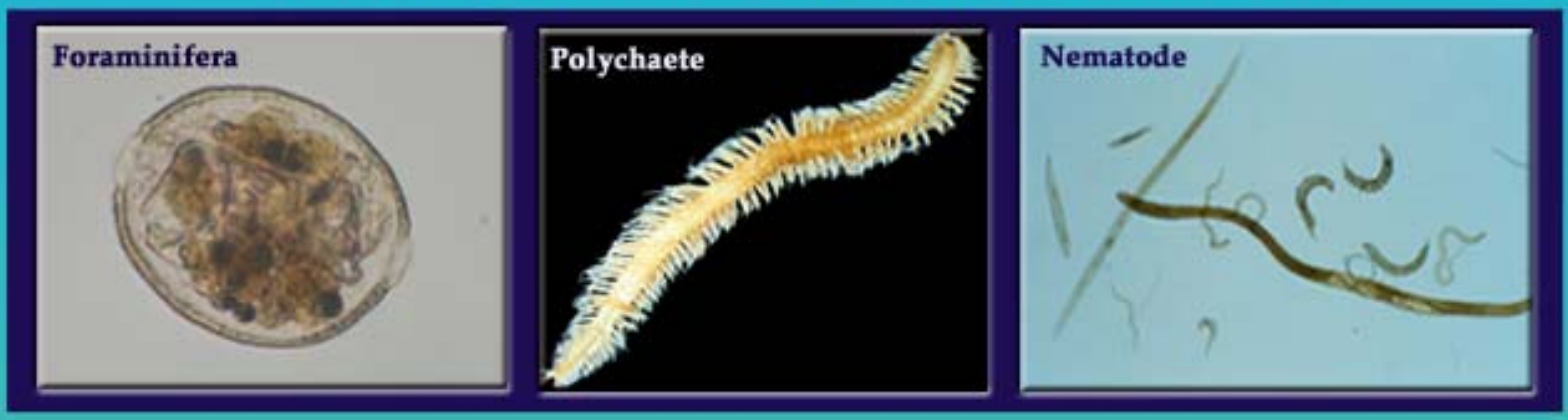
International Seabed Authority

Biodiversity, Species Ranges, and Gene Flow in the Abyssal Pacific Nodule Province: *Predicting and Managing the Impacts of Deep Seabed Mining*



ISA Technical Study: No. 3

Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province: predicting and managing the impacts of deep seabed mining

ISA Technical Study: No. 3

International Seabed Authority

Cover:

Pictures of the three main fauna studied by the Kaplan Project were provided courtesy of Nina Okhawara of JAMSTEC, Japan *(foraminifera)*; Adrian Glover *(polychaete)* and John Lambshead *(nematode)* both of the Natural History Museum, United Kingdom

International Seabed Authority

Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province: predicting and managing the impacts of deep seabed mining

This document is the final report to the International Seabed Authority of the "Kaplan Project" (1 March 2002 to 30 June 2007) designed to assess the Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province. The project was funded jointly by the J.M. Kaplan Fund and the International Seabed Authority. Principal investigators were Dr. Craig R. Smith, University of Hawaii at Manoa, USA; Drs. Gordon Paterson, John Lambshead and Adrian Glover, Natural History Museum of London, UK; Dr. Alex Rogers, Zoological Society of London, UK; Dr. Andrew Gooday, National Oceanography Centre, Southampton, UK; Dr. Hiroshi Kitazato, JAMSTEC, Japan; Drs. Myriam Sibuet, Joëlle Galéron, and Lenaïck Menot, IFREMER, France

International Seabed Authority

National Library of Jamaica Cataloguing-in-Publications Data

Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province: predicting and managing the impacts of deep seabed mining: report / compiled for the International Seabed Authority by Craig R. Smith … [et al.]

p. : ill., map; cm. – (ISA technical study ; no.3)
Includes bibliography
ISBN: 978-976-95217-2-8

1. Marine biodiversity 2. Biological diversity conservation
3. Ocean mining – environmental aspects
I. Smith, Craig R II. Series
333.95164– dc 21

International Seabed Authority
14-20 Port Royal Street
Kingston, Jamaica
Tel: (876) 9229105, Fax: (876) 9220195
URL: http://www.isa.org.jm

TABLE OF CONTENTS

Executive Summary

Abyssal Pacific sediments in the Clarion-Clipperton Zone (CCZ) harbour abundant mineral resources, in the form of nickel- and copper-rich manganese nodules, that are of increasing commercial and strategic interest. Abyssal sediments may also be major reservoirs of biodiversity. It has been extremely difficult to predict the threat of nodule mining to biodiversity (in particular, the likelihood of species extinctions) because of very limited knowledge of (a) the number of species residing within areas potentially perturbed by single mining operations, and (b) the typical geographic ranges of species within the nodule province. In this project, we have used state-of-the-art molecular and morphological methods to evaluate biodiversity and geographic ranges of three key faunal groups in the abyssal Pacific nodule province: polychaete worms, nematode worms, and protozoan foraminifera. Together, these groups constitute >50% of faunal abundance and species richness in abyssal sediments, and represent a broad range of ecological and life history types.

The primary goals of this project, involving scientists and collaborators from five countries, have been as follows:

1) To estimate, using modern molecular and morphological methods, the number of polychaete, nematode and foraminiferal species at two to three stations spaced at ~1,500 km intervals across the Pacific nodule province.

2) To evaluate, using state-of-the-art molecular and morphological techniques, levels of species overlap and, if possible, rates of gene flow, over scales of 1,000 - 3,000 km for key components of the polychaete, nematode and foraminiferan fauna.

3) To broadly communicate our findings to the scientific and mining-management communities, and make specific recommendations to the International Seabed Authority on minimising risks to biodiversity resulting from mining.

To address these project goals, we collected macrofaunal and meiofaunal samples, using special 'DNA-friendly techniques', at three sites spaced at intervals of ~1,000–2,000 km across the Pacific region targeted for nodule mining. The sampling programme involved three research cruises in which project personnel (8-20 people per cruise) spent 83 days at sea and collected a total of 40 box core and 32 multiple cores samples. Collected samples of foraminifera, nematodes and polychaetes were then transported to laboratories in the USA, the United Kingdom, Japan and

France for sorting and detailed morphological and molecular analyses. Our analyses, results and syntheses have been reported as 16 presentations at international scientific meetings and workshops, and as 20 publications in the peer-reviewed scientific literature. Many more presentations and publications are planned for the near future.

Our results indicate high, unanticipated, and still poorly sampled levels of species diversity for all three sediment-dwelling faunal components (foraminifera, nematodes and polychaetes) at our individual study sites. Cryptic speciation (i.e., the presence of multiple species previously identified as single species) appears to be very common in the polychaetes and nematodes. Habitat heterogeneity also appears to be higher than previously appreciated. We speculate that the total species richness of sediment-dwelling foraminifera, nematodes and polychaetes (a subset of the total fauna) at a single site in the CCZ could easily exceed 1,000 species. Our results from all faunal components suggest that there is a characteristic fauna of the abyss, i.e., that abyssal habitats have sustained species radiations and are not merely sinks of non-reproducing individuals transported from ocean margins. In addition, there is significant evidence that the community structure of the foraminifera and polychaetes differs substantially over scales of 1,000–3,000 across the CCZ.

Our findings suggest that marine protected areas (MPAs) should be erected to safeguard biodiversity in the CCZ in the face of nodule mining. We recommend that MPAs should be set up as follows.

1) MPAs should be placed at multiple locations across the CCZ, at the very least in the eastern, central and western portions of the region of mining claims.

2) Because of the steep latitudinal gradients in productivity and community structure within the equatorial Pacific, the MPAs should be designed to protect biodiversity across the entire width of the CCZ, i.e., from 7o–17o N latitude.

3) The MPAs should be large enough to encompass major areas of the known benthic habitat types in the CCZ, including abyssal hills with and without nodules, rocky ridges, and multiple seamounts of various elevations above the seafloor.

4) Each MPA must be large enough for most of its area to be buffered from the direct and indirect impacts of nodule mining activities, including influences from sediment plumes in the water column and at the seafloor.

5) Because benthic processes and community structure in the CCZ are strongly influenced by processes in the water column above, it is highly desirable for management of the MPAs to include control of substantial human activities (mining, energy exploitation, waste disposal, and commercial fishing) from the abyssal seafloor to the ocean surface. This recommendation is consistent with the concept of ecosystem based management (Pikitch et al., 2004).

It is critically important to recognise that our recommendations are based on a limited, albeit rapidly growing, database on biodiversity and species ranges in the CCZ, and should be applied using the precautionary principle. Specifically, where data are inadequate to exclude

potential harm to the environment from a particular human activity (in this case nodule mining), the activity should be conservatively managed to ensure environmental protection.

Our ongoing studies, collaborative projects (e.g., the Census of Diversity of Abyssal Marine Life), and workshops (specifically the Pew-funded workshop to *Design Marine Protected Areas for Seamounts and the Abyssal Nodule Province in Pacific High Seas,* which took place Oct 23-26, 2007) are helping to better resolve biodiversity levels and species ranges in the abyssal Pacific and provide explicit guidance in the near future to the International Seabed Authority in the conservation of abyssal Pacific ecosystems.

1 May 2007

INTRODUCTION

Deep-sea sediments appear to be major reservoirs of biodiversity, by some estimates harbouring 10-100 million species of worms, crustaceans and mollusks (Grassle and Maciolek, 1992; Snelgrove and Smith, 2002; Smith et al., in review). These estimates remain extremely controversial because truly vast regions of the deep-sea are very poorly sampled, taxonomic expertise required to identify and describe deep-sea species is dwindling rapidly, and modern molecular techniques have not been applied to most deep-sea animal groups. Thus, we can only guess at the number of species, or the typical geographic ranges of species in the abyssal North Pacific, the largest ecosystem on the Earth's solid surface.

In addition to high biodiversity, abyssal Pacific sediments harbour mineral resources of potential commercial and strategic interest, in particular polymetallic nodules. These nodules are potato-sized concretions of manganese, iron, cobalt, copper and nickel, which occur on the seafloor over broad areas of the Pacific beneath waters deeper than 4000 m (Smith and Demopoulos, 2003; Glover and Smith, 2003). The region of maximum commercial interest for polymetallic nodule mining (called the Pacific nodule province) lies in international waters, and stretches from 118°–157° W, and from 9°–16° N, an area of >3 million square kilometers (Figure 1). At present, eight contractors are licensed by the International Seabed Authority (the international body charged by the United Nations Convention on the Law of the Sea with managing deep-sea mining) to explore nodule resources and to test mining techniques in eight claim areas, each covering 150,000 km^2 (i.e., an area roughly the size of Florida). When mining ultimately begins, each mining operation is projected to directly disrupt, through nodule 'harvesting', 300-800 km^2 of seafloor per year and disturb the sediment-dwelling fauna over an area 5-10 times that size due to redeposition of suspended sediments (data from the International Seabed Authority, Hannides and Smith, 2003). Thus, over the 15-year of a single operation, nodule mining might severely damage abyssal seafloor communities over areas of 20,000 to 45,000 km^2 (a zone at least the size of Massachusetts).

It has been virtually impossible to evaluate the threat of nodule mining to biodiversity (in particular, the likelihood of species extinctions) without better knowledge of the following:

1) The number of species residing within areas potentially perturbed by single mining operations.

2) The typical geographic ranges of species (and rates of gene flow) within the general nodule province.

If biodiversity levels were very low within mining claims, or if most species ranges were very large compared to the scales of claim areas and potential mining disturbance, one might conclude that rates of species extinctions caused by nodule mining would be low. In fact, levels of biodiversity are likely to be very high in claim-area sediments, and species ranges are not well known. Both biodiversity and species ranges remain poorly understood within the nodule province for three reasons: (i) many areas of the nodule province, and dominant groups of seafloor animals (especially nematode worms and foraminifera), remain grossly undersampled. (ii) while various expeditions have sampled the seafloor biota of the nodule province, each sampling programme has, generally, used different specialists to identify the animals from their collections - most species collected are new to science and have not been formally described in the scientific literature, there is no way to relate the species list of one study to that of another, and thus to compare species lists across the entire nodule province. (iii) all biodiversity studies in the nodule province have used traditional morphological methods for identifying species. However, recently

