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**Abundance of small individuals influences the effectiveness of
processing techniques for deep-sea nematodes**

Leduc D.^{1*}, Probert P.K.¹, Berkenbusch K.¹, Nodder S.D.², Pilditch C.A.³

¹Portobello Marine Laboratory, Department of Marine Science, University of Otago,
P.O. Box 8, Portobello 9048, New Zealand

²National Institute of Water and Atmospheric Research (NIWA) Limited, Private Bag
14-901, Kilbirnie, Wellington, New Zealand

³Department of Biological Sciences, University of Waikato, Private Bag 3105,
Hamilton, New Zealand

*Corresponding author. Tel.: +64 3 479 5828; fax: +64 3 479 8336

Email address: daniel.leduc@otago.ac.nz

ABSTRACT

Nematodes are the most abundant metazoans of deep-sea benthic communities, but knowledge of their distribution is limited relative to larger organisms. Whilst some aspects of nematode processing techniques, such as extraction, have been extensively studied, other key elements have attracted little attention. We compared the effect of (1) mesh size (63, 45, and 32 μm) on estimates of nematode abundance, biomass, and body size, and (2) microscope magnification (50 and 100 \times) on estimates of nematode abundance at bathyal sites (250-3100 m water depth) on the Challenger Plateau and Chatham Rise, south-west Pacific Ocean. Variation in the effectiveness of these

techniques was assessed in relation to nematode body size and environmental parameters (water depth, sediment organic matter content, %silt/clay, and chloroplastic pigments). The 63- μm mesh retained a relatively low proportion of total nematode abundance (mean \pm SD = $55 \pm 9\%$), but most of nematode biomass ($90 \pm 4\%$). The proportion of nematode abundance retained on the 45- μm mesh in surface (0-1 cm) and subsurface (1-5 cm) sediment was significantly correlated ($P < 0.01$) with %silt/clay ($R^2 = 0.39$) and chloroplastic pigments ($R^2 = 0.29$), respectively. Variation in median nematode body weight showed similar trends, but relationships between mean nematode body weight and environmental parameters were either relatively weak (subsurface sediment) or not significant (surface sediment). Using a low magnification led to significantly lower (on average by 43 %) nematode abundance estimates relative to high magnification ($P < 0.001$), and the magnitude of this difference was significantly correlated ($P < 0.05$) with total nematode abundance ($R^2_p = 0.53$) and the number of small ($\leq 250 \mu\text{m}$ length) individuals ($R^2_p = 0.05$). Our results suggest that organic matter input and sediment characteristics influence the abundance of small nematodes in bathyal communities. The abundance of small individuals can, in turn, influence abundance estimates obtained using different mesh sizes and microscope magnifications.

Keywords: continental slope, productivity, chloroplastic pigments, water depth, sediment granulometry, meiofauna, magnification, morphometry, size spectra

1. Introduction

Deep-sea benthic invertebrate communities perform important ecosystem services such as the regulation of nutrient fluxes and the provision of food to higher trophic levels (Ridgwell and Hargreaves, 2007; Jones, 2008). Metazoan meiofauna (i.e., animals that pass through a 0.5-1.0 mm mesh but are retained on a 20-63 μm mesh), and nematodes in particular, are the most abundant animals in these communities and make a substantial contribution to deep-sea ecosystem functioning (Pequegnat et al., 1990; Tietjen, 1992; Baguley et al., 2008; Danovaro et al., 2008). Meiofauna are well-suited for monitoring human impacts on deep-sea ecosystems because of their widespread occurrence, permanent contact with the sediment, and high population turnover rate (Giere, 2009). Knowledge of meiofauna distribution in the deep sea, however, is limited compared with that of macro- and megafauna (Gage and Tyler, 1992).

Processing of meiofauna samples is generally time-consuming owing to their small size, high abundance, and high diversity (Schratzberger et al., 2000). These characteristics, however, mean that more information can be generated from the study of meiofaunal communities than from analyses restricted to larger, but less abundant and less diverse organisms (Giere, 2009). This greater amount of information is particularly useful for deep-sea studies, where the number of sampling opportunities and the number of samples that can be obtained is limited by high research costs and logistical constraints. It is important, therefore, to assess the effectiveness of processing methods to help maximise the amount of information that can be obtained from deep-sea samples (Gage et al., 2002). During the last four decades, meiofaunal research techniques have been simplified and standardised (McIntyre, 1969; Pfannkuche and Thiel, 1988; Somerfield et al., 2005). Some aspects of meiofaunal

sample processing methods, such as extraction, have been extensively studied (de Jonge and Bouwman, 1977; Schwinghamer, 1981; Burgess, 2001), but others, such as the choice of mesh size and sorting, have attracted less attention (but see de Bovée et al. 1974; Escobar-Briones et al. 2008).

Results from early deep-sea investigations led Thiel (1975) to suggest that food limitation in the deep sea favours small body size. Several studies also reported trends of decreasing nematode body size with water depth (e.g., Pfannkuche, 1985; Soetaert and Heip, 1989). Consequently, most subsequent meiofaunal studies used relatively fine ($\leq 45 \mu\text{m}$) mesh sizes (see review by Soltwedel 2000). Trends of decreasing nematode body size with water depth, however, are far from universal (Shirayama, 1983; Vanhove et al., 1995; Soltwedel et al., 2003; Udalov et al., 2005). In addition, depth-related trends in nematode body size are usually described using univariate metrics (e.g., mean body weight, Udalov et al. 2005), which do not accurately describe variation in size spectra. The effectiveness of different mesh sizes (e.g., 32 versus 63 μm) for the characterisation of nematode abundance, for example, is likely to depend on the abundance of small individuals, and may not be related to variation in mean body size (Vanreusel et al., 1995).

