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Instruments and Methods

Influence of mesh size and core penetration on estimates of deep-sea nematode abundance, biomass, and diversity

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ABSTRACT

It is essential to maximize the information that can be gathered in deep-sea studies by thoroughly assessing sample processing methods. Nematodes are commonly used for the study and monitoring of deep-sea floor habitats, but the potential effects of different methods on the quantification of community attributes remain to be quantified. Here, we consider key methodological elements by comparing the effect of sediment depth and mesh size (63, 45, and 32 μm) on: (1) estimates of nematode community attributes, and (2) the sampling effort required to detect changes in these attributes at a bathyal site on the Chatham Rise, south-west Pacific Ocean. The 63 μm mesh retained most (95%) of the nematode biomass but a lower proportion (53–71%) of the nematode abundance. Retention efficiency of common species on this mesh ranged from 12 (*Hapalomus* sp.) to >88% (*Comesomatidae* spp.). The 63 μm mesh yielded significantly lower diversity estimates than the finer meshes, and failed to detect differences in community structure observed using the 45 and 32 μm mesh sizes. Sediment depth had a substantial effect on all measured community attributes, highlighting the importance of sufficient core penetration into the sediment ($\geq$5 cm) for adequately characterizing nematode distribution. Power analysis showed that using a 32 μm mesh and deepest core penetration led to relatively few (3–8) samples being required to detect significant changes in nematode diversity indices relative to coarser mesh sizes. Characterization of nematode diversity and community structure using appropriate and robust methods of sampling is suggested as a sensitive and efficient tool for the assessment of anthropogenic impacts on deep-sea ecosystems.

1. Introduction

The deep sea is a difficult ecosystem to study as a result of its large size and remote nature. Our understanding of deep-sea processes is limited by our ability to obtain reliable quantitative estimates of faunal abundance, biomass, and diversity at a variety of spatial and temporal scales. It is, therefore, essential to maximize the information that can be gathered in deep-sea studies by assessing the effectiveness of different methods (Gage et al., 2002).

Nematodes account for 80–90% of the abundance of all metazoans (Lambshead, 2004), and are among the most diverse and widespread organisms in benthic habitats (Giere, 2009). The characterization of nematode assemblages requires only small (1–10 cm^2) samples compared to those needed for larger organisms, such as macrofauna (0.1–0.25 m^2) (Eleftheriou and Moore, 2005). Detecting changes in nematode diversity may also require fewer samples than for macro- and megafauna (Rogers et al., 2008). Nematodes are ideal organisms for the study and monitoring of deep-sea soft sediments, but comparisons between studies and geographical locations have been hampered by the variety of sampling and processing methods used (Soltwedel, 2000).

The perceived smaller size of meiofauna in deep-sea sediments relative to coastal ecosystems has led to the use of progressively smaller mesh sizes ranging from 76 μm in early investigations (McIntyre, 1964), to <45 μm in most recent studies (Soltwedel, 2000; Mokievskii et al., 2007). A review of published literature, however, revealed that the water-depth-related decrease in nematode size is evident only in oligotrophic waters, and that other factors, such as sediment granulometry and trophic conditions, play determinant roles in shelf and upper slope habitats (Udalov et al., 2005). In addition, patterns of decreasing body size with water depth are not universal. Soetaert et al. (2002), for example, observed that nematode size varied little between surface sediments of productive shelf habitats and the deep sea, and Shirayama (1983) found a trend of increasing nematode body size with water depth in the western Pacific Ocean.

The choice of mesh size depends largely on study objectives (Bachelet, 1990). Whilst smaller mesh sizes give more accurate

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abundance and diversity data than a coarser mesh, they increase sample processing time and associated costs. This increased effort per sample in turn limits the number of replicates that can be processed within a realistic timescale. Some investigators may be primarily interested in obtaining accurate biomass estimates, in which case a relatively coarse mesh may be more cost-effective than a fine mesh (Grove et al., 2006). Several authors have argued that the main contribution of smaller mesh sizes is the retention of more individuals rather than more species (Rodrigues et al., 2007). Using a very fine mesh (e.g. 32 μm) may not, therefore, always be necessary for investigating bathyal ecosystems. Although several studies have compared the extraction efficiency of different mesh sizes for deep-sea meiofauna (Pfannkuche, 1985; Vanreusel et al., 1995; Schewe and Soltwedel, 1999; Soltwedel et al., 2003), few have compared the efficiency of the finer mesh sizes (e.g., 45 or 32 μm) now used by most researchers (Mokievskii et al., 2007). To our knowledge, the effects of mesh size on estimates of nematode diversity and community structure have not been examined, and few studies have quantified the effect of mesh size on nematode biomass estimates (Vanreusel et al., 1995).

In addition to mesh size, other factors, such as sediment depth, can also influence estimates of faunal parameters. Nematodes, for example, show clear sediment-depth-related gradients in density and size (Heip et al., 1985; Soetaert et al., 2002). These vertical changes are most pronounced in highly productive areas where marked geochemical gradients develop in the sediments (Soetaert et al., 2002). Core penetration into the sediment can have a substantial effect on macrofaunal community attributes (Rodrigues et al., 2007), but few such data are available for meiofauna (Boucher and Lambshead, 1995). Moreover, the potential effects of sediment depth on the efficiency of different mesh sizes have not been investigated.