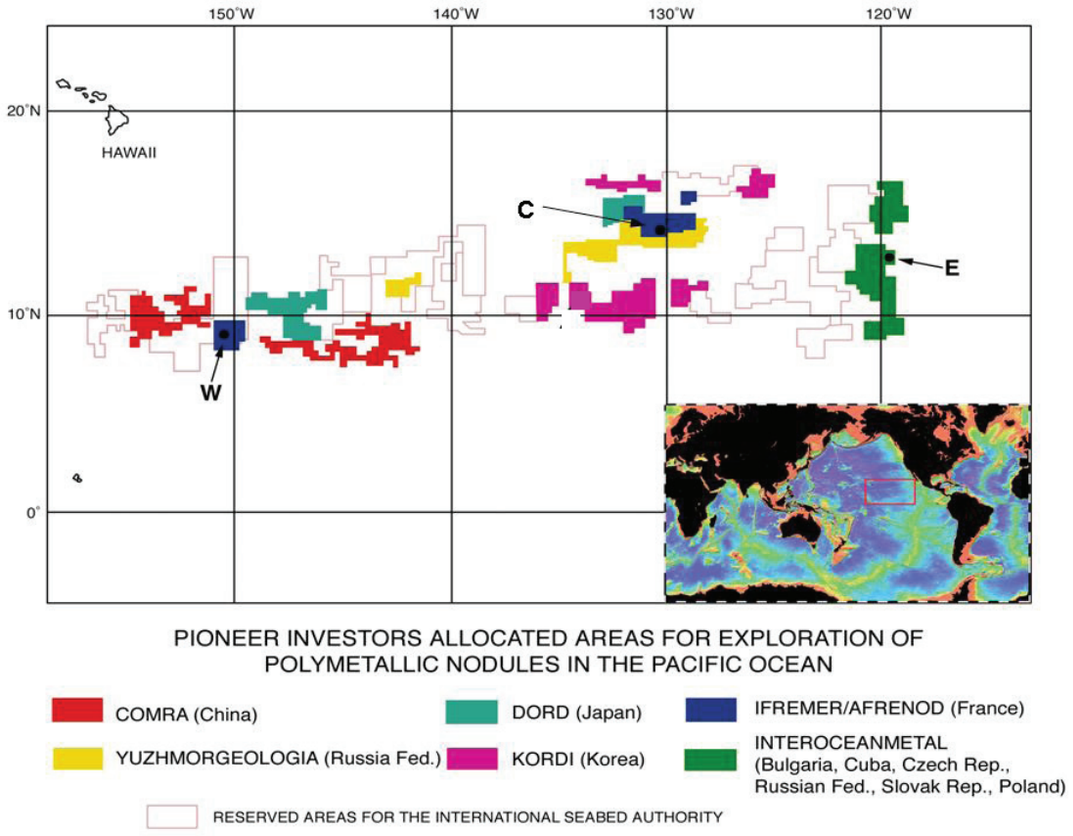


Figure 1. The region of maximum commercial interest in the Pacific nodule province (box in inset) and claim areas licensed to exploration contractors. The sites at which samples were collected for this project are indicated by E, C and W (the Kaplan East, Central and Western Sites, respectively). Site E is located in the IOM claim area at ~15° N, 119° W (water depth = 3,990–4,096 m), Site C is located in the eastern IFREMER/AFRENOD claim area at ~ 14°5' N, 130° 5' W (water depth 4,997–5,054 m) and Site W is located in the western IFREMER/AFRENOD claim area at 9° 33' N, 150° 0.5' W (water depth 5,043–5,059 m).

developed molecular methods (e.g., using the genetic information in DNA sequences) suggest that morphological techniques typically underestimate the number of species and overestimate species ranges in marine habitats (e.g., Knowlton, 1993, 2000; Van Soosten et al., 1998; Creasey and Rogers, 1999). Until biodiversity levels and species ranges in the Pacific nodule province are much better understood, the impacts of nodule mining (or other large-scale anthropogenic disturbances) on deep-sea biodiversity cannot be predicted.

In this project, we have begun to rigorously evaluate biodiversity levels, geographic ranges, and rates of gene flow for three key faunal components of the seafloor communities within the abyssal Pacific nodule province. This has involved a research programme that combines rigorous field sampling with state-of-the-art laboratory analyses. The primary goals of the programme are to:

3) Estimate, using modern molecular and morphological methods, the number of polychaete, nematode and foraminiferal species at two to three stations spaced at ~1,500 km intervals across the Pacific nodule province.

4) Evaluate, using state-of-the-art molecular and morphological techniques, levels of species overlap and, if possible, rates of gene flow, over scales of 1,000–3,000 km for key components of the polychaete, nematode and foraminiferan fauna.

5) Broadly communicate our findings to the scientific and mining-management communities, and make specific recommendations to the International Seabed Authority on minimising risks to biodiversity resulting from mining.

During this project, we have focused on polychaetes, nematodes and foraminifera for the following reasons. *Polychaete worms* dominate the abyssal macrofauna (animals between 0.3 to 30 mm in smallest dimension), constituting 60-75% of macrofaunal abundance and species richness (Hessler and Jumars, 1974; Paterson et al., 1998; Glover, 2001; Smith and Demopoulos, 2003; Hannides and Smith, 2003). Polychaetes exhibit a broad range of feeding types and life-history strategies, and they are frequently used to evaluate anthropogenic disturbance in shallow-water habitats (e.g., at dredge-spoil dumps, sewer outfalls, and sites of anoxia)(Pearson and Rosenberg, 1978; Diaz and Rosenberg, 1995). The *nematode worms* make up the bulk of the meiofauna (animals 0.03 to 0.30 mm in smallest dimension) and may be the most abundant and species-rich, multi-cellular animals in deep-sea sediments (Boucher and Lambshead, 1995; Brown et al., 2001; Lambshead and Boucher, 2003). Nematodes are used to monitor anthropogenic stress in shallow-water habitats (Bhadury et al., 2005), and their limited dispersal abilities suggest they may be particularly sensitive to extinction resulting from large-scale nodule-mining disturbance. *Foraminifera* (amoeboid animals ranging in size from ~0.03 mm to 10's of centimeters) are the most abundant protozoans in deep-sea sediments, and substantially influence seafloor habitat structure and energy flow in the Pacific nodule province (Gooday, 1994). Foraminiferan community structure is frequently used to infer disturbance and climate-change impacts in fossil marine habitats. While exhibiting very high local species diversity in abyssal sediments (often > 100 species at a single site), many foraminifera are thought to have very broad species ranges (Gooday et al., 1998).

FIELD PROGRAMME

To address the project goals, we collected macrofaunal and meiofaunal samples at three sites along a latitudinal transect crossing the abyssal Pacific nodule province (Figure 1). The three sites were spaced at intervals ~1,000–2,000 km and spanned the areas in the abyssal Pacific potentially impacted by nodule mining. Sampling was conducted during three cruises aboard the oceanographic research vessels *RV New Horizon*, the *RV Umitaka-Maru*, and the *RV L'Atalane* (during the *Nodinaut* cruise). Macrofaunal samples were generally collected with a USNEL-type box corer (Gage and Tyler, 1991), which collects a sediment sample 0.25 m^2 in plan area (Fig. 2). Meiofaunal samples were generally collected with a multiple corer (Gage and Tyler, 1991), which collects 12 tubes (or 'cores') of relatively undisturbed sediment, each sampling a plan area of 80 cm^2 (Fig. 3).

***RV New Horizon* Cruise to Site E, 4 Feb – 7 Mar 2003, San Diego to San Diego.** During this 32-day cruise, we spent five days surveying and sampling our eastern study area. Based on data obtained from the International Seabed Authority, we selected a study area of 10^o x 10^o centred on 15^o N, 119^o W (Fig. 1), with 15-25 kg m^{-2} of nodule cover. We surveyed the area with 12-Khz sonar

Figure 2. The USNEL type box corer in the process of sample box removal. Insert: Vertical view of the box core sample (0.25 m^2 in area), with manganese nodules on the sediment surface. Photos: P. Martinez and C. Smith (inset).

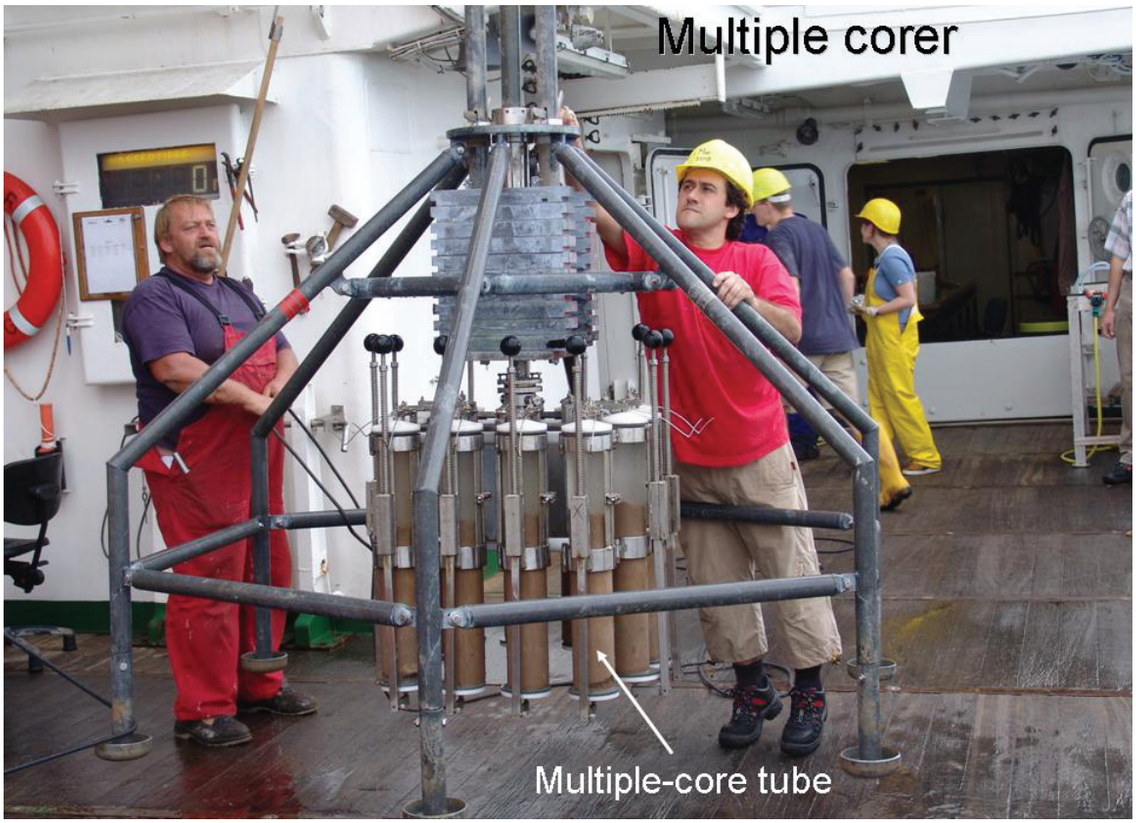


Figure 3. Multiple corer during recovery from sampling. The multiple corer collects 12 tube samples (each 80 cm^2) in area. Photo courtesy of P. Martinez.

and found it to be slightly rolling (i.e., with abyssal-hill topography) with occasional moderate slopes, and water depths ranging from 4,014 to 4,124 m. Most areas appeared to be suitable for coring. We then collected, at random points within this square, 13 box cores (nine using DNA-friendly techniques (see below) and four using formalin preservation techniques) for infaunal macrobenthos, and 10 multiple cores for nematode, foraminiferan and microbial studies.

***RV Umitaka-Maru* Cruise to Site W, 14 - 21 Feb 2004, Hilo to Honolulu**. During this cruise to Site W (the western IFREMR claim area), a total of nine multiple cores were collected for meiofauna at random locations centred on rectangle of 10^o x 10^o centred approximately on 9° 33′ N, 150° 0.5′ W, with a water depth of approximately 5,050 m (Fig. 1).

***RV L'Atalante (Nodinaut)* Cruise to Sites C and W, 17 May – 28 June 2004, Manzanillo, Mexico to Noumea, New Caledonia.** During this cruise, funded by IFREMER, samples for macrofauna and meiofauna (nematoda and foraminifera) were collected at Sites C and W (Fig. 1), as follows.

Site C. Macrofaunal samples included seven quantitative box cores collected at random locations within a 10^o x 10^o rectangle in nodule facies zero (an area with few nodules, where

macrofaunal abundances were expected to be highest). These box cores were processed using DNA-friendly techniques. In addition, two "very disturbed" (i.e., non-quantitative) box cores and 14 multiple core tubes (a total of two multiple core deployments) were also collected at random locations from facies zero and processed with DNA-friendly techniques. The total macrofaunal material collected for DNA studies was thus roughly equivalent to eight to nine box core samples. In addition, IFREMER scientists collected eight box core samples at random locations in nodule facies B and C (areas of dense cover of small nodules and sparse cover of large nodules, respectively) for formalin preservation of macrofauna.

Nematode samples were collected from seven multiple core lowerings in Facies 0, from four multiple core lowerings in Facies B, and from one multiple core lowering in Facies C.

Samples for foraminifera were collected from five multiple core lowering in Facies 0 and from four multiple core lowerings in Facies B. One to three tubes were collected from each lowering for foraminiferal samples, and processed for species identification (fixed in formalin) and for molecular analyses (fixed in chilled ethanol).

Site W. For macrofauna, four quantitative and two semi-quantitative box cores were collected at random locations in the IFREMER Western Area and processed using DNA-friendly techniques. In addition, three blade-core samples collected by the research submersible *Nautile* were processed similarly, after removal of any megafauna atop the cores. The total area sampled using DNA-friendly techniques was equivalent to approximately 6.5 box core samples. In addition, IFREMER scientists collected four box core samples at random locations for formalin preservation of macrofauna. One multiple core lowering was sampled for nematodes and foraminifera, with three tubes taken for each type of analysis.

Sample processing for all cruises. Upon recovery, box core samples were immediately drained of top water. Nodules and the top 0-5 cm and 5-10 cm layers of sediment were then quickly removed and transferred into a cold van (4° C) for processing to prevent temperature deterioration of DNA in sampled animals. The sediments from box core samples were washed on a 300-µm sieve using chilled seawater (4-6° C) and fixed in 95% ethanol (for DNA analyses) or in 10% formaldehyde-seawater solution (for morphological analyses).