Environmental factors such as organic matter (OM) input and sediment granulometry can influence nematode body size (Wieser, 1959; Tita et al., 1999; Udalov et al., 2005), but few studies have assessed their potential impact on the effectiveness of different mesh sizes. Brown et al. (2001), for example, suggested that increasing OM input leads to lower abundance of small nematodes in the equatorial Pacific. Increased organic matter input, however, could also have the opposite effect if it led to greater proportion of juveniles (Shirayama, 1983). To our knowledge, potential relationships between sediment granulometry and the effectiveness of

different mesh sizes have not been investigated. Further assessment of the effectiveness of different mesh sizes (e.g., 63, 45, and 32 μm) in relation to environmental parameters and commonly used measures of body size (e.g., mean body weight) is needed to help make informed decisions about the optimal mesh size to use in deep-sea studies.

Choosing a mesh size depends on study objectives and involves trade-offs between accuracy and pragmatic considerations (Schlacher and Wooldridge, 1996). Using a relatively coarse mesh, for example, may be adequate when characterising meiofaunal biomass (Grove et al., 2006), but may be unsuitable for studies on diversity (Leduc et al., 2010). In contrast, finer mesh sizes retain more individuals, but increase the cost of sample processing which, in turn, limits the number of replicates that can be analysed (Bachelet, 1990).

Another aspect of meiofaunal research techniques that has received little attention is the choice of magnification for abundance estimates. Pfannkuche and Thiel (1988) and Westheide and Purschke (1988) recommend using a stereomicroscope with 25-50 \times magnification, whereas Somerfield et al. (2005) recommend using a compound microscope with 100 \times magnification. With few exceptions, most researchers use the lower magnification (e.g., Pfannkuche 1985; Soltwedel et al. 2003), unless they intend to identify specimens to genus or species, or obtain biomass estimates (e.g., Schratzberger et al. 2000; Grove et al. 2006). Mounting specimens for observation under the compound microscope increases processing time substantially, but may provide more accurate counts than those obtained using a stereomicroscope. The difference in abundance estimates between high and low magnifications is likely to be most pronounced when many small individuals are present in the samples, but this potential bias has not yet been

quantified. Moreover, magnification is frequently not specified in the methods, making comparisons between studies difficult.

The first objective of this study was to quantify the effect of mesh size (63, 45 and 32 μm) on estimates of nematode abundance, biomass, and body size on the Chatham Rise and Challenger Plateau, south-west Pacific Ocean. Differences in the effect of mesh size on nematode abundance estimates were examined in relation to variation in body size (i.e., mean and median body weight) and environmental parameters. The second objective was to determine the effect of magnification (50 versus 100 \times) on estimates of nematode abundance at several locations across the Chatham Rise. Differences in nematode counts between magnifications were examined in relation to nematode abundance and relative abundance of small individuals. The present study provides the first assessment of common methods used for processing nematode samples across gradients of productivity (from low to high chloroplastic pigment concentrations in the sediments), sediment granulometry (6-93% silt/clay), and water depths (~250-3100 m). Results from a study on the effects of mesh size and core penetration depth on nematode community structure and diversity at a single study site are reported elsewhere (Leduc et al., 2010).

2. Materials and methods

2.1 Sampling and laboratory methods

Samples were obtained as part of a larger study of variation in the abundance, biomass and diversity of benthic organisms on the continental margin of New Zealand. The present study focused on two main bathymetric features of the New Zealand Exclusive Economic Zone, the Chatham Rise and the Challenger Plateau (Figure 1). The Chatham Rise is a broad submarine ridge extending eastwards from the South

Island of New Zealand at depths ~350-3000 m. It lies under the Subtropical Front (STF), a region where warm subtropical surface water to the north meets cold, high nutrient-low chlorophyll subantarctic surface water to the south (Boyd et al., 1999). The STF appears to be bathymetrically locked onto the southern flank of the Rise near 44°S (Uddstrom and Oien, 1999; Sutton, 2001), and is associated with heightened primary productivity (Bradford-Grieve et al., 1997; Murphy et al., 2001). The Challenger Plateau encompasses water depths ranging from 400 to ~3000 m in subtropical waters in an area of generally low biological productivity to the northeast of the South Island, New Zealand (Murphy et al., 2001).

Samples for studying the effect of mesh size on nematode community parameters (i.e., abundance, biomass, and body weight) were collected from 23 locations between 240 and 1300 m water depth on the Chatham Rise and Challenger Plateau in March-April and May-June 2007, during National Institute of Water and Atmospheric Research (NIWA) cruises TAN0705 and TAN0707, respectively, as part of the Ocean Survey 20/20 initiative. Samples for comparing the effect of magnification on estimates of nematode abundance were collected along a transect at 178°30' E across the Chatham Rise (nine stations, 350-3100 m water depth) in September-October 2001 (NIWA cruise TAN0116).

Samples were taken using an Ocean Instruments MC-800A multicorer (MUC; core i.d. = 9.52 cm). For meiofaunal analyses, one to two replicates (i.e., samples from different MUC deployments) per site were obtained during the 2007 cruises, and 3-5 replicates per site were obtained in the 2001 cruise. Each meiofaunal sample consisted of a subcore (i.d. = 2.6 cm) taken to a sediment depth of 5 cm. Subcores obtained in 2007 were divided into 0-1 and 1-5 cm sections (surface and subsurface

samples, respectively), whereas subcores obtained in 2001 were not divided. All samples were preserved in 10% buffered formalin and stained with Rose Bengal.