The purpose of the present study is to quantify and compare the effect of sediment depth and mesh size (63, 45, and 32 μm) on estimates of nematode abundance, biomass, diversity, and community structure. The sampling effort (i.e., number of replicates) required to detect changes in these attributes was also compared between mesh sizes and sediment depths. We used samples from a single bathyal site, at 1240 m water depth on the southern flank of the Chatham Rise in the south-west Pacific Ocean, to remove potential confounding effects of geographical area and water depth on nematode diversity attributes.

2. Methods

2.1. Study site

The study site (1240 m water depth; 44°29.1′S, 177°8.6′E) is on the southern flank of the Chatham Rise, a broad, ~800 km long submarine ridge, extending eastwards from the South Island of New Zealand (Fig. 1). The rise lies beneath the Subtropical Front (STF), a region where warm, saline, macronutrient-depleted subtropical surface water to the north meets cold, fresh, macronutrient-replete, micronutrient-poor subantarctic surface water to the south. The STF appears to be bathymetrically locked onto the Chatham Rise, with pronounced temperature gradients and strong currents on the northern and southern flanks of the rise, especially near 44°S (Uddstrom and Oien, 1999; Nodder et al., 2007), and is associated with heightened primary productivity (Murphy et al., 2001). The benthic community on the crest and southern flank of the Chatham Rise is characterized by elevated macro- and meiofaunal biomass (Probert and McKnight, 1993; Grove et al., 2006), and elevated sediment community oxygen consumption (Nodder et al., 2003, 2007). Sediments at the study

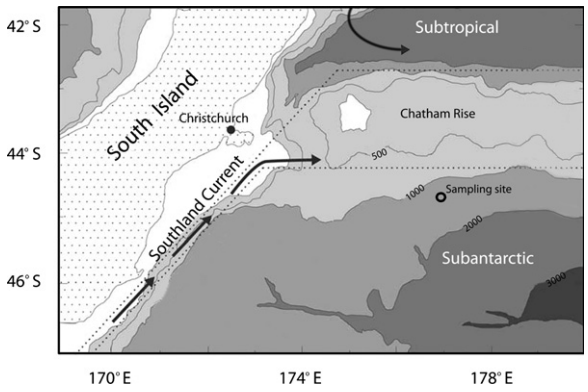

Fig. 1. Map of study area showing location of Subtropical Front (dotted line) and location of study site (black circle).

site are characterized by high silt and clay contents (~50% and 26%, respectively) with moderate levels of calcium carbonate (38%) and organic matter (~2%, as total organic matter (loss-on-ignition), S. Nodder, Ocean Survey 20/20 programme, unpublished data).

2.2. Sampling and laboratory procedures

Samples were collected in April 2007 (NIWA voyage TAN0705) using an Ocean Instruments MC-800A multicorer (MUC). Three cores for faunal analysis were obtained from each of two MUC deployments (MUC 1 and 2). From each of these cores, one subcore of internal diameter 26 mm was taken to a depth of 5 cm. Each subcore was divided into 0–1 and 1–5 cm sediment depth layers and preserved in 10% buffered formalin and stained with Rose Bengal. Sediment samples were washed through a 1 mm sieve to remove macrofauna and through a set of sieves of 63, 45, and 32 μm mesh sizes to retain nematodes and other meiofauna. Meiofauna were extracted from the sieved sediment by Ludox flotation, transferred to pure glycerol, and mounted on slides (Somerfield and Warwick, 1996). Meiofauna were counted and identified to major taxa using a compound microscope (100 × magnification).

The length (excluding filiform tail) and maximum width of at least 150 nematodes from each sample were determined for biomass determination. The number of nematodes measured from each mesh size in each sample was determined based on the proportion of individuals found on each sieve. Meiofaunal biomass was measured using video image analysis (Grove et al., 2006). Body volumes were converted to dry weight by assuming a relative density of 1.13 and a dry:wet weight ratio of 0.25 (Feller and Warwick, 1988). Length and maximum body width were measured for 10 adults (or all adults if fewer were encountered) of each putative species.

Nematode community structure and diversity were examined based on the identification of 120 and 190 individuals from the 0–1 and 1–5 cm sediments layers, respectively. To assess the effect of mesh size on nematode diversity and community structure, nematodes from the 63, 45, and 32 μm fractions were combined in proportions reflecting their respective abundance on each sieve. If, for example, nematodes in a given sample at 0–1 cm sediment depth were found in proportion 0.6, 0.3, and 0.1 on the 63, 45, and 32 μm mesh, respectively, then a sample of 120 individuals was assembled by combining the first 0.6(1 2 0), 0.3(1 2 0), and 0.1(1 2 0) individuals identified from each size fraction (i.e., 72+36+12=120). Samples reflecting the use of a 45 μm mesh were created in a similar way by combining nematodes from the >63 and 45–63 μm fractions.