Upon recovery, all multiple core samples were immediately transferred into a cold van (4° C) for processing, to prevent deterioration of the DNA in the fauna. Two to three multiple core tubes (each 80 cm^2 in sample surface area) from each multiple core deployment were processed for nematodes and foraminifera. The top 3 cm of sediment from each of the nematode/foraminiferan tubes were processed using a series of water decantations through various sieves, the smallest size being 45-µm for the nematode, and 32 µm for the foraminifera. All material retained on the sieve was then preserved in 95% ethanol or fixed in 4% formaldehyde solutions. The tubes for foraminiferans were processed using a combination of 'DNA-friendly' techniques and formaldehyde preservation for morphological analyses.

See the specific papers cited for details of sample processing and laboratory analyses.

RESULTS

Foraminifera – *Andrew Gooday, Fusae Nozawa, Nina Ohkawara, Hiroshi Kitazato*

The analysis of deep-sea, sediment-dwelling foraminifera involves some special problems. The only reliable way to extract individuals from sediment residues is to hand sort them, an extremely time-consuming task, particularly in the case of the very fine (>32 µm) residues. Deep-sea foraminifera are also very diverse. At the Kaplan sites, they are dominated by rather featureless, single-chambered (monothalamous) species which are difficult to differentiate and difficult to correlate between samples. These factors placed constraints on the number of Kaplan samples that could be sorted. Despite these limitations, a total of 20 syringe subcores (3.45 cm^2 surface area, 0-1 cm) from seven multiple core tubes taken at three stations (CRS 824, 827, 838) at Kaplan Site E (KE) site, and six subcores from Stn CRS-866 (taken from two multiple core tubes) at the Kaplan Site C (KC), have been analysed. In the presentation of results, multiple core stations (or deployments) are indicated by 'CRS numbers'. The main results are as follows.

1. Densities

The Site E subsamples together yielded 12,513 rose-Bengal-stained (i.e., live at time of sampling) benthic foraminifera. Agglutinated taxa (foraminifera forming tests or external structures of cemented sediment grains), consisting mostly of monothalamous types or komokiaceans, were dominant (see Kaplan Project Image Catalogues). Almost two-thirds (65%) of specimens were either obvious fragments, mainly of komokiaceans and tubular foraminifera, or single chambers or small groups of chambers believed to be fragments of very fragile komokiaceans. The remaining 4,438 specimens (35%) were complete individuals, most (78%) of them indeterminate agglutinated spheres (termed 'psammosphaerids') that constituted 27.6% of all specimens (complete plus fragments). Nine hundred and eighty three of the complete individuals (22% of complete tests, 7.6% of all specimens) could be assigned to either described or undescribed species . Only 26 specimens (0.59% of complete individuals) were calcareous and these had invariably lost their tests through dissolution.

Work on the Site C material is ongoing. So far, 3,686 complete individuals have been picked from the six multicore subcores (CRS 866, subcores A1, A2, A3, B4, B5, B6) and 1,586 fragments have been picked from subcores A2 and B4 (fragments were not extracted from the other three subcores). The proportion of fragmented specimens was again high; 33.4% and 73.2% in A2 and B4, respectively. Psammosphaerids were extremely abundant in subcores A2, B5, and B6. In A2, there were 1,035 psammosphaerid specimens, constituting 94.4% of complete individuals and 62.9% of all specimens (complete plus fragments). B5 yielded 1,100 psammosphaerids, equaling 91.9% of all complete individuals, and B6 contained 375 psammosphaerids, constituting 70.5% of all complete individuals.

2. Species richness

A total of 252 morphospecies was recognised in the 20 Site E syringe subsamples analysed, with 170 species represented by complete individuals and 84 by fragments. Between 41 and 65 morphospecies (complete and fragmented) occurred in individual syringe subcores and 66-110 in

individual multiple core tubes (three syringe subcores per tube). The numbers of species shared between tubes from the same station (CRS 824 A, B, and C) and between different stations (CRS 824, 827, 838) at Site E are shown in Fig. 4. Only 17 species were common to all three stations and 34 occurred at two stations. The top-30-ranked species included a mixture of *Lagenammina* species (6), soft-walled saccamminids (5), psammosphaerids (2) and other monothalamous forms (2), organic-walled allogromiids (2), *Nodellum*-like allogromiids (6), and hormosinaceans (5) and other multilocular agglutinated forms (3) (Nozawa et al., 2006, Table 5). None was calcareous. Although the same species tended to appear among the ten top-ranked ones in each core, there was considerable variation in the ranking (Nozawa et al., 2006, Table 6).

A total of 202 morphospecies are recognised in the six Site C syringe subcores analysed so far; 140 represented by complete individuals and 62 by fragmented specimens. The number of species in individual subcores was 42, 33, 55, 61, 47 and 62 (in syringe subcores A1, A2, A3, B4, B5, B6 respectively). By far the most abundant species was the tiny Psammosphaerid sp. 11 which accounted for 89.8%, 87.5% and 53% of all complete foraminifera in syringe subcores A2, B5 and B6, respectively, and 61.5% in all four subcores combined. The second-ranked species overall, Allogromiid sp. 9, was an order of magnitude less abundant and represented only 3.2% of specimens. These two top-ranked species were very unevenly distributed between subcores (see below). Four other psammosphaerid species each accounted for around 2% of the assemblage but the percentage abundance of all other species was <1% of the densities in all four subcores combined.

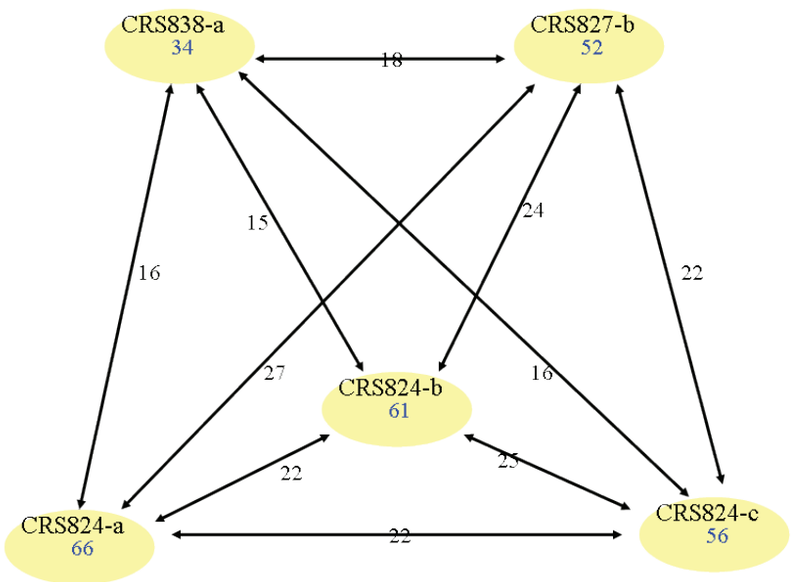


Figure 4. Foraminiferan morphospecies (excluding fragmented species) shared between multiple samples at Site E. CRS 824, CRS 827, and CRS 838 each represent separate stations (i.e., deployments) of the multiple core at random locations with the 10° x 10° sampling area. CRS824-a, CRS824-b and CRS824-c represent separate tube cores from station CRS 824. Blue numbers indicate the total number of species from a sample shared with all any other sample.

3. Small-scale heterogeneity

Some groups exhibited considerable spatial heterogeneity. At Site E, 45% of the 3,455 indeterminate psammosphaerids and 45% of the 3,087 komokiacean-like chambers occurred in single syringe subcores. Some of the fragments exhibited a similar degree of patchiness. For example, in the case of tube C from deployment 824, syringe subcore C7 yielded 1,473 komoki-like chambers whereas only three and four chambers were present in the two other syringe subcores from this multiple core drop. At Site C, the two top-ranked species were represented in syringe subcores A1, A2, A3, B4, B5 by, respectively, 2, 984, 1, 3, 1,047, 283 (Psammosphaerid sp. 11) and 0, 3, 0, 118, 0, 2 (Allogromiid sp. 9) individuals.

4. Species distributions

4.1. Distribution within Site E. Between 66 and 100 species (complete and fragmented) were found in each of the individual cores (each represented by three subcores). However, few species were widely distributed among these samples. Only four species occurred in all seven cores and another four in six cores, whereas 12 of the top 30 species occurred in three or fewer of the seven samples (Nozawa et al., 2006, Table 5). The number of species shared between cores from the same station (CRS824A, B, C) and between different stations (CRS 824, 827, 838) are shown in Fig. 4. Only 17 species were common to all three sites and 34 occurred at two sites.

4.2 Distribution between Kaplan sites. Our list for Sites E and C combined presently totals 300 species represented by complete individuals. Of these, we have currently identified only 29 species, 10% of this total, which occur at both Sites C and E. This number is undoubtedly underestimated and reflects, in part, the fact that samples from the two sites were analysed by different students, and the large volume of work involved in correlating the two separate species lists. It will increase as the comparison of species datasets progresses. Nevertheless, some of the most striking differences are certainly real. In particular, psammosphaerids are very abundant at both Sites E and C, but the species are different. The overwhelmingly dominant species at Site C, a distinctive psammosphaerid with a fairly coarse-grained test composed of mineral grains and scattered large biogenic fragments, is certainly not present at Site E. Similarly, the psammosphaerids that are abundant at Site E (indeterminate types 1-9) are rare at Site C. Allogromiid sp. 9, second-ranked at Site C and also a distinctive species, is not present at Site E.

4.3. Taxonomic studies. The foraminiferal material from both Kaplan sites includes a large number of undescribed species. We are currently working on the description of the morphologically simple but distinctive psammosphaerid that is very abundant in some of the Kaplan central samples (Fig. 5) (Ohkawara et al., in prep.). This species forms an agglutinated sphere, around 100 µm diameter, composed of a jumble of agglutinated particles of different types, including quartz grains, diatom fragments and radiolarian tests. The test is loosely held together with sparse organic cement and fine organic strands that appear to be pseudopodia.

4.4. Wider comparison. In a general sense, our results are consistent with the conclusion of Saidova (1965) that agglutinated foraminifera account for almost 100% of the foraminiferan fauna at depths >3,500-4,500 m in the Pacific Ocean. Almost two-thirds (64.5%) of the 12,513 foraminifera picked from the Site E samples were fragments, or presumed fragments. Bernstein et al. (1978) also

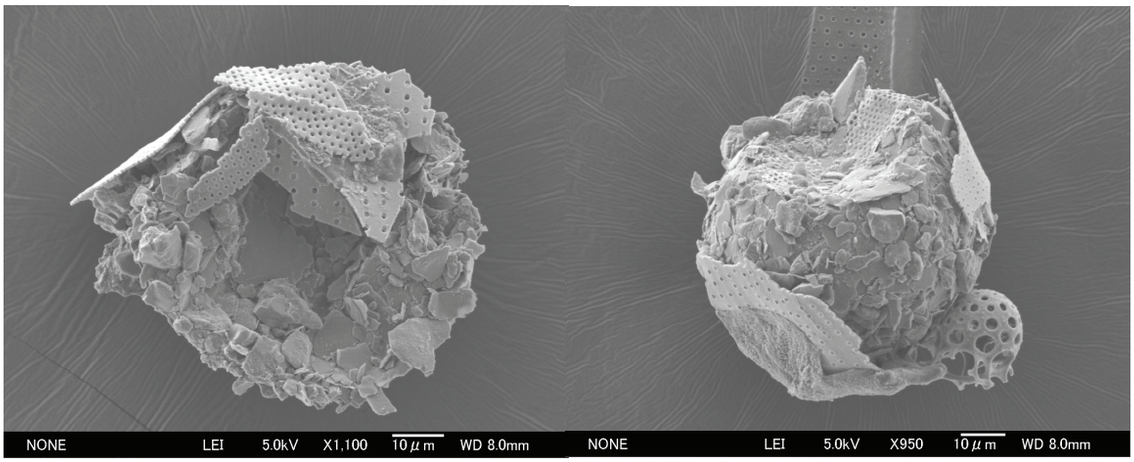


Figure 5. Tiny, undescribed agglutinated sphere (psammosphaerid) that is very abundant in some samples from the Kaplan Central Site C (SEM photographs by Ms Nina Ohkawara, JAMSTEC)

reported a predominance of agglutinated foraminiferal fragments at the abyssal CLIMAX II site (centred on 28° 28' N, 155° 20'W; 5,500-5,800 m water depth) beneath the oligotrophic central North Pacific gyre in a manganese nodule region. An important difference is that we examined the >32-µm fraction of small samples, whereas Bernstein et al. (1978) used larger box corer samples sieved on 297-µm mesh screens. Our results compare most closely to those of Snider et al. (1984) who reported that almost 70% of foraminiferal biovolume in box corer subcores (>42 µm fraction) from another central North Pacific nodule containing site (MPG-I; ~30° N, 158° W, ~5850 m) consisted of agglutinated fragments. Snider et al. (1984) also found that 'sac-shaped' forms, possibly similar to our psammosphaerids, were the most important foraminiferal morphogroup (31% of numbers) in their samples.

The best comparative species-level Pacific data are from previous studies by our research group (Okamoto, 1988; Gooday et al., 2001, 2004; Hori 2001; Todo 2003; Nozawa, 2003, 2005; Todo et al., 2005; Ohkawara, 2006). These reveal the presence at abyssal North and equatorial Pacific sites of a core group of widely distributed, largely undescribed, monothalamous species (Table 1). Many of these have also been recognised in the Atlantic and Indian oceans. Some of the hard-shelled morphospecies in our samples are also widely distributed in the Pacific and other oceans. They include *Adercotryma glomeratum, Spiroplectammina biformis, Lagenammina tubulata, L. difflugiformis* and some of the *Reophax* morphotypes (e.g. *R. helenae, 'R. bilocularis'* and *'R. scorpiurus'*). However, these widely distributed species represent a small proportion (~7%) of the species recognised at Kaplan Sites E and C. Most of the Kaplan species were rare and we cannot say whether they were confined to particular cores, stations or sites because they have small geographical ranges or because they were undersampled. Nonetheless, there are at least 10 species of foraminifera that are abundant at one of the Kaplan Sites C or E, but rare or absent at the other C; this suggests significant turnover of major components of the foraminiferan faunal over scales of roughly 1,000 km across the CCZ.