Samples for studying the effect of mesh size on nematode community parameters (TAN0705 and TAN0707) were rinsed through a 1-mm mesh to remove macrofauna, and through a set of nested sieves of 63-, 45-, and 32- μm mesh size to retain meiofauna. Meiofauna from each mesh size was extracted from the remaining sediment by Ludox flotation (Somerfield and Warwick, 1996). Meiofaunal samples were then rinsed with a mixture of dilute ethanol and glycerol, transferred to a cavity block, and left under a fume hood for at least 48 h to allow water and ethanol to evaporate, leaving the sample material in pure glycerol (Somerfield and Warwick, 1996). Samples were mounted on 1-2 permanent slides (depending on the amount of material in the sample) and sealed with paraffin wax. The surface area of the sample on each slide was approximately 10 cm^2 . All nematodes present in the sample were counted using a compound microscope (100 \times magnification).

Nematode body volumes were estimated from length and maximum body width measurements obtained by video image analysis (Nodder et al., 2003; Grove et al., 2006). Body volumes were converted to dry weight (DW) based on a relative density of 1.13 and a dry:wet weight ratio of 0.25 (Feller and Warwick, 1988). Estimates of mean and median body weight were based on a minimum of 50 and 100 nematodes (or all individuals if fewer were present) in surface (0-1 cm) and subsurface (1-5 cm) sediment, respectively. Mean and median body size estimates based on the 32- and 45- μm mesh sizes included individuals from the coarser meshes.

The efficiency of the 63- μm mesh in characterising nematode abundance was expressed as the number of nematodes retained by the 63- μm mesh divided by the number of nematodes retained on all three mesh sizes. Similarly, the efficiency of the

45- μm mesh was expressed as the sum of nematodes retained by the 63- and 45- μm mesh sizes divided by the total number of nematodes in the sample. Calculations were done in the same manner for nematode biomass.

Samples for studying the effect of magnification on nematode abundance (TAN0116) were rinsed through a 500- μm mesh to remove macrofauna and through a 45- μm mesh to retain nematodes. Meiofauna was extracted using Ludox flotation (Somerfield and Warwick, 1996), rinsed in freshwater, and transferred to a Bogorov tray for counting under a stereomicroscope at 50 \times magnification. The counted specimens were not removed from the sample. Samples (including all meiofaunal organisms and detritus) were subsequently transferred to glycerol and mounted onto slides as described above. Nematodes were re-counted with a compound microscope (100 \times magnification) connected to a computer screen. The magnification of objects viewed on the screen was approximately 220 \times . The length of at least 50 nematodes per sample was measured using video image analysis to estimate the abundance of small (≤ 250 and 350 μm in length) individuals.

Physical and biogeochemical sediment parameters at each site were measured for the surface (0-5 mm) sediment layer from one or two cores of the same MUC deployment (2007 data only). These parameters were: %silt/clay (sum of silt and clay particles), %total organic matter (TOM), and chloroplastic pigments ($\mu\text{g g}^{-1}$ DW sediment, sum of chlorophyll *a* and phaeopigments). Methods for the determination of environmental parameters are given in Nodder et al. (2003) and Grove et al. (2006). Briefly, silt/clay content was determined by wet-sieving subsamples at 63 μm and analysing the <63- μm fraction using Sedigraph techniques, chloroplastic pigment content was estimated using standard spectrophotometric techniques after freeze-

drying and extraction in 90% acetone (Sartory, 1982), and TOM content was determined by loss-on-ignition (500°C for 4 h) (Eleftheriou and Moore, 2005).

2.2 Statistical analyses

Unless specified otherwise, statistical analyses of mean and median nematode body weight were carried out using total population estimates (i.e., estimates based on the 32- μm mesh). Mean and median nematode body weight and the proportion of nematode abundance and biomass retained on the 63- and 45- μm mesh sizes were compared between surface and subsurface sediments using paired *t*-tests.

Relationships between nematode body weight and the proportion of nematode abundance retained on the 63- and 45- μm mesh sizes were investigated using Pearson correlation coefficients (Quinn and Keough, 2009).

The influence of environmental parameters on nematode body weight and the proportion of nematodes retained on the 63- and 45- μm mesh sizes were analysed using multiple linear regressions (Minitab v. 15) with the following dependent variables: mean nematode body weight, median nematode body weight, and the proportion of nematode abundance retained on the 63- and 45- μm mesh sizes in surface and subsurface sediment. Four predictors (independent variables) were used in multiple regressions: water depth, %silt/clay, TOM, and chloroplastic pigments. Owing to the limited number of observations, models with three predictors or less were used to avoid overfitting (Quinn and Keough, 2009). For each dependent variable, all possible combinations of models with three predictors or less were compared and the model with the greatest coefficient of determination (R^2) was selected. As a result, some regression models have different numbers and/or combinations of predictors. The relative importance of each predictor in each model

was evaluated by comparing coefficients of partial determination (R^2_p , Quinn and Keough, 2009).

Data were assessed for normality and homogeneity of variance using Anderson-Darling normality test and Levene's test, respectively (Anderson and Darling, 1952; Levene, 1960). When necessary, data were \log_{10} -transformed to meet assumptions for parametric analyses. The absence of collinearity was verified by comparing tolerance values of predictors (Quinn and Keough, 2009). The relationship between selected dependent variables and predictors was illustrated graphically using partial regression plots (Quinn and Keough, 2009).

The effects of nematode abundance, and relative abundance of small (≤ 250 or ≤ 350 μm in length) individuals, on the difference in nematode counts between high ($100\times$) and low ($50\times$) magnification were also investigated using multiple linear regression. Separate multiple regressions were performed using each size class of small individuals and regression assumptions verified as described above. The analysis was originally done using the relative abundance of nematodes smaller than 150, 250, 350, 450, and 550 μm in length. Preliminary analysis showed a significant relationship for nematodes <250 μm in length, but non-significant relationships for nematodes <350 μm in length. The size threshold where nematodes began to be overlooked due to their small size was, therefore, between 250 and 350 μm . Hence only results for these two size classes were shown.

