MacDonald IR, Debenham C, Iken K (2005) Macro- and meegabenthic communities in the high Arctic Canada Basin: initial findings. Polar Biol 28: 218–231.
2. Rowe GT (1983) Biomass and production of the deep-sea macrobenthos. In: Rowe GT, ed. The Sea, vol 8:Deep-Sea Biology. New York: Wiley. pp 97–122.
3. Mare MF (1942) A study of a marine benthic community with special reference to the micro-organisms. J Mar Biol Ass UK 25: 517–554.
4. Dinet A, Desbruyères D, Khripounoff A (1985) Abondance des peuplements macro- et méio-benthiques: répartition et stratégie d’échantillonnage. In: Laubier L, Monniot C, eds. Peuplements profonds du Golfe de Gascogne: campagnes Biogas. Brest: IFREMER. pp 121–142.
5. Eleftherious E, Moore DC (2005) Macrofauna techniques. In: Eleftherious E, McIntyre A, eds. Methods for the Study of Marine Benthos, 3rd ed. Oxford, UK: Blackwell Publishing. pp 160–22.
6. Gage JD, Hughes DJ, Gonzalez Vecino JL (2002) Sieve size influence in estimating biomass, abundance and diversity in samples of deep-sea macrobenthos. Mar Ecol Prog Ser 225: 97–107.
7. Rex MA, Ettier RJ, Morris JS, Crouse J, McClain CR, et al. (2006) Global bathymetric patterns of standing stock and body size in the deep-sea benthos. Mar Ecol Prog Ser 317: 1–9.
8. Thiel H (1979) The size structure of the deep-sea benthos. Int Rev Gesamthydrobiol 60: 576–606.
9. Baguley JG, Hyde LJ, Montagna PA (2004) A semi-automated digital microphotographic approach to measure meiofaunal biomass. Limnol Oceanoogr: Methods 2: 181–190.
10. Soetaert K, Heip C (1995) Nematode assemblages of the deep sea and shelf break sites in the North Atlantic and Mediterranean sea. Mar Ecol Prog Ser 125: 171–183.
11. Sharma J, Bluhm BA (2010) Diversity of free-living nematodes from macrobenthos in the Arctic deep-sea Canada Basin. Mar Biodiv: DOI 10.1007/s12526-010-0060-1.
12. Roland GS, Rowe GT (1991) Deep-sea benthic sampling with the GOMEx box corer. Limnol Oceanogr 36: 1015–1020.
13. MacDonald IR, Bluhm BA, Iken K, Gagaev S, Strong S (2010) Benthic Macrofauna and meiofauna assemblages in the Arctic deep-sea. Deep-Sea Res II 57: 136–152.
14. Sanders HL, Hessler RR (1969) Diversity and composition of abyssal benthos. Science 166: 1074.
15. Scinorsh JW (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 4: 67–69.
16. Soetaert K, Franco M, Lampadarios N, Muthumbi A, Steyaert M, et al. (2009) Factors affecting nematode biomass, length and width from the shelf to the deep sea. Mar Ecol Prog Ser 392: 125–132.
17. Wieser W (1960) Benthic studies in Buzzard’s Bay II. The meiofauna. Limnol Oceanogr 5: 128–137.
18. Clarke KR, Gorley RN (2006) PRIMER v6: User Manual/Tutorial. PRIMER.
19. Clarke KR, Warwick RM (2001) Change in marine communities: An approach to statistical analysis and interpretation, 2nd ed. Plymouth: Primer-E Ltd. 169 p.
20. Moens T, Vinex M (1997) Observations on the feeding ecology of estuarine nematodes. J Mar Biol Ass UK 77: 231–227.
21. Wieser W (1955) Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. Arch Zool 2: 439–484.
22. Warwick RM (1984) Species size distribution in marine benthic communities. Oecologia 61: 32–41.
23. Thiel H (1975) The size structure of the deep-sea benthos. Int Rev Ges Hydrobiol 60: 575–606.
24. Solvvedel T, Piankuche O, Thiel H (1996) The size structure of deep-sea meiofauna in the north-eastern Atlantic: nematode size spectra in relation to environmental variables. J Mar Biol Ass UK 76: 327–344.
25. Gage JD, Tyler PA (1991) Deep-sea biology: a natural history of organisms at the deep-sea floor. Cambridge, UK: Cambridge University Press. 504 p.
26. Shirayama Y (1983) Size structure of deep-sea meio- and macrobenthos in the Western Pacific. Int Rev Ges Hydrobiol 68: 799–810.
27. Schwinghammer P (1983) Characteristic size distributions of integral benthic communities. Can J Fish Aquatic Sci 38: 1253–1263.
28. Bazzanti M (2000) Macrobenthic nematodes as biological indicators in a Mediterranean lowland river in Central Italy: a case study. Arch Hydrobiol 148: 59–70.
29. Escobar-Briones EG, Diaz C, Legendre P (2008) Meiofauna community structure of the deep-sea Gulf of Mexico: variability due to the sorting method. Deep-Sea Res II 55: 2627–2633.
30. Hope WD (2005) An annotated checklist of marine nematodes of the Western North Atlantic and Gulf of Mexico. J Nematol 37(8): 1–200.
31. Jensen P (1988) Nematode assemblages in the deep-sea benthos of the Norwegian Sea. Deep-Sea Res 35: 1173–1184.
32. Tietjen JS (1989) Ecology of deep-sea nematodes from the Puerto Rico Trench area and Hatteras Abyssal Plain. Deep-Sea Res 36: 1579–1594.
33. Moens T, Yeates GW, DeLay P (2004) Use of carbon and energy sources by nematodes. Nematol Mon Pers 2: 529–545.
34. Wei C-L, Rowe GT, Hubbard GF, Scheltema AH, Wilson GDF, et al. (2010) Bathymetric zonation of deep-sea macrofauna in relation to export of surface phytoplankton production. Mar Ecol Prog Ser 399: 1–14.

Author Contributions

Conceived and designed the experiments: BAB GTR. Performed the experiments: JS JB BAB GTR. Analyzed the data: JS JB BAB. Wrote the paper: JS BAB.