Table 1. Distributions Of Consistently Identifiable Monothalamous (Single-chambered) Foraminiferal Morphotypes At Kaplan East and Central Sites and Other Pacific, Atlantic and Indian Ocean Localities.

	North Pacific			W. Pacific	Equatorial Pacific					SE Pacific	North Atlantic	Indian Ocean
Station/locality	**2/3**	**6**	**15**	**40**	**JET**	**KC**	**KE**	**IOM**	**64**	**AT**	**PAP**	
Water depth (m)	**5350**	**5289**	**4263**	**7132**	**5300**	**5100**	**4100**	**4400**	**5570**	**7800**	**4850**	**3400**
Allogromiid sp. 1	X	X	X	-	X	**X**	**X**	-	X	-	X	X
Chitinosiphon sp.	-	X	X	X	X		**X**	-	X	X	X	X
Nodellum-like 1	X	X	X	X	X	**X**	**X**	X	X	X	X	X
Nodellum-like 2	X	X	X	X	X	**X**		-	X	-	X	X
Nodellum-like 3	-	X	-	X	X		**X**	X	X	X	X	X
R. moniliforme	X	X	X	-	X	**X**	**X**	X	X	-	-	-
Resigella-like 1	X	X	X	X	X	**X**	**X**	-	X	-	X	-
Resigella-like 2	-	-	-	X	-	**-**	**X**	-	X	X	-	-
Placopsilinella sp.	X	X	X	-	X	**-**	**X**	-	X	-	X	X
Saccamminid 2	-	X	-	-	X	**X**	**X**	-	-	-	X	-
Saccamminid 4	X	X	-	X	X	**X**	**X**	X	X	X	X	-
Saccamminid 5	X	X	X	X	X	**X**	**X**	X	X	-	X	X
Saccamminid 6	-	X	-	-	-	**-**	**?**	-	-	-	X	-
Psammsphaerid sp. 5	X	-	-	-	-	**-**	**?**	-	X	-	-	-
Vanhoeffenella	X	X	-	X	X		**X**	**X**	X	X	X	-

Nematoda – *P. John D. Lambshead, David H. Lunt, Robin M. Floyd, Brian Elce, Craig R. Smith and Alex D. Rogers*

We amplified 18S rRNA genes from 97 nematode specimens fixed in chilled 95% ethanol sampled from soft sediments from Kaplan Sites E (15°N, 119°W at 4,050 m depth), and W (9.5°N, 150°W at 5000 m depth)(Fig. 1). Nematodes were the most abundant metazoan in the meiofaunal samples from these sites.

The polymerase chain reaction (PCR) was carried out in a volume of 25 µl per reaction, comprising: Qiagen PCR buffer at 1x concentration (including $MgCl_2$ at 1.5 mM); dNTPs at a concentration of 0.2 mM for each nucleotide; primers at 0.5 µM each; and Qiagen *Taq* DNA polymerase at 0.025 units/µl. The primers used were Nem_18S_F (5'-CGCGAATRGCTCATTACAACAGC-3') and Nem_18S_R (5'-GGGCGGTATCTGATCGCC-3'), which were designed to amplify nematode 18S sequences only (Floyd et al., 2005).

The 97 specimens could be classed into 73 MOTUs (molecular operational taxonomic units, i.e. putative species) that differed by three or more base pairs (b.p.) over the approximately 500 b.p. examined. Only three of those MOTUs could be assigned to an already barcoded shallow water genus, *Daptonema* (Monhysteroidea). In this group, the deep-sea MOTUs are interspersed phylogenetically with shallow species (Fig. 6). Most of the deep-sea MOTUs show a different pattern, exemplified by the Oncholaimoidea (Fig. 6). Here, the MOTUs are divided into two monophyletic groups. The first is a sister taxon to the five barcoded shallow water genera (from two families, the Oncholaimidae and the Enchelidiidae). The second is a sister taxon to both the first group and the shallow water genera. Similar patterns were found in the Enoploidea and the Chromadoroidea.

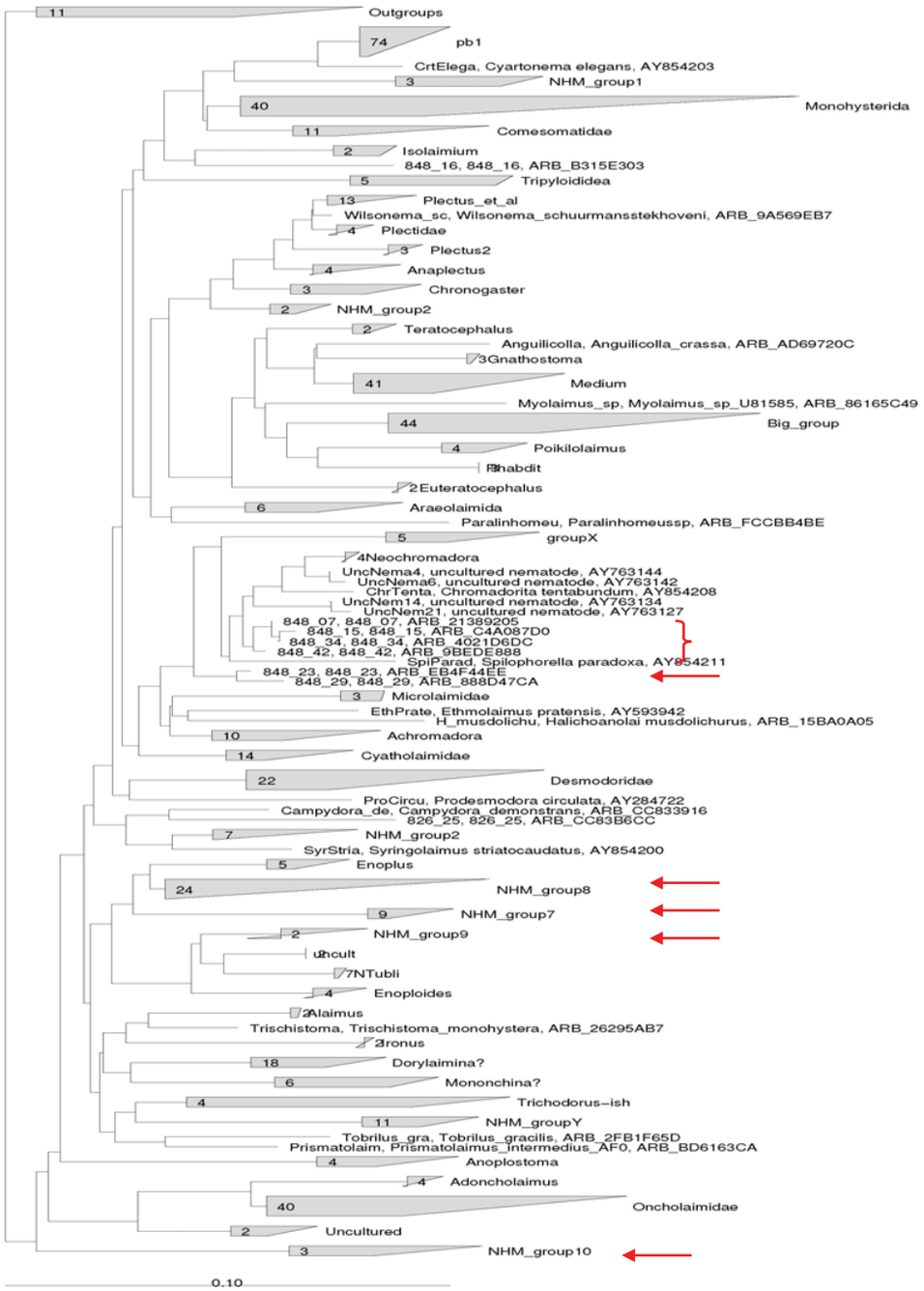


Figure 6. Amplified 18S rRNA genes (500 bps) from 97 nematode specimens from Kaplan E and W Sites (with designations beginning with “826”, “848”, or “NHM”). There are 73 MOTUs (putative species) differing by ≥ 3 bps. Only 3 of these MOTUs fall within a bar coded shallow-water genus *Daptonema* (Monhysteroidea). Most of the deep-sea MOTUs (examples indicated by red arrows and brackets) fall into distinct, monophyletic groups: e.g., clusters in Oncholaimoidea, Enoploidea and Chromadoroidea.

These results are important for at least two reasons. First, they demonstrate that barcoding can reveal novel lineages in small invertebrates that are hidden by cryptic morphology. In contrast with our barcoding results, previous orphological analysis, using characters that can be discerned under the light microscope, has found genera and higher taxa in the deep sea largely identical to those found in shallow water (Lambshead et al., 2003). Our barcoding approach appears to be resolving a large number of morphologically cryptic taxa, a recurrent theme in the application molecular techniques to invertebrate taxonomy (Knowlton, 1993, 2000; Van Soosten et al., 1998; Creasey and Rogers, 1999).

Second, our results indicate the presence of numerous unique abyssal nematode taxa, suggesting that the abyss has sustained adaptive radiation, and is not primarily a sink for non-reproductive nematode individuals derived from slope habitats (in contrast to the 'abyssal sink hypothesis' of Rex et al., 2005). Molecular analysis of single-celled oceanic (Moon-van der Staay et al., 2001) and deep-sea (Lopez-Garcia et al., 2001) eukaryotes has also revealed unexpected and novel diversity of lineages, suggestive of species radiation in the abyss.

Molecular analyses are ongoing in the laboratory of Dr. Alex Rogers, and we anticipate at least doubling the number of barcoded nematodes from our CCZ study sites within the next two to three months. This should provide better resolution on the presence of unique abyssal nematode taxa.

Polychaeta – *Adrian G. Glover, Gordon L.J. Paterson, Iris Altamira, Patricia Dyal, Lenaïck Menot, Robin Floyd, and Craig R. Smith*

Polychaetes, which are segmented worms within the phylum Annelida, are an ubiquitous and diverse component of marine soft-sediment habitats at all depths. There are at least 10,000 known species and although the majority of these are described from shallow water, it is now accepted that the polychaetes are also extremely diverse in the deep sea. For example, a previous study from the central Pacific has shown that over 170 species were recovered from just $3m^2$ of seafloor (Glover et al., 2002). Nonetheless, there are very few data available on polychaete species ranges and degrees of gene flow across the abyssal plains over geographic scales 1,000-3,000 km. The principal goal of the polychaete component of this Kaplan-ISA funded project has been to use a combined morphological and molecular approach to study polychaete biodiversity across the Clarion-Clipperton Fracture Zone (CCFZ) region, and to specifically test the following null hypotheses:

1) The abundance, species richness and community structure of the polychaete fauna shows little variation at 1,000-3,000 km scales across the CCZ.

2) The majority of polychaetes are broadly distributed at 1,000-3,000 km scales along the CCZ.

Our polychaete studies are still in progress, with ongoing efforts devoted to molecular analyses and to morphological intercalibration of the collections of working species of polychaetes identified by different taxonomists for the various studies in the CCZ and the abyssal North Pacific. The intercalibration efforts are benefiting substantially from an international workshop, titled *APIP - Abyssal Polychaete Inter-calibration Project* funded by the Census of the Diversity of Abyssal Marine Life (CeDAMar) and other sources, held at the Natural History Museum in London 8-11 January 2007 (see website http://www.cedamar.org/ for Workshop report).

1. Polychaete Specific Methodology

The polychaete fraction of the macrofaunal samples taken during the three cruises was sorted from bulk-fixed samples at the University of Hawaii (CRS lab) and shipped to the NHM for morphological analysis. Initially, the molecular component of the project was to be undertaken at the British Antarctic Survey (Alex Rogers laboratory) following morphological studies at the NHM. However, the significant problems encountered in extracting DNA from the abyssal material and funding constraints led to a project over-run. Two additional grants were obtained from the Natural History Museum to continue the molecular project and develop new techniques for the extraction of DNA from bulk-fixed abyssal polychaete samples. These grants totaled ~$60,000 (staff time and consumables).

Polychaete specimens received at the NHM were initially identified to family level, placed into individually numbered 2-ml microtube vials, and stored in temperature-controlled conditions to restrict damage to DNA. A database was created to identify all individuals with a unique number, which has been continually updated with data from morphological investigations (photographs, slide preparations, line drawings, electron microscopy images) and molecular studies (hyperlinks to the sequences). This database has allowed four different taxonomists and two molecular biologists to work on the same samples in synchrony without significant problems.

Selected families thought to potentially contain widespread species were chosen for more detailed investigation. Identification to species-level was carried out by morphotyping, using drawings, slide preparations and electron microscopy. For larger animals, tissue samples were excised for DNA studies, while smaller animals were lysed whole and sequenced after morphological data had been obtained. Molecular protocols are given in Appendix 2.

Genes targeted during molecular studies were 18S rRNA, ITS2 (nuclear DNA) and 16S rRNA, CO1 and cytB (mitochondrial DNA).