3. Results

3.1 *Effect of mesh size on estimates of nematode community parameters*

Nematode abundance at the 23 study sites ranged from 132 to 3085 individuals (ind.) 10 cm^{-2} , and biomass ranged from 7.7 to 473.6 $\mu\text{g DW } 10\text{ cm}^{-2}$. The 63- μm mesh

retained a relatively low proportion of nematode abundance (mean = 55%, SD = 9%), but retained most of nematode biomass ($90 \pm 4\%$). Overall, the 63- and 45- μm mesh sizes were less efficient in surface than subsurface sediment. The proportion of nematode abundance and biomass retained by the 63- μm mesh was significantly lower in surface than in subsurface sediment (paired t -test, $n = 36$, $t = 6.01$, $P < 0.001$) (Table 1). The same pattern was observed for 45- μm mesh size ($t = 3.74$, $P \leq 0.001$).

Coarse mesh sizes led to higher estimates of mean and median nematode body weight relative to finer mesh sizes (Table 2). Estimates based on the 45- and 63- μm mesh sizes were up to 38 and 155% higher, respectively, than estimates based on the 32- μm mesh. Estimates of mean and median nematode body weight based on the 63-, 45-, and 32- μm mesh sizes were significantly higher for subsurface than for surface sediment (paired t -test, $n = 36$, $P < 0.05$).

There was a positive relationship between the proportion of nematode abundance retained on the 63- μm mesh and mean nematode body weight in subsurface sediment ($P \leq 0.01$) (Table 3). A similar relationship was found between the proportion of nematode abundance on the 45- μm mesh and mean nematode body weight in subsurface sediment. No significant relationships were found for mean nematode body weight in surface sediment or depth-integrated (0-5 cm) samples ($P > 0.1$). Median nematode body weight was significantly correlated with the proportion of nematodes retained on the 63- and 45- μm mesh sizes in both surface and subsurface sediment ($P < 0.05$).

There was no significant relationship between environmental parameters and the proportion of nematode abundance retained on the 63- μm mesh screen in surface sediment. There was, however, a significant negative relationship between the proportion of nematode abundance retained on the 45- μm mesh in surface sediment

and %silt/clay ($R^2_p = 0.39$, $P = 0.001$, Table 4, Figure 2). Median nematode body weight in surface sediment was negatively correlated with %silt/clay ($R^2_p = 0.44$, $P = 0.004$) and positively correlated with chloroplastic pigments ($R^2_p = 0.19$, $P = 0.041$), but no significant relationship was found for mean nematode body weight in surface sediment.

The concentration of chloroplastic pigments accounted for most of the variation for all dependent variables in subsurface sediment (Table 4, Figure 2). The proportions of nematode abundance retained on the 63- and 45- μm mesh screens in subsurface sediment were also significantly correlated with TOM and %silt/clay ($P < 0.05$).

Water depth was retained in only one of the six significant regression models. No significant relationship was found between water depth and any of the dependent variables in surface or subsurface sediment after the effect of the other variables was taken into account ($P > 0.05$).

3.2 Effect of magnification on estimates of nematode abundance

Nematode abundance across the Chatham Rise transect (nine sites) ranged from 11 to 1098 ($50\times$ magnification) and from 58 to 1867 ind. 10 cm^{-2} ($100\times$ magnification). Estimates of nematode abundance obtained using low ($50\times$) magnification were significantly lower than estimates obtained using high ($100\times$) magnification (paired t -test, $n = 31$, $t = 7.27$, $P < 0.001$). The former yielded values that were 43% lower on average ($SD = 28$) than values based on the latter.

There was a significant, positive relationship between nematode abundance and the difference in nematode counts between high ($100\times$) and low ($50\times$) magnification (Table 5, Figure 3). The proportion of small nematodes ($\leq 250\ \mu\text{m}$ in

length) was also significantly correlated with differences in counts between magnifications, but no significant relationship was found for nematodes $\leq 350 \mu\text{m}$ in length. No significant relationship was found between the percentage difference in nematode counts between magnifications and any of the independent variables.

4. Discussion

4.1 Relationships between nematode body size and environmental parameters

The size of meiofaunal taxa, such as nematodes, can vary considerably depending on environmental parameters (Tita et al., 1999; Soetaert et al., 2009). Water-depth-related trends in nematode body size have been the subject of many studies (see Udalov et al., 2005 for an overview), but the mechanisms responsible for these trends are still debated. Several studies found a positive relationships between organic matter (OM) input and nematode body size (Vanreusel et al., 1995; Sommer and Pfannkuche, 2000; Brown et al., 2001; Soetaert et al., 2009), supporting Thiel's (1975) hypothesis that food limitation favours small organisms. Not all meiofaunal studies, however, are consistent with this view (Pfannkuche and Thiel, 1987; Vanreusel et al., 1995; Schewe and Soltwedel, 1999; Soltwedel et al., 2003). Recent evidence suggests that increased OM input has an indirect effect on nematode body size through the generation of reduced conditions in subsurface sediment, which favours larger individuals (Soetaert et al., 2002). In this study, the presence of a positive relationship between chloroplastic pigments and nematode body size in surface sediment suggests that OM input can have a direct impact on body size. A similar relationship was also found for subsurface nematodes, however, suggesting that OM input can also have an indirect effect on body size through the creation of pronounced biogeochemical gradients (Jensen, 1987). The relationship between chloroplastic pigments and

nematode body size was much stronger for subsurface than surface sediment, and variation in nematode body weight was much greater for subsurface than surface sediment (see Tables 2 and 4). Organic matter input is, therefore, most likely to impact nematode body size through its effect on sediment biogeochemistry.