Table 2 illustrates the success rate of our molecular approach, with a 70% success rate for 18S and a trend of reduced success rate in mitochondrial and coding genes. These are the first sequences to be obtained from abyssal polychaetes, and we are continuing with our attempts to improve the quality of sequences.

Table 2. Percentage success rate for PCR on abyssal polychaete samples.

Gene	% PCR success
Nuclear 18S rRNA	70
Nuclear ITS2	51
Mitochondrial 16S rRNA	29
Mitochondrial cytB	19
Mitochondrial CO1	11

2. Polychaete abundance and community structure

Specimens were analysed from 48 separate samples across the three Kaplan Sites E, C and W (Table 3). A total of 485 individuals (excluding fragments without heads) were identified and entered into the database. Excluding those samples that were deemed non-quantitative (due to

TABLE 3. POLYCHAETE SPECIES LIST INDICATING THE PRESENCE/ABSENCE AND ABUNDANCE OF SPECIES AT THE THREE SAMPLING SITES IN THE CCZ. BOLD TYPE INDICATES SPECIES WITH POTENTIALLY WIDESPREAD DISTRIBUTIONS.

		Site		
Family	**Species**	**E**	**C**	**W**
Acrocirridae	Acrocirrid sp. indet.	2		
	Acrocirrid sp. PIP#6	3		
	Acrocirrid sp. PIP#7	3		
	Acrocirrid sp. PIP#8	1		
	Acrocirrid sp. PIP#9		1	
Ampharetidae	Ampharetid sp. indet.	3	1	
Amphinomidae	Amphinomid sp.	3		
	Amphinomid sp. A	11		
Capitellidae	Capitellid sp. indet.		1	1
Chaetopteridae	Chaetopterid sp. A	1		
Chrysopetalidae	Chrysopetalum sp. A	1		
	Dysponetus sp. A	6	2	
Cirratulidae	Aphelochaeta sp. A	2		
	Aphelochaeta sp. B	2	1	
	Aphelochaeta sp. C	3		1
	Aphelochaeta sp. D	1		
	Aphelochaeta sp. E	1		
	Aphelochaeta sp. F		2	
	Aphelochaeta sp. indet.	1		
	Chaetozone sp. A	3	1	
	Chaetozone sp. indet.	1	1	
	Cirratulidae sp. indet.	4	9	1
	Cirriformia sp. indet. ?		1	
	Monticellina sp. A	4	3	2
Euphrosinidae	Euphrosinid sp. A		1	
Flabelligeridae	Diplocirrus sp. PIP#10	1		
	Flabelligerid sp. indet.	1		
Glyceridae	Glycera sp. PIP#17	1	4	
	Glycerid sp. PIP#19		1	
Goniadidae	Bathyglycinde c.f profunda		1	
	Goniada sp. PIP#16	2		
	Goniadid sp. PIP#20	1		

TABLE 3 CONT'D. POLYCHAETE SPECIES LIST INDICATING THE PRESENCE/ABSENCE AND ABUNDANCE OF SPECIES AT THE THREE SAMPLING SITES IN THE CCZ. BOLD TYPE INDICATES SPECIES WITH POTENTIALLY WIDESPREAD DISTRIBUTIONS.

		Site		
Family	**Species**	**E**	**C**	**W**
Hesionidae	Acrocirrid sp. PIP#9		1	
	Hesionid sp. indet.		1	
	Hesionid sp. PIP#13	1		
	Hesionid sp. PIP#14		1	
	Hesionid sp. PIP#15		1	
Lumbrineridae	Lumbrinerid sp. indet.			1
	Lumbrinerides cf. laubieri	26		
	Lumbrineris sp. A	10		
	Lumbrineris sp. indet.	4		
Nereididae	Ceratocephale sp. A	5		1
Paraonidae	Aricidea sp. A	1		
	Aricidea sp. B		2	
	Aricidea sp. C		6	
	Aricidea sp. D		1	
	Aricidea sp. E		1	
	Aricidea sp. indet.	1	1	
	Paraonis sp. A	1		
	Paraonis sp. indet.	1		
Phyllodocidae	Pseudomystides sp.	1		
Pilargidae	Ancistrosyllis groenlandica	1		
	Pilargid sp. indet.		1	
	Pilargis sp. PIP#12		1	
Spionidae	Auospio sp. nov. PIP#21		1	
	Aurospio cf. dibranchiata	5	26	6
	Prionospio sp. indet.	1		
	Prionospio sp. PIP# 2	4	9	1
	Prionospio sp. PIP# 3	2	2	
	Prionospio sp. PIP# 5	15	6	
	Spionidae sp. indet.	4	12	4
	Spiophanes sp. PIP# 4	1	2	
Syllidae	Eusyllinae sp. A	1	13	1
	Eusyllinae sp. B	3		
	Sphaerosyllis sp. A	4		
	Sphaerosyllis sp. B	4		
	Sphaerosyllis sp. C	4		
	Sphaerosyllis sp. indet.		1	
	Syllidae sp. indet.		2	

Table 4. Total number of polychaetes collected in all samples from the Kaplan-ISA abyssal project. Quantitative samples are highlighted in bold.

Site	Sample #	No. of individuals
E	822	16
	825	**27**
	826	**36**
	829	**30**
	830	**16**
	833	**16**
	834	13
	841	**29**
	842	**12**
	843	9
	844	**18**
	846	**19**
	847	**9**
	817/T3	12
	824/T6	1
	827/T1	2
	831/T1	1
	831/T7	2
	832/T6	1
	838/T8	1
E Total		**270**
C	**859/KGS5**	**20**
	868/MTB9/TC1	1
	868/MTB9/TC4	1
	868/MTB9/TC7	2
	869/MTB10/TC1-7	5
	870/MTB11/TC1-7	13
	875/KGS16	**17**
	876/KGS17	**21**
	877/KGS19	**21**
	878/KGS20	**20**
	879/KGS26	29
	880/KGS27	**26**
	871/MTB13/TC1-7	4
C Total		**180**
W	855/T1	1
	855/T3	1
	855/T4	1
	855/T5	1
	857/T2	1
	857/T5	1
	857/T6	1
	857/T8	2
	885/KGS28	**6**
	886/KGS32	**4**
	887/KGS33	**2**
	888/KGS37	**3**
	889/KGS39	**8**
	890/KGS40	2
	PL1614	1
W Total		**35**
Grand Total		**485**

partial loss of sample during recovery), we have calculated an abundance of 84.8 ind m^{-2} (s.e. = 11.1) at Site E, 83.3 ind m^{-2} (s.e. = 4.8) at Site C, and 18.4 ind m^{-2} (s.e. = 4.3) at Site W. This indicates a substantial decline in polychaete abundance, and, by inference, seafloor standing crop and productivity, in moving from Site E to Site W, i.e., from the eastern to the western end of the CCZ.

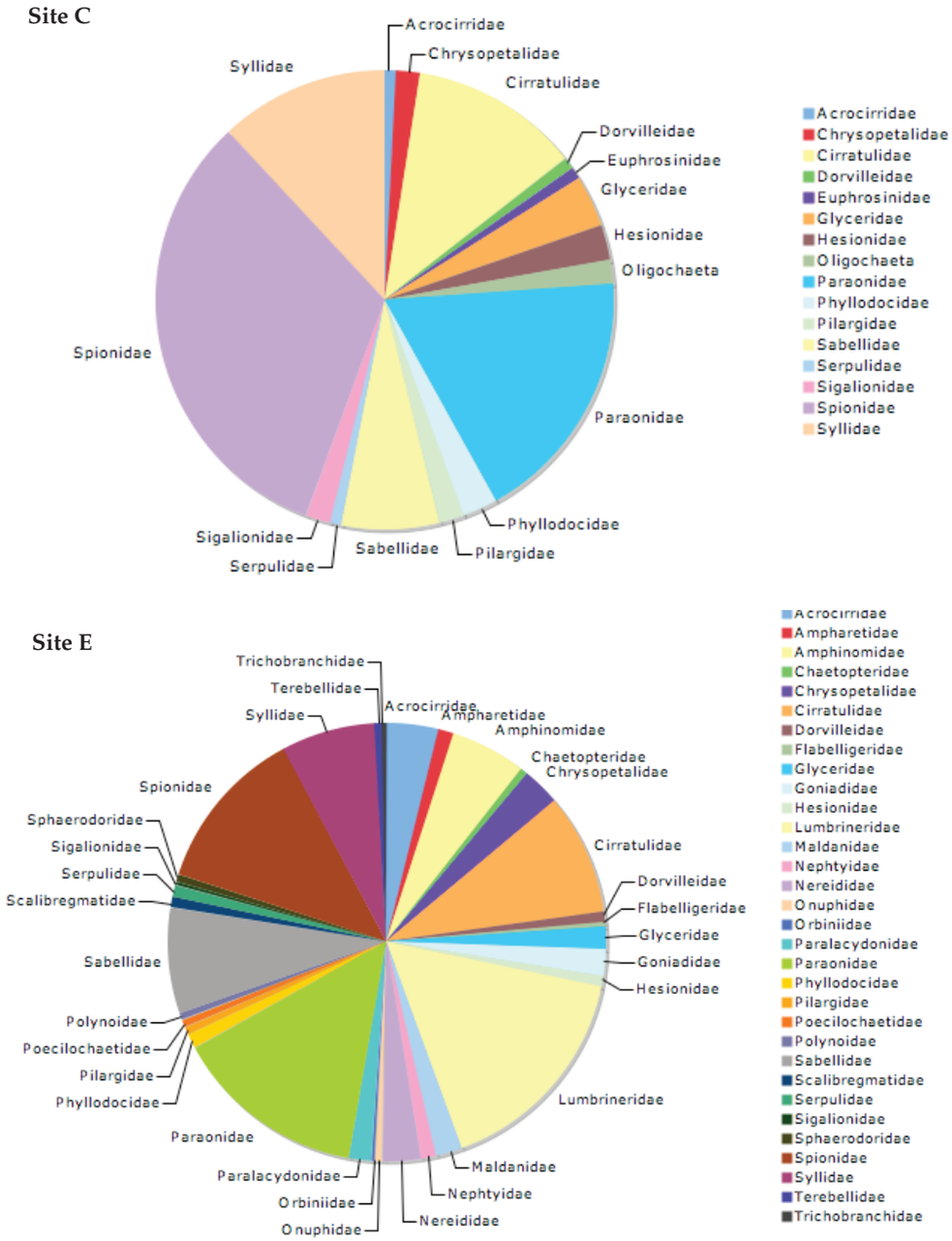


Figure 7. Percentage dominance of polychaete families at Sites C and E.

Analysis of the polychaete composition at the family level strongly suggests reduced diversity at Sites C and W, compared to Site E. The polychaete families Lumbrineridae and Amphinomidae make up substantial proportions of the polychaete fauna at Site E, but are nearly or completely absent at Sites C and W (Figs. 7 and 8). These differences in proportions of Lumbrineridae and Amphinomidae between Site E and Sites C and W are highly statistically significant ($p < 0.0005$ in all cases, percentage tests, Sokal and Rohlf, 1981). In contrast, Sites C and W are dominated by Spionidae, Cirratulidae, Syllidae and Paraonidae only (Figure 7). Polychaetes in the families Lumbrineridae and Amphinomidae are jawed motile predator/omnivores and may be rare or absent at Sites C and W because the lower productivity at these stations limits the development of higher trophic levels (i.e., the abundance of potential prey may be too low to support most predators). Thus, the familial-level data strongly indicate that at least some species (i.e., those in the family Amphinomidae) are restricted to only the eastern end of the CCZ.

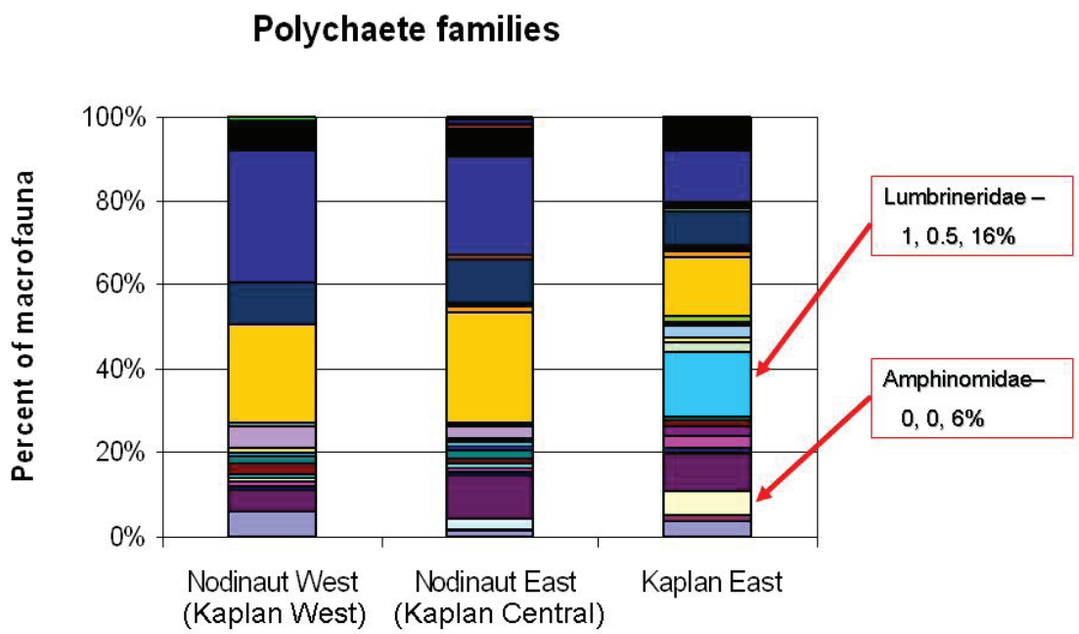


Figure 8. Familial composition of macrofaunal polychaetes in box core samples, collected along the Kaplan transect. Samples sizes are: 99,345 and 253 Kaplan West, Central and East, respectively.

For selected, relatively abundant polychaete families, species-level identifications were carried out using morphological characters to determine whether species may have widespread distributions. Sixty-nine different species were identified from these selected families, illustrated in Table 4. Identification based on morphology suggested that only 12 of these species were widespread in their distributions, being present in more than one site, and that the remainder were either endemic to single sites or missed due to small sample sizes.

Table 5 compares distributions from previous CCFZ Domes studies (Paterson et al.,1998) with the results from this project. CCFZ Domes sites cover a similar geographic spread as Kaplan, comprising three sites, Domes A in the west of the CCFZ and the same site as Kaplan W, PRA in

Table 5. Analysis of the geographic spread of species from selected families. Numbers to the left of the slash are from the Domes study; those to the right of the slash are from the Kaplan samples. The families represent a range of trophic types and species from these families are also the subject of molecular-morphological analysis

Spionidae		**Cirratulidae**	
Total No of species	**13/5**	Total No of species	**9/8**
Endemics	**7/ 1**	Endemics	**4/7**
2 sites adjacent	**1/ 0**	2 sites adjacent	**2/ 1**
2 sites	**1/ 0**	2 sites	**2/ 0**
All three sites	**4/ 4**	All three sites	**1/ 0**
Lumbrineridae		**Syllidae**	
Total No of species	**9/2**	Total No of species	**11 / 5**
Endemics	**6/ 0**	Endemics	**4 / 5**
2 sites adjacent	**0/ 0**	2 sites adjacent	**2/ 0**
2 sites	**1/ 0**	2 sites	**3/ 0**
All three sites	**2/ 2**	All three sites	**2/ 0**
Paraonidae			
Total No of species	**8/ 6**		
Endemics	**0 / 6**		
2 sites adjacent	**2/ 0**		
2 sites	**1/ 0**		
All three sites	**5/ 0**		

toward the central region of the CCFZ of the CCFZ and ECHO slightly further east of PRA (both PRA and ECHO were in the same region as Kaplan C). There was a difference in sampling intensity between Domes and Kaplan; Domes A comprised 60 boxes cores, PRA 20 and ECHO 15.

The Kaplan results indicate that there is a high degree of endemism in all the families with the exception of the Lumbrineridae and Spionidae, where species appear to be more widespread. However, caution needs to be applied in over-interpreting these results because the Domes results indicate that endemism is much lower as a proportion of the total number of species in each family. At least one family, the Paraonidae, show no endemicity in the Domes data. It is likely that endemicity will be a feature of all sites, but that it will be lower than indicated by the Kaplan results.

These results indicate that, because of the low densities and relatively high sample diversity of the CCZ, a robust sampling programme has to be undertaken to ensure that sufficient specimens are sampled. This is, and will continue to be, problematic from a logistic perspective. (The Kaplan-funded project had, by normal scientific criteria, a good sampling programme with at least eight replicate box cores taken at each station). Unifying the datasets of Kaplan and Domes is obviously an important step to assessing morphological patterns, and work on this aspect continues.

The high 'endemicity' found in the Kaplan samples poses a problem in the molecular analyses as species with limited distributions provide limited data with which to test the original hypotheses.

3. Molecular diversity of abyssal polychaetes

Major problems with the quality of template DNA recovered from the worm tissues have severely hampered the molecular part of this project. However, we have been continuing our efforts to improve the quality of 18S rRNA gene sequencing, and we now have reliable sequences from 15 specimens. It should be noted that these are the first molecular data from any abyssal polychaete study. Initial results suggest that in general terms, there is a high degree of cryptic speciation in abyssal polychaetes (Figure 9). The genetic heterogeneity within our morphotypes for the 18S gene suggests that levels of species diversity calculated from earlier

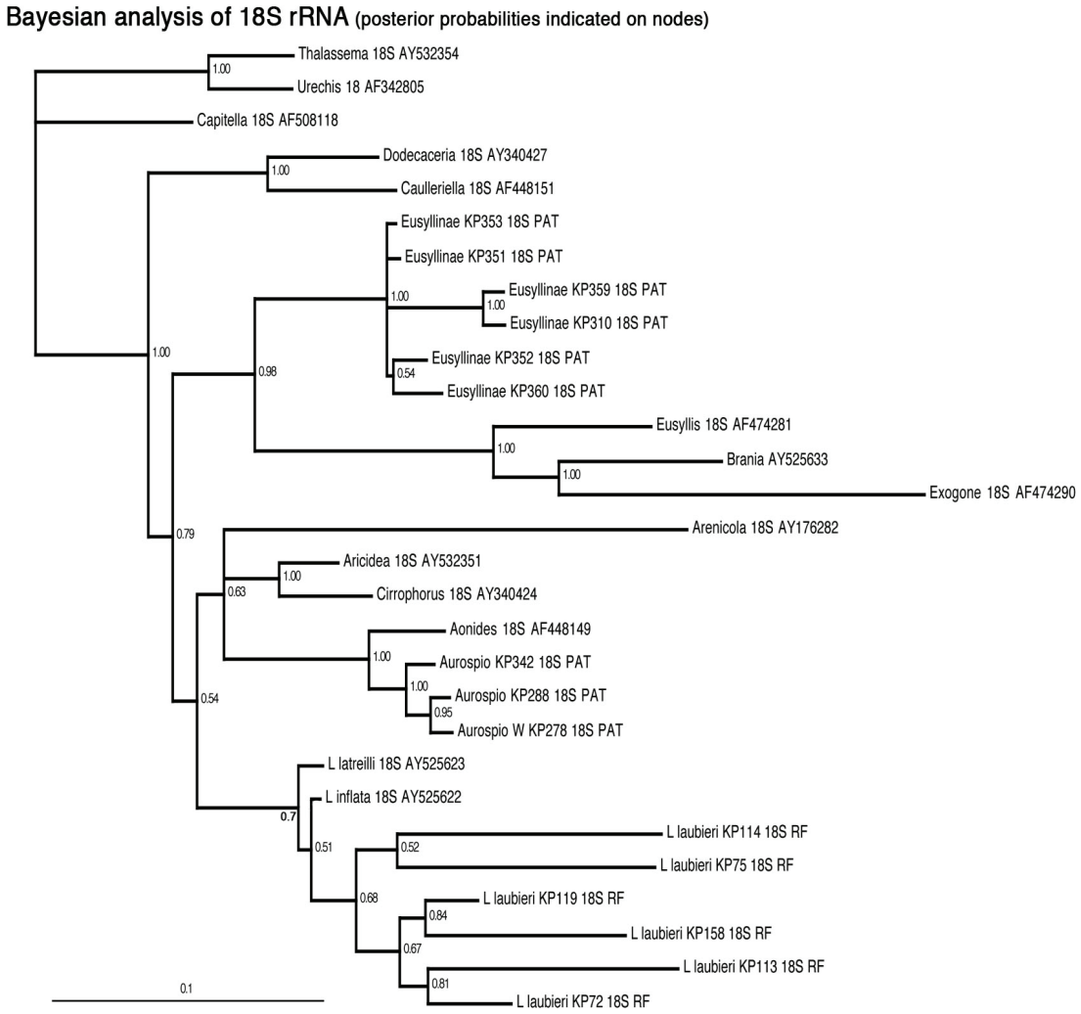


Figure 9. Bayesian analysis of 18S rRNA data from abyssal polychaetes with posterior probabilities marked on nodes. Specimens from this project are indicated by numbers with the letters 'KP'. Sequenced data for additional taxa are from GenBank.

studies in the CCZ are substantial underestimates. For a single syllid morphotype, at least two, possibly three molecular haplotypes (or putative species) were recovered. For the species *Aurospio dibranchiata*, molecular evidence suggests the presence of discordantly-distributed haplotypes. Within the species *Lumbrinerides* c.f *laubieri*, multiple cryptic haplotypes have been discovered.

As a result of the APIP workshop, it will be possible to expand the comparison from within the CCZ, basically extending a within-basin comparison to an inter-ocean comparison. Species of syllids and glycerids from the Atlantic have been recognised as morphologically similar to Kaplan specimens. The Atlantic specimens collected during the DIVA project have also been sequenced. A collaboration has been established which will enable us to compare sequences and thus provide another scale of morphological and molecular comparisons.

A second set of polychaete specimens in three families (Glyceridae, Cirratulidae, Paraonidae) are currently undergoing DNA sequencing. New methods are now being deployed and an increase in DNA product has been isolated. Better success rates for a number of genes have been achieved and it should also be possible to reanalyse samples from the first attempt, increasing the number of genes analysed giving better resolution. This work is still in progress and is due for completion in the summer of 2007 when the results will be presented at the International Polychaete Conference in Maine.

4. Polychaete conclusions

In general, our data do not support hypothesis no. 1, i.e., that the abundance, species richness and community structure of the polychaete fauna are essentially homogeneous on scales of 1,000–3,000 km across the CCZ. Both abundance and familial diversity declined from Site C to Site W. This is indicative of a potentially high degree of heterogeneity at broad scales in the central Pacific abyss. This is supported by evidence from the community composition of polychaetes which showed broad-scale changes at scales of 1,000-3,000 km.

In general, our data also fail to support hypothesis no. 2, i.e., that the majority of polychaetes are broadly distributed at 1,000-3,000 km scales across the CCZ. Although there have been major obstacles to obtaining the molecular data, the molecular data thus far suggests both a higher than expected degree of molecular heterogeneity and cryptic speciation in the abyss. While morphotypes may be broadly shared across 1,000-3,000 km scales, as yet we find no evidence for high levels of gene flow. Rather, our data suggest that morphologies may be relatively conserved and slowly evolving in abyssal polychaetes, yet speciation and isolation with distance may still be occurring.

Clearly, our data represent only a first step towards understanding molecular diversity in the abyss, and further attempts must be made to resolve the methodological problems associated with working on these very small specimens.

Collaborative studies with IFREMER through Project Nodinaut – *Joëlle Galéron and Lenaïck Menot*

Preliminary outcomes of the Nodinaut cruise

In May-June 2004, the *Nodinaut* cruise was conducted by IFREMER. This cruise constituted part of the activities agreed upon in the contract for nodule exploration in the Clarion-Clipperton

Zone (CCZ), drawn up between the French authorities and the International Seabed Authority (ISA). Cruise goals were to gather environmental reference data in two out of the three French claim areas, respectively the IFREMER/AFRENOD east and west zones, which include Kaplan Sites C and W (Figure 1).

Predictions of the effects of nodule mining impacts as well as recommendations on biodiversity conservation suffer from many gaps in our understanding of benthic community patterns and dynamics in the abyssal realm. In addition to collection of baseline study, the *Nodinaut* cruise aimed at addressing two of the issues within the CCZ: elucidating (a) regional-scale patterns of macrobenthic community structure, and (b) local influences of nodules on benthic community structure.

Regional-scale patterns of macrobenthic community structure. There are major gaps in our knowledge of the biogeography, species ranges and patterns of gene flow across abyssal benthic habitats of the Pacific Ocean. Owing to the large-scale impact anticipated from nodule mining, it is of fundamental interest to know over what spatial scales benthic population are distributed, and thus the likelihood of disrupting a substantial proportion of species ranges from mining activities. In addition, we need to know over what spatial scales benthic populations are genetically connected (i.e., sustain gene flow) and, thus, could effectively provide new settlers to areas of mining disturbance. These issues are specifically addressed by the Kaplan project. The comparison of macrobenthic community patterns between the eastern and western French claim areas constitutes a contribution of the Nodinaut project to this topic. The two areas are likely to be subjected to different trophic inputs because of an overall westward trend of reduced primary productivity in the central Pacific (Smith and Demopoulos, 2003). Indeed, the abundance of the megafauna, macrofauna and meiofauna is lower in the western than in the eastern area. The two areas also differ according to their substrates, with the western area being characterised by a higher heterogeneity, which may contribute to the observed increase in richness of megafaunal assemblages from east to west. On the other hand, the small encrusting protozoans, mainly foraminiferans, living on nodules are more diverse in the Eastern than in the Western area (Veillette et al., submitted).