Sediment granulometry is another factor that can have an important influence on nematode size distribution through its effect on the size of interstitial spaces (Wieser, 1959; Tita et al., 1999). The potential impact of sediment granulometry on nematode size distribution in the deep sea has not been studied extensively, however, partly because particle size is often highly correlated with water depth and/or OM input (e.g., Soetaert et al., 2009). The absence of correlations between these variables in the present study allowed their effects to be studied separately. There was a negative correlation between %silt/clay and median nematode body weight in both sediment layers, but this relationship was much more pronounced for surface than subsurface sediment. The effect of %silt/clay on median nematode body weight in surface sediment mostly reflected variation in the abundance of small individuals, as indicated by the lack of a significant correlation in the abundance of large nematodes in the >63- μm size fraction. This finding indicates that small nematodes are more susceptible to changes in silt/clay content than larger ones. Higher silt/clay content reduces the size of interstitial spaces, which may improve the mobility of small nematodes by increasing the efficiency of undulatory propulsion (Wallace, 1968). Larger nematodes, however, may move equally well in sandy or muddy sediment owing to their ability to displace sediment particles, especially in fluid deep-sea oozes. Alternatively, the low abundance of small nematodes at the surface of coarse sediment may be the result of strong hydrodynamic conditions at sandy sites (Nodder et al., 2007). Near-bottom current speed at a site 750 m deep on the southern flank of

the Chatham Rise, for example, can exceed 10 cm s^{-1} and cause periodic resuspension of fine particles (Nodder et al., 2007).

Data from the present study suggest that the effect of environmental factors on nematode body size depends on sediment depth. Sediment granulometry was the main factor affecting body size in surface sediment, while chloroplastic pigment concentration was more important in subsurface sediment. Constraints on body size associated with reduced conditions in subsurface sediment, therefore, appear to be stronger than constraints imposed by the size of interstitial spaces. Overall, our results are consistent with a recent meta-analysis, which showed that food supply and sediment granulometry are the main predictors of nematode body size in marine sediment (Udalov et al., 2005). The present study also supports the analysis of Udalov et al. (2005), who found no relationship between water depth and nematode body size after the effect of sediment granulometry and food input were taken into account.

Body size is likely to influence the effectiveness of different mesh sizes when determining meiofaunal abundances, but the magnitude of this effect has seldom been quantified (de Bovée et al., 1974). The present study showed that the proportion of nematodes retained on the 63- and 45- μm mesh screens was not correlated with mean nematode body weight in depth-integrated (0-5 cm) samples. Hence variation in mean nematode body weight cannot be used to predict the efficiency of these mesh sizes in deep-sea habitats. In contrast, median nematode weight was significantly correlated with the proportion of nematodes retained on the 63- and 45- μm mesh screens in both surface and subsurface sediments. Median nematode body weight, therefore, reflects changes in the proportion of nematodes in the 32-45, 45-63, and $> 63 \mu\text{m}$ size fractions more accurately than mean nematode body weight. This difference is probably related to the lower sensitivity of median values (compared to mean values)

to the presence or absence of large individuals (Soetaert and Heip, 1989). Studies of depth-related trends in nematode body size therefore should include information on median body size and/or size spectra rather than focus solely on mean body size (e.g., Vanreusel et al. 1995).

4.2 Effect of mesh size on estimates of nematode community parameters

The proportion of nematode abundance retained on the 63- μm mesh was relatively low for both surface (30-66%) and subsurface sediment (40-82%). As a result, this mesh size is not considered suitable for characterising deep-sea nematode abundance, although it provides reasonably accurate biomass estimates (retaining 78-96% of total nematode biomass). The 45- μm mesh retained a relatively high (72-91%) proportion of nematode abundance in depth-integrated (0-5 cm) samples, and may be considered suitable for deep-sea investigations depending on the degree of accuracy required.

Few data are available for comparing the proportions of nematodes retained on different mesh sizes. The mean proportion of nematodes retained on the 32-63 μm size fraction in surface sediments at the Chatham Rise and Challenger Plateau study sites (54%) was high compared with surface (0-1 cm) sediment of two abyssal sites (~4750 m water depth) in the NE Atlantic (23 and 26%) (Vanreusel et al., 1995). Values obtained for depth-integrated (0-5 cm) samples (29-60%) were, however, comparable to the proportion of total meiofauna (including Foraminifera) in the 32-63 μm size fraction (24-40%) in the oligotrophic central Arctic Ocean (~900-4200 m depth) (Schewe and Soltwedel, 1999). Pfannkuche (1985) and Pfannkuche and Thiel (1987) used a range of mesh sizes with a lowest mesh size of 42 μm and found that nematodes in the 42-63 μm size fraction represented 8- 26% of total nematode abundance in the NE Atlantic (500-4850 m depth) and High Arctic (226-3920 m

depth). Excluding nematodes in the 32-45 μm size fraction, nematodes in the 45-63 μm size fraction in the present study represented a relatively high proportion (20-47%) of total abundance across the entire sediment depth (0-5 cm). These results are somewhat unexpected given that the focus was on relatively shallow (250-1300 m depth) and productive areas (the Chatham Rise especially), which are usually associated with larger mean nematode body size relative to deeper, less productive locations (Soetaert et al., 2009). Nevertheless, mean body size does not necessarily reflect the proportion of small individuals present in a community, as shown in the present study. Differences between this and other studies are unlikely to reflect differences in sediment grain size, since we sampled a wide range of sediment grain sizes (6-93% silt/clay). Differences in sample processing techniques (such as sorting magnification), however, may help explain the observed discrepancy.