Local influences of nodules on benthic community structure. A second gap in knowledge about the ecology of abyssal benthic communities concerns the factors structuring these communities and maintaining a high local species diversity. Rex et al. (2005) depict deep-sea biodiversity patterns for molluscs as a source-sink system in which bathyal populations are a source of juveniles for reproductively unviable abyssal populations. According to this hypothesis, the threat for biodiversity of human activities in the abyssal realm would be rather low. True abyssal species are, however, known for some groups belonging to the macrofauna, such as the Isopoda (Hessler, 1970), and the megafauna, especially Echinodermata (Billet, 1991). In addition, ovigerous polychaetes were also found in samples from the *Nodinaut* cruise, suggesting reproductive viability for these species in the CCZ (pers. obs.). Another explanation for the unexpectedly high local diversity of the resource-poor abyssal sediments relies on the heterogeneity of the deep-sea floor. Some of this heterogeneity may lie in long-lasting small patches of decomposing detritus (Grassle, 1989) and some in spatially complex topographic features, like biogenic structures or nodule facies. In coastal environments, topographically heterogeneous systems such as polychaete tubes (Zuhlke, 2001), seagrass beds (Bostroem and Bonsdorff, 2000) or boulder fields (Guichard and Bourget, 1998, Cruz Motta et al., 2003), are known to influence the structure and composition of the infauna. Similar patterns may be expected for nodule fields, which would have implications for sampling and

conservation strategies. The Nodinaut project aimed at testing this hypothesis on meio- and macro- and megabenthic communities, owing to the previous knowledge and detailed maps of nodule facies on the eastern French claim area (Du Castel, 1985). Indeed, the Nodinaut results indicate that the presence of nodules influences all three size classes. In nodule fields, megafaunal and macrofaunal densities are enhanced whereas meiofaunal densities are reduced. Nodules clearly provide a habitat for fixed megafaunal assemblages which feed on suspended organic particles, like sponges and actiniarians, whereas areas without nodules are dominated by deposit feeders like holothurians for which more than 20 species have been identified. Overall, the presence of nodules enhances the richness of megafaunal assembles. The higher densities of the macrofauna observed in presence of nodules was unexpected as free space at the sediment surface is rather low in the nodule field studied. It clearly suggests that abyssal macrobenthic populations are not space limited. The inverse trend of lower meiofaunal densities in the nodule field may suggest some sort of competition between meio- and macro-infauna. Both assemblages of spionids for the macrofauna and nematodes for the meiofauna shows a high species richness but dissimilar species compositions in areas with and without nodules.

Results of the *Nodinaut* cruise confirm that nodule fields constitute a special habitat for a sessile epifauna otherwise rare in the deep sea (Mullineaux, 1987; Tilot, 1992). They also indicate that nodule fields likely constitute a distinct habitat for infaunal communities. Nodule influences on sessile epifauna and infauna thus appear to contribute to enhanced biodiversity of the abyssal benthos at regional scales, which has significant ramifications for biodiversity conservation in regions of nodule removal due to mining.

DISCUSSION AND INITIAL RECOMMENDATIONS TO THE ISA

The results of this project are still accumulating and undergoing analyses, so our recommendations to the ISA concerning managing of nodule-mining environmental impacts must be considered to be in a 'draft' form. More detailed synthesis and recommendations will be made in February 2008 following the workshop funded by the Pew Fellows Program in Marine Conservation, to *Design Marine Protected Areas for Seamounts and the Abyssal Nodule Province in Pacific High Seas.* This workshop convened ~20 scientific and legal experts and ISA managers to provide specific guidance to the ISA in marine conservation in the CCZ.

At present, the substantial advances in our understandings of patterns of biodiversity and potential species ranges in the CCZ resulting from this project provide important new insights into biodiversity and its conservation in nodule-mining activities. In our discussion and recommendations we will focus on answering four major questions:

1) *What are the diversity levels of foraminifera, nematodes and polychaetes at our three Kaplan Sites (E, C, and W) spanning the CCZ?*

2) *Is there evidence of a characteristic abyssal fauna in this region?*

3) *What are the levels of species overlap (and rates of gene flow) across our Kaplan Sites - E, C, W - spanning 3,000 km of the CCZ?*

4) *What are the resulting ramifications and recommendations for the ISA for the management of nodule mining and the designing of marine protected areas?*

1. What are the levels of diversity at individual sites?

Our results indicate high, unanticipated levels of species diversity for all three sediment-dwelling faunal components studied at our individual sites - E, C, and W. The foraminifera contain at least 252 species (based on morphological analyses) at Site E and at least 180 species at Site C. Many of these species are new to science and do not appear to have been collected elsewhere. In addition, the foraminifera are characterised by extreme, small-scale heterogeneity in community structure, with dramatic differences between core tubes from individual multiple core lowerings. The nematodes also exhibit very high, within-site diversity, with 73 putative species (or MOTUs) from only 97 sequenced individuals. Because of the high ratio of species to individuals within the nematodes (i.e., one new species was found for every 1.3 individuals sequenced), the total nematode species richness is still grossly undersampled. We can be certain that far more species remain to be collected at each site. The polychaetes also exhibit very high within-site diversity for the families studied in detail; for example, Site E contains at least 48 polychaete species within 16 families. The high abundance of apparently cryptic species found with our molecular studies indicates that earlier estimates of polychaete species richness within abyssal Pacific sites, based on morphological studies (e.g., the 170 species from 3 m^2 by Glover et al. (2002)), are likely to be low by at least a factor of two. We speculate that, based even on the relatively limited number of samples we have been able to analyse thus far, the total species richness of sediment-dwelling foraminifera, nematodes and polychaetes (a subset of the total fauna) at a single site in the CCZ could easily exceed 1,000 species.

The preliminary IFREMER outcomes from the *Nodinaut* cruise provide still further evidence of very high, undersampled biodiversity in the CCZ. IFREMER has found high within-site heterogeneity in infaunal community structure, which appears to covary with the abundance and size of manganese nodules, on scales of 0.1-10 kilometers at Sites C and W. Different components of the fauna (i.e., the macrofauna and meiofauna) respond differently to this apparent habitat heterogeneity. In addition, we have very little understanding of the ecological factors driving these community changes, with some trends (e.g., greater abundance of infaunal macrobenthos in areas of higher nodule abundance) contradicting our expectations. Thus, it is very difficult to predict the upper limit to biodiversity within and across sites in the CCZ, or to predict, precisely, how nodule mining activities might influence habitat variability.

In summary, all faunal components studied in this project exhibit very high, poorly sampled, within-site species diversity. Cryptic speciation (i.e., the presence of multiple species previously identified as single species) appears to be very common in the faunal components studied with molecular approaches, i.e., the polychaetes and nematodes. Habitat heterogeneity also appears to be higher than previously appreciated. All these lines of evidence indicate that there are very high levels of biodiversity potentially impacted by mining in the CCZ.

2. Is there evidence of a characteristic abyssal fauna in this region?

Our results from all faunal components suggest that there is a characteristic fauna of the abyss: i.e., the abyss is not merely a sink of non-reproducing individuals transported from the

continental margins (Rex et al., 2005; Smith et al., in review). Many of the hundreds of species of foraminifera identified from our samples appear to be restricted to, or at least characteristic of, the abyss. Seventy out the 73 MOTUs of nematodes appear to be new genera distinct from shallow-water genera and, thus, may well have evolved in the abyss. The molecular data for the polychaetes also suggest numerous cryptic new species in our Kaplan abyssal samples, again suggesting that the abyssal polychaete fauna contains much higher species diversity than previously appreciated and is likely to include numerous species evolved in the abyss. All of these results suggest that the abyss harbours a specially adapted, diverse fauna distinct from the fauna of the continental margins. It seems very unlikely that most, or even many, species found in the CCZ abyss are protected from extinction by populations residing many thousands of kilometers away at much shallower depths on the continental margins, as predicted in the 'abyssal sink' hypothesis of Rex et al. (2005).

3. What are the levels of species overlap (and rates of gene flow) across our Kaplan Sites E, C, W spanning 3,000 km of the CCZ.

Our data concerning species distributions and gene flow from this study are still limited by relatively small sample sizes and incomplete intercomparisons, so any conclusions must be drawn with caution. Nonetheless, there is significant evidence that community structure of the foraminifera and polychaetes differ substantially on scales of 1,000–3,000 across the CCZ. Within the foraminifera, at least 10 species are abundant at either Site E or C, but are rare or absent at the other site; this is strong evidence of turnover of some of the foraminiferan fauna over a distance of $\leq$ 1,000 km. The polychaete fauna exhibits major differences at the familial level between Site E at the eastern end of our transect and Sites C and W in the central and western areas, suggesting major turnover in species, and changes in community complexity, over scales of 1,000–3,000 km across the CCZ. The molecular data for polychaetes also suggest that while morphotypes may be widely distributed across the CCZ, many actual species may not be distributed across our three sites, E, C and W. These apparent patterns of faunal turnover seem likely be driven in part by the east to west decline in productivity across the CCZ, but may also be driven, in part, by varying habitat heterogeneity documented by the IFREMER studies.

4. What are the resulting ramifications and recommendations for the ISA for the management of nodule mining and the designing of marine protected areas?

Any recommendations made to the ISA at this point must be considered preliminary and used with the precautionary principle (i.e., our understanding of biodiversity levels, species ranges and gene flow in the CCZ is still limited so we must err on the side of over-protection of the environment). It is clear that novel taxa, and evolutionary novelty, occur in the abyss so we cannot assume that protection only of the ocean margins will preserve abyssal biodiversity. There is also substantial evidence from our studies that the CCZ is not one continuous habitat harbouring a single biotic assemblage; species appear to turnover, and community structure varies substantially over spatial scales of 1,000 km or less (i.e., of scales $\leq$ the distance between our Kaplan Sites E and C, Fig. 1). This suggests that marine protected areas (MPAs) designed to safeguard biodiversity in the CCZ, in the face of nodule mining, should be set up as follows.

- MPAs should be placed at multiple locations across the CCZ. At the very least, major MPAs should be established in the regions of Sites E, C, and W.

- Because of the steep latitudinal gradients in productivity and community structure within the equatorial Pacific (Smith et al., 1997; Smith and Demoupoulos, 2003; Hannides and Smith, 2003), the MPAs should be set up to protect biodiversity across the width of the CCZ from 7°–17° N latitude.

- The MPAs should be large enough to encompass major areas of the known benthic habitat types in the CCZ, including abyssal hills with and without nodules, rocky ridges, and multiple seamounts of various elevations above the seafloor.

- Each MPA must be large enough for it core area to be buffered from the direct and indirect impacts of nodule mining activities, including influences from sediment plumes in the water column and at the seafloor.

- Because benthic processes and community structure in the CCZ (including biomass, growth rates, biodiversity, and species composition) are strongly influenced by processes in the water column above (e.g., primary production and organic carbon export), it would be highly desirable for management of the MPAs to include control of substantial human activities (mining, energy exploitation, waste disposal, and commercial fishing) from the abyssal seafloor to the ocean surface. This is consistent with the concept of ecosystem based management (Pikitch et al., 2004), in which human activities are managed to maintain ecosystems in (a) healthy, productive and resilient condition and (b) to explicitly account for the interconnectedness among systems (e.g., the export or particulate organic carbon and nekton carcasses from the upper ocean to the abyssal seafloor).

It is critically important to recognise that these recommendations are based on a limited, albeit rapidly growing, database on biodiversity and species ranges in the CCZ, and should be applied using the precautionary principle (Pikitch et al., 2004). Specifically, where data are inadequate to exclude potential harm to the environment from a particular human activity (e.g., nodule mining), the activity should be conservatively managed to ensure environmental protection.

Ongoing studies, collaborative projects (e.g., the Census of Diversity of Abyssal Marine Life), and workshops (specifically the Pew-funded workshop to *Design Marine Protected Areas for Seamounts and the Abyssal Nodule Province in Pacific High Seas* with recommendations to be made to the ISA in February 2008) will help to better resolve biodiversity levels and species ranges in the abyssal Pacific and provide guidance to the ISA in the conservation of abyssal Pacific ecosystems.

FUTURE DIRECTIONS

Based on our results to date, we can make several recommendations for future studies to improve understanding of patterns of biodiversity and gene flow in the CCZ.

(1) Despite the intensive sampling efforts of the Kaplan Project and other research programmes, the biodiversity of CCZ benthos remains substantially undersampled. Major effort should be devoted to additional sampling across the region (both longitudinally and latitudinally)

using DNA-friendly techniques to better constrain levels of species richness, levels of endemism, and gene flow. Development of an epibenthic sled (Gage and Tyler, 1991) capable of sampling sediment infauna in nodule fields would be extremely useful to such a sampling effort. Emphasis should be placed on collecting very large numbers of macrofaunal individuals (>10,000) at multiple sites across the region to allow accurate estimates of species richness, and to allow adequate sample sizes for gene-flow analyses for a large number of species.

(2) The reproductive capabilities of most meiofaunal and macrofaunal species found within the CCZ remain unevaluated. Is reproduction common among the CCZ macrobenthos, or are species in this region primarily represented by non-reproductive sink populations transported from more productive oceanic regions (e.g., island slopes and the equatorial upwelling zone)? Intensive sampling with an epibenthic sled, combined with evaluation of reproductive status (Gage and Tyler, 1991) for a broad range of species, would help to determine whether the CCZ is generally a sink for many species of macrobenthos (Rex et al., 2005).

(3) Substantial progress must be made in the description and phylogenetic analysis of novel taxa from a range of phyla and size classes found in the CCZ and the abyssal Pacific generally. A combination of molecular with morphological approaches, as used in the Kaplan Project and in Brandt et al. (2007), is critical to progress in this area.

(4) Because the many hundreds of new species from the CCZ will require many years for formal description, intercalibration of working-species collections from disparate sampling efforts is critical to biogeographic syntheses across the CCA and the Pacific basin. With support from CeDAMar, intercalibration of working species collections is ongoing with the Kaplan material, but intercalibration will require substantial effort beyond the end date of CeDAMar (December 2010). Such intercalibrations are critical to evaluation of species ranges and faunal turnover across the CCZ, and in the abyssal Pacific in general.

(5) Finally, modeling efforts are needed to assess the errors in estimating abyssal endemism from various levels of sampling effort. Such efforts are crucial for placing confidence limits on estimates of regional endemism (Glover et al., 2002) and for predicting sampling effort required to sufficiently characterise regional species pools.