4.3 Effect of magnification on estimates of nematode abundance

There was a marked difference (on average almost two-fold) in nematode abundance estimates obtained using high (100 \times) and low (50 \times) magnifications. The relative magnitude of this difference (i.e., expressed as percentage of total abundance) did not vary as a function of nematode abundance or abundance of small (i.e., ≤ 250 or ≤ 350 μm in length) nematodes. The absolute difference in nematode counts between high and low magnification, however, increased significantly with nematode abundance (as determined under high 100 \times magnification). These results suggest that a relatively constant proportion of individuals is overlooked when enumerating nematodes at low magnification, leading to greater absolute error in counts for high abundance samples. The number of small (≤ 250 μm in length) nematodes was also a significant contributing factor, indicating that small nematodes are more likely to be overlooked

at low magnification than at high magnification. Using a finer mesh (e.g., a 32- μm instead of a 45- μm mesh) could have led to even greater differences between high and low magnification counts since it would have increased the abundance of small nematodes in the samples. The proportion of nematodes $\leq 300 \mu\text{m}$ in length in surface sediment, for example, was greater on the 32- μm mesh ($83 \pm 8\%$) than on the 45- μm mesh ($49 \pm 18\%$) (data not shown).

Since small nematodes are more likely to be overlooked at low magnification than are large ones, some bias will be introduced in the characterisation of the size spectra of the nematode population. Previous studies investigating the size spectra of deep-sea meiofauna using different mesh sizes have quantified meiofaunal/nematode abundances using a stereomicroscope (magnification not specified), which may have led to an underestimation of nematode abundance in the small size classes (Pfannkuche, 1985; Pfannkuche and Thiel, 1987; Vanreusel et al., 1995; Soltwedel et al., 1996; Schewe and Soltwedel, 1999; Soltwedel et al., 2003). This difference in processing method could partly explain the high number of individuals retained by the 32- and 45- μm mesh sizes in the present study relative to previous studies.

5. Conclusions

The present study assessed common processing techniques used for the study of deep-sea nematodes across gradients of productivity, sediment granulometry, and water depth. The 63- μm mesh retained a low proportion ($< 60\%$ on average) of nematode abundance, and, therefore, is not considered suitable for characterising accurately nematode abundance at bathyal sites. The proportion of nematode abundance on the 45- μm mesh screen (72-91% of total abundance) was significantly correlated with chloroplastic pigments and silt/clay content. Hence the choice of mesh size (e.g., 32

versus 45 μm) for the study of bathyal nematodes should take into account these environmental parameters; a 32- μm mesh, for example, may be preferable when studying nematodes at sites with high silt/clay content and low productivity.

Differences in nematode counts between high (100 \times) and low (50 \times) magnification were positively correlated with the abundance of small individuals ($\leq 250 \mu\text{m}$ length), which suggests that small nematodes are overlooked at low magnification.

Underestimating the abundance of small nematodes may also bias the characterisation of nematode size spectra. High (100 \times) magnification should, therefore, be used to study the abundance and size spectra of deep-sea nematodes.

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Fig. 1. Map of New Zealand showing sampling locations on (A) Challenger Plateau and (B) Chatham Rise, including sites for investigating the effects of mesh size (circles) and magnification (crosses) on nematode community attributes.

Fig. 2. Partial regression plots showing relationship between (A) % silt/clay and proportion of nematodes retained on the 45- μm mesh in surface (0-1 cm) sediment, (B) %silt/clay and median nematode body weight in surface sediment, (C) chloroplastic pigments and proportion of nematodes retained on the 45- μm mesh in subsurface (1-5 cm) sediment, and (D) chloroplastic pigments and median nematode body weight in subsurface sediment (see Table 4 for summary statistics).

Fig. 3. Relationship between the difference in nematode counts between high (100x) and low (50x) magnifications and nematode abundance estimated using high magnification (see Table 5 for summary statistics).

Table 1. Mean (SD) percentage of total nematode abundance and biomass retained on 63- and 45- μm mesh sizes at different sediment depths (0-1, 1-5, and 0-5 cm). Different superscript letters (a, b) indicate significant difference between sediment depths (paired t -tests, $P \leq 0.001$).

Mesh size (μm)	Sediment depth (cm)		
	0-1	1-5	0-5
Abundance			
63	46 (10) ^a	61 (10) ^b	55 (9)
45	78 (7) ^a	83 (6) ^b	81 (6)
Biomass			
63	83 (8) ^a	91 (5) ^b	90 (4)
45	94 (4) ^a	97 (3) ^b	97 (3)

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Table 2. Estimates of mean and median (SD) nematode body weight at different sediment depths (0-1, 1-5, and 0-5 cm) based on different mesh sizes (63, 45, and 32 μm). Different superscript letters (a, b) indicate significant difference between sediment depths (paired t -tests, $P \leq 0.001$).

Mesh size (μm)	Sediment depth (cm)	Mean body weight ($\mu\text{g DW}$)	Median body weight ($\mu\text{g DW}$)
63	0-1	0.080 (0.040) ^a	0.035 (0.010) ^a
	1-5	0.120 (0.081) ^b	0.049 (0.024) ^b
	0-5	0.104 (0.050)	0.043 (0.004)
45	0-1	0.052 (0.025) ^a	0.019 (0.004) ^a
	1-5	0.096 (0.066) ^b	0.031 (0.012) ^b
	0-5	0.069 (0.029)	0.026 (0.007)
32	0-1	0.043 (0.020) ^a	0.014 (0.004) ^a
	1-5	0.081 (0.060) ^b	0.023 (0.009) ^b
	0-5	0.062 (0.027)	0.019 (0.002)

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Table 3. Relationship between mean or median nematode body weight and the percentage of total nematode abundance retained on 63- and 45- μm mesh sizes at different sediment depths (0-1, 1-5, and 0-5 cm). Numbers in bold indicate significant correlation ($P < 0.05$). All significant relationships are positive.

Mesh size (μm)	Sediment depth (cm)	Mean nematode body weight		Median nematode body weight	
		<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²
63	0-1	0.298	<0.01	0.003	0.42
	1-5	0.001	0.29	0.008	0.35
	0-5	0.081	0.07	0.003	0.42
45	0-1	0.575	<0.01	0.006	0.37
	1-5	0.01	0.17	0.022	0.26
	0-5	0.280	<0.01	0.031	0.23

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Table 4. Summary statistics of multiple linear regression analyses between environmental parameters and the proportion of nematode abundance retained on 63- and 45- μm mesh sizes, and mean/median nematode body weight in surface and subsurface sediment. Only results of significant regressions are shown, and for each multiple regression, the predictor variable with the greatest coefficient of partial determination (R^2_p) is shown in bold.

Predictor	<i>F</i> ratio	<i>t</i>	R^2_p	<i>P</i>
Surface (0-1 cm) sediment				
<i>45 μm Abundance</i>				
Total organic matter		-1.85	0.08	0.081
%silt/clay		-4.09	0.39	0.001
Total regression	$F_{2,17} = 12.36$		0.58	<0.001
<i>Median nematode body weight</i>				
Total organic matter		-1.30	0.06	0.215
Chloroplasic pigments		2.27	0.19	0.041
%silt/clay		-3.44	0.44	0.004
Total regression	$F_{3,16} = 4.57$		0.51	0.021
Subsurface (1-5 cm) sediment				
<i>63 μm Abundance</i>				
Total organic matter		-2.52	0.19	0.022
Chloroplasic pigments		3.70	0.41	0.002
%silt/clay		-2.58	0.20	0.020
Total regression	$F_{3,16} = 5.28$		0.48	0.009
<i>45 μm Abundance</i>				
Total organic matter		-2.29	0.18	0.035
Chloroplasic pigments		2.89	0.29	0.010
%silt/clay		-2.60	0.23	0.019
Total regression	$F_{3,16} = 4.08$		0.42	0.024
<i>Mean nematode body weight</i>				
Water depth		1.14	0.04	0.271
Chloroplasic pigments		2.58	0.19	0.019
%silt/clay		1.84	0.10	0.084
Total regression	$F_{3,16} = 6.56$		0.54	0.004
<i>Median nematode body weight</i>				
Chloroplasic pigments		6.81	0.74	<0.001
%silt/clay		-3.84	0.25	0.002
Total regression	$F_{2,17} = 23.23$		0.77	<0.001

Table 5. Multiple linear regression summary statistics for examining the effects of nematode abundance and the proportion of small (≤ 250 or $350 \mu\text{m}$ in length) individuals on the difference between nematode counts using high ($100\times$) and low ($50\times$) magnification (significant total regressions could not be obtained for percentage difference in nematode counts between high and low magnification).

Predictor	<i>F</i> ratio	<i>t</i>	R^2_p	<i>P</i>
Number of small individuals ($\leq 250 \mu\text{m}$)		2.08	0.05	0.048
Nematode abundance		8.05	0.53	<0.001
Total regression	$F_{2,28} = 47.25$		0.78	<0.001
Number of small individuals ($\leq 350 \mu\text{m}$)		0.98	0.01	0.338
Nematode abundance		5.91	0.33	<0.001
Total regression	$F_{2,28} = 40.56$		0.76	<0.001

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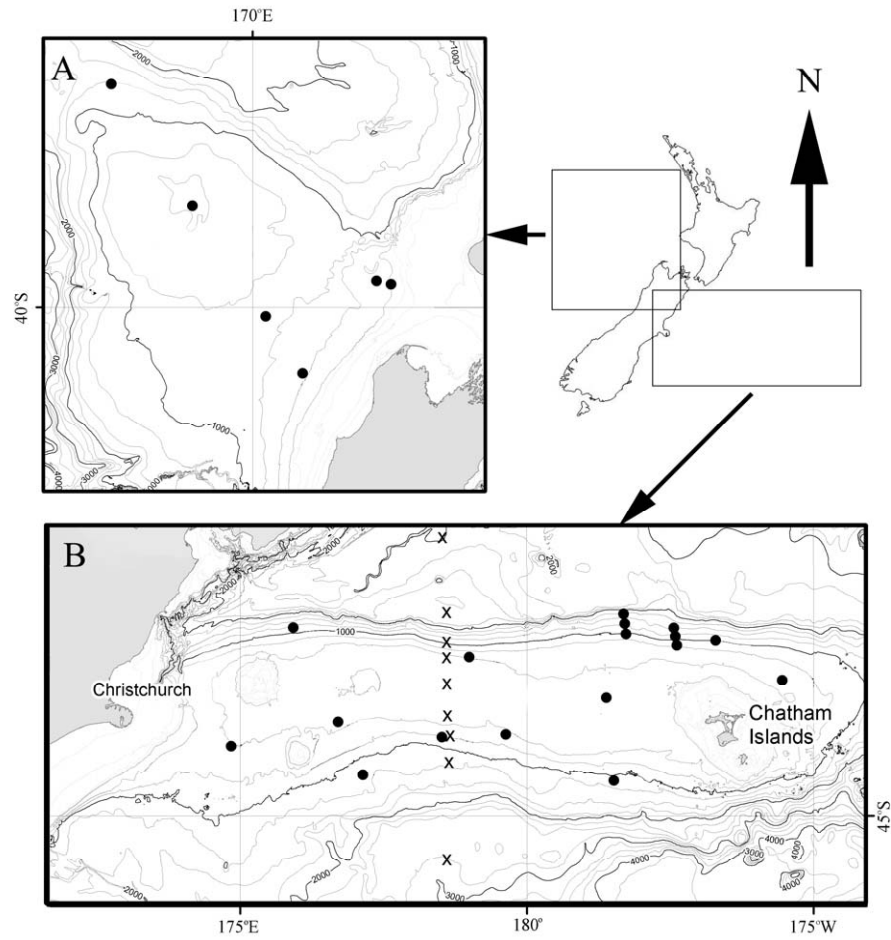


Figure 1 Map of New Zealand showing sampling locations on the (A) Challenger Plateau and (B) Chatham Rise. Sites used for investigating the effect of mesh size and magnification on meiofaunal community attributes are shown by circles and crosses, respectively.

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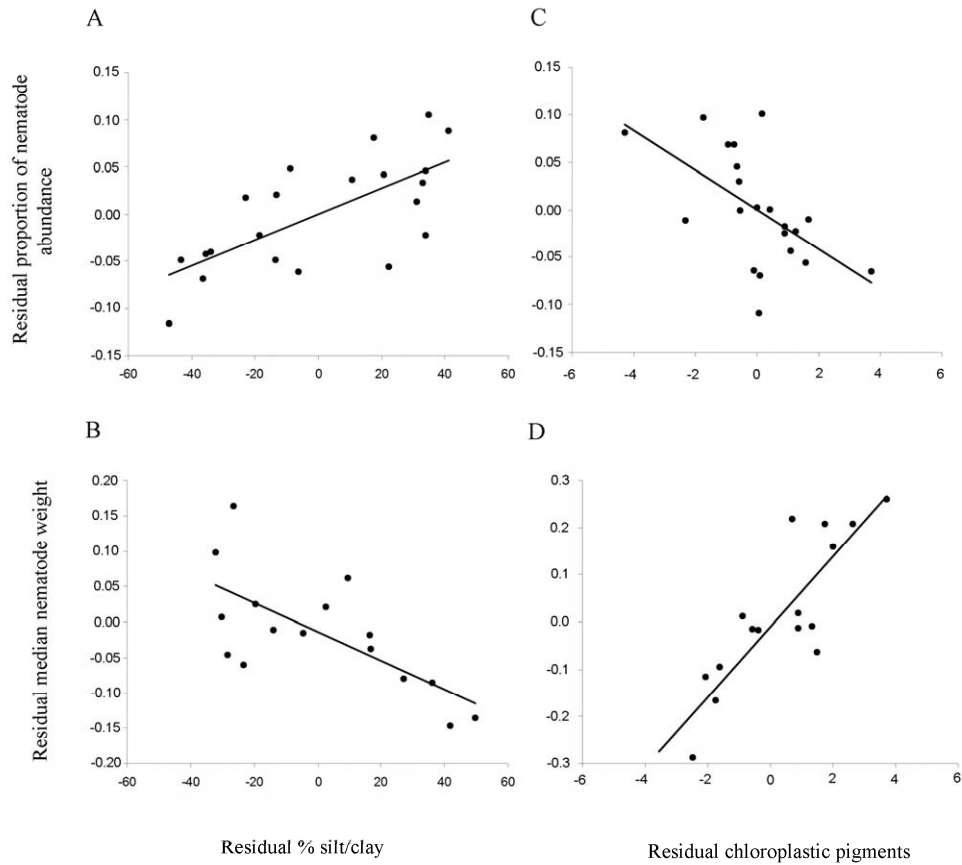


Figure 2. Partial regression plots showing relationship between (A) % silt/clay and proportion of nematodes retained on the 32- μ m mesh in surface (0-1 cm) sediment, (B) %silt/clay and median nematode body weight in surface sediment, (C) chloroplastic pigments and proportion of nematodes retained on the 32 μ m mesh in subsurface (1-5 cm) sediment, and (D) chloroplastic pigments and median nematode body weight in subsurface sediment. See Table 4 for summary statistics.



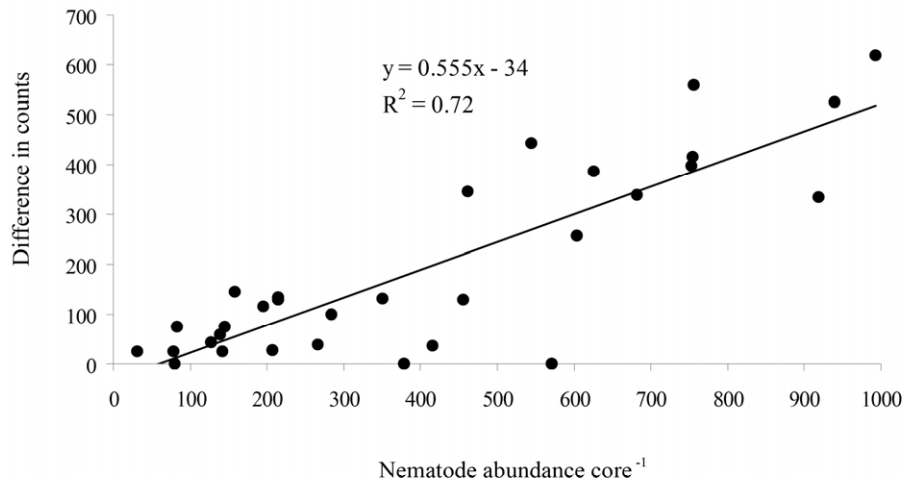


Figure 3. Difference in nematode counts between high (100x) and low (50x) magnifications plotted against nematode abundance estimated using high magnification.