(6) The biota of hard substrates of the CCZ, especially rock outcrops associated with fracture zones and seamounts, remains very poorly studied. Is the manganese-nodule biota distinct from that on other hard substrates, or is there a common fauna on rocky surfaces throughout the region? *In situ* studies, using ROV's or manned submersibles of the biota living on a broad range of rocky substrates throughout the CCZ would help to determine whether the nodule fauna is unique and/or narrowly distributed within subregions of the nodule province.

LITERATURE CITED

Bhadury, P., M.C. Austen, D.T. Bilton, P. John D. Lambshead, A.D. Rogers, G.R. Smerdon. 2005. Combined morphological and molecular analysis of individual nematodes through short-term preservation in formalin. *Molecular Ecology Notes* 5, 965-968.

Bernstein, B.B., R.R. Hessler, R. Smith, P.A. Jumars. 1978. Spatial dispersion of benthic Foraminifera in the abyssal central North Pacific. *Limnology and Oceanography* 23, 401-416.

Billet, D.S.M. 1991. Deep-sea holothurians. *Oceanogr Mar Biol Annu Rev*. 29, 259-317.

Brandt, A., *et al.* 2007. The Southern Ocean deep sea: first insights into biodiversity and biogeography. *Nature,* 447, 307–311.

Bostroem, C., E. Bonsdorff. 2000. Zoobenthic community establishment and habitat complexity - the importance of *seagrass* shoot-density and physical disturbance for faunal recruitment. *Mar. Ecol. Prog. Ser.* 205, 123-138.

Boucher, G. and P.J.D. Lambshead. 1995. Marine nematode ecological biodiversity in samples from temperate, tropical and deep-sea regions. *Conservation Biology* 9, 1594–1604.

Brown, C.J., P.J.D. Lambshead, C.R. Smith, L.E. Hawkins, R. Farley. 2001. Phytodetritus and the abundance and biomass of abyssal nematodes in the central, equatorial Pacific. *Deep-Sea Research I* 48, 555-565.

Cook, A.A., P. Bhadury, N.J. Debenham, B.H.M. Meldal, M.L. Blaxter, G.R. Smerdon, M.C. Austen, P.J.D. Lambshead, A.D. Rogers. 2005. Denaturing Gradient Gel Electrophoresis (DGGE) as a tool for the identification of marine nematodes. *Mar. Ecol. Prog. Ser.* 291, 103-113.

Creasey, S.S. and A.D. Rogers. 1999. Population genetics of bathyal and abyssal organisms. A*dvances in Marine Biology* 35, 1-151.

Cruz Motta, J.J., A.J. Underwood, M.G. Chapman, F. Rossi. 2003. Benthic assemblages in sediments associated with intertidal boulder-fields. *J. Exp Mar Biol Ecol* 285-286, 383-401.

Diaz, R.J. and R. Rosenberg. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. *Oceanography and Marine Biology Annual Review* 33, 245–303.

Du Castel, V. 1985. Etablissement d'une carte géologique au 1/20.000 d'un domaine océanique profond dans une zone riche en nodules polymétalliques du Pacifique Nord (zone Clarion-Clipperton). Université de Bretagne Occidentale.

Floyd, R.M., A.D. Rogers, P.J.D. Lambshead, C.R. Smith. 2005. Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes* 5 (3), 611-612.

Folmer, O. M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek.1994. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3, 294-299.

Gage, J.D., P.A. Tyler. 1991. *Deep-sea biology: a natural history of organisms at the deep-sea floor*. Cambridge University Press.

Glover, A.G. and C.R. Smith. 2003. The deep seafloor ecosystem: current status and prospects for change by 2025. *Environmental Conservation* 30 (3), 1-23.

Glover, A.G., G.L.J. Paterson, B. Bett, J. Gage, M. Sibuet, M. Sheader, L. Hawkins. 2001. Patterns in polychaete abundance and diversity from the Madeira Abyssal Plain, northeast Atlantic. *Deep-Sea Research I* 48, 217–236.

Glover, A.G., *et al.* 2002. Polychaete species diversity in the central Pacific abyss: local and regional patterns, and relationships with productivity. *Mar. Ecol. Prog. Ser.* 240, 157-170.

Gooday, A.J., 1994. The biology of deep-sea foraminifera: a review of some advances and their applications in paleoceanography. *Palaios*, 9, 14-31.

Gooday, A.J., B.J. Bett, R. Shires, P.J.D. Lambshead. 1998. Deep-sea benthic foraminiferal species diversity in the NE Atlantic and NW Arabian area: a synthesis. *Deep-Sea Research II* 45 (1-3),165-201.

Gooday, A.J., S. Hori, Y. Todo, T. Okamoto, H. Kitazato, A. Sabbatini. 2004. Soft-walled, monothalamous benthic foraminiferans in the Pacific, Indian and Atlantic Oceans: aspects of biodiversity and biogeography. *Deep-Sea Research I* 51, 33-53.

Gooday, A.J., H. Kitazato, S. Hori, T. Okamoto. 2001. Monothalamous soft-shelled foraminifera at an abyssal site in the North Pacific: a preliminary report. *Journal of Oceanography* 57, 377-384.

Grassle, J.F. and N.J. Maciolek. 1992. Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *American Naturalist* 139 (2), 313-341.

Grassle, J.F. 1989. Species diversity in deep-sea communities. *Trends Ecol Evol* 4, 12-15.

Guichard, F., E. Bourget. 1998. Topographic heterogeneity, hydrodynamics, and benthic community structure: a scale-dependent cascade. *Mar Ecol Prog Ser* 171,59-70.

Halanych, K.M., R.A. Lutz, and R.C. Vrijenhoek. 1998. Evolutionary origins and age of vestimentiferan tube-worms. *Cah. de Biol. Mar.* 39, 355-358.

Hannides, A. and C.R. Smith. 2003. The northeast abyssal Pacific plain. In: *Biogeochemistry of Marine Systems,* K.B. Black and G.B Shimmield, eds. CRC Press, Boca Raton, Florida, 208-237.

Hessler, R.R. and P.A. Jumars. 1974. Abyssal community analysis from replicate box cores in the central north Pacific. *Deep Sea Research* 21, 185-209.

Hessler, R.R. 1970. The Desmosomatidae (Isopoda, Asellota) of the Gay Head-Bermuda transect. *Bulletin of the Scripps Institution of Oceanography* 15, 1-185.

Hori, S. 2001. Deep-sea benthic foraminiferal assemblages at three abyssal sites: comparison of North Pacific, Equatorial Pacific and North Atlantic assemblages. Unpublished dissertation, Shizuoka University, Japan, 36 pp., pls 1-24. (in Japanese with English abstract)

Knowlton, N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* 24, 189-216.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* 420, 73-90.

Lambshead, P.J.D., G. Boucher. 2003. Marine nematode deep-sea biodiversity – hyperdiverse or hype? *Journal of Biogeography* 30 (4), 475-485.

Lambshead, P.J.D., C.J. Brown, T. Ferrero, N. Mitchell, L.E. Hawkins & C.R. Smith. 2003. Biodiversity of nematode assemblages from the Clarion-Clipperton Fracture Zone, an area of commercial mining interest. *BMC Ecology* 3, 1.

López-García, P., F. Rodríguez-Valera, C. Pedrós-Alió, D. Moreira. 2001. Unexpected diversity of small eukaryotes in deep-sea Antarctic plankton. *Nature* 409, 603–607.

Mincks, S.L., P.L. Dyall, G.L.J. Paterson, C.R. Smith, A.G.G. Glover. (submitted) A new species of *Aurospio* (Annelida, Spionidae) from Antarctica, with an analysis of its ecology, reproductive biology and evolutionary history. *Marine Ecology.*

Moon-van der Staay, S.Y., R.D. Wachter, D. Vaulot. 2001. Oceanic 18S rDNA sequences from picoplankton reveal unsuspected eukaryotic diversity. *Nature* 409, 607–610

Mullineaux, L.S. 1987. Organisms living on manganese nodules and crusts: Distribution and abundance at three North Pacific sites. *Deep-Sea Res* 34, 165-184.

Nozawa, F. 2003. Research on recovery processes of deep-sea benthic foraminifera at re-deposition area during JET disturbance experiment. Unpublished Undergraduate. dissertation, School of Science, Chiba University, 21pp., 15 figures, 1 table, 21 Plates. (in Japanese)

Nozawa, F. 2005. Deep-sea benthic foraminifera in the equatorial Pacific Ocean. Unpublished Master's dissertation, Graduate School of Natural Sciences, Chiba University, 35 pp., 20 figures, 9 tables, 39 Plates. (in Japanese)

Nozawa, F., H. Kitazato, M. Tsuchiya, and A.J. Gooday. 2006. 'Live' benthic foraminifera at an abyssal site in the equatorial Pacific nodule province: abundance, diversity and taxonomic composition. *Deep-Sea Research I* 53 (8), 1406-1422.

Okamoto, T. 1998. Responses of deep-sea benthic foraminiferal distribution to artificial disturbance during JET. Unpublished dissertation, Shizuoka University, Japan, 35 pp., pls 1-19. (in Japanese with English abstract)

Ohkawara, N. 2006. Deep-sea benthic foraminiferal fauna in the central Equatorial Pacific. Unpublished undergraduate thesis, Hirosaki¤ University, Japan, 26 pp, figs 1-16, tables 1-5, pls 1-25. (in Japanese)

Paterson, G.L.J., G.D.F. Wilson, N. Cosson and P.A. Lamont. 1998. Hessler and Jumars (1974) revisited: abyssal polychaete assemblages from the Atlantic and Pacific. *Deep-Sea Research II* 45 (1-3), 225-251.

Pearson, T.H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Review* 16, 229-311.

Pikitch, E.K., C. Santora, E.A. Babcock, A. Bakun, R. Bonfil, D.O. Conover, P. Dayton, P. Doukakis, D. Fluharty, B. Heneman, E.D. Houde, J. Link, P.A. Livingston, M. Mangel, M.K. McAllister, J. Pope, K.J. Sainsbury. 2004. Ecosystem-based fishery management. *Science* 305, 346-347.

Rex, M.A., C.R. McClain, N.A. Johnson, R.J. Etter, J.A. Allen, P. Bouchet, A. Waren. 2005. A source-sink hypothesis for abyssal biodiversity. *American Naturalist* 165, 163-178.

Rogers, A.D., P.J.D. Lambshead. 2004. Molecular studies of nematode diversity; past, present and future. In: Cook, R. & Hunt, D.J., Eds. *Proceedings of the Fourth International Congress of Nematology, 8 - 13 June 2002, Tenerife, Spain*. Nematology Monographs and Perspectives Volume 2. Brill Academic Publishing.

Saidova, Kh. M. 1965. Distribution of benthic foraminifera in the Pacific. *Oceanology* 5, 72-83.

Smith, C.R., W. Berelson, D.J. DeMaster, F.C. Dobbs, D. Hammond, D.J. Hoover, R.H. Pope, M. Stephens. 1997. Latitudinal variations in benthic processes in the abyssal equatorial Pacific: control by biogenic particle flux. *Deep-Sea Research II* 44 (9-10), 2295-2317.

Smith, C.R., F. De Leo, A. Bernardino, A. Sweetman, P. Martinez Arbizu. (In review) Abyssal biodiversity, ecosystem function and climate change. *Trends in Ecology and Evolution.*

Smith, C.R., A. Demopoulos. 2003. The deep Pacific Ocean floor. In: Tyler, P.A., ed. *Ecosystems of the World 28: Ecosystems of the deep oceans*. Elsevier, pp. 179-218.

Smith, C.R., L.A. Levin, A. Koslow, P.A. Tyler, A.G. Glover. 2008. The near future of deep seafloor ecosystems. In: *Aquatic Ecosystems: trends and global prospects,* N. Polunin, ed., Cambridge University Press, in press.

Snelgrove, P.V.R. and C.R. Smith. 2002. A riot of species in an environmental calm: the paradox of the species-rich deep-sea floor. *Oceanography and Marine Biology Annual Review*, 40, 311-342.

Snider, L.J., B.R. Burnett, R.R. Hessler. 1984. The composition and distribution of meiofauna and nanobiota in a central North Pacific deep-sea area. *Deep-Sea Research* 31, 1225-1249.

Sokal, Robert R., F.J. Rohlf. 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Co., New York, second edition.

Tilot, V. 1992. La structure des assemblages mégabenthiques d'une province à nodules polymétalliques de l'océan Pacifique tropical Est. Université de Bretagne Occidentale.
Todo, Y. 2003. Deep-sea benthic foraminiferal fauna that dwell at abyssal depths in the Pacific Ocean. Unpublished dissertation, Shizuoka University, Japan, 27 pp, pls 1-18. (in Japanese with English abstract)
Todo, Y., H. Kitazato, J. Hashimoto, A.J. Gooday. 2005. Simple foraminifera flourish at the ocean's deepest point. *Science* 307, 689.
van Soosten C. H. Schmidt and W. Westheide. 1998. Genetic variability and relationships among geographically widely separated populations of *Petitia amphophthalma* (Polychaeta: Syllidae). Results from RAPD-PCR investigations. *Marine Biology,* 131, 659-669.
Wiklund, H., A. Nygren, F. Pleijel and P. Sundberg. 2005. Phylogeny of Aphroditiformia (Polychaeta) based on molecular and morphological data. *Molecular Phylogenetics and Evolution* 37 (2), 494-502.
Zuhlke, R. 2001. Polychaete tubes create ephemeral community patterns: Lanice conchilega (Pallas, 1766) associations studied over six years. *Journal of Sea Research* 46, 261-272.

APPENDIX 1

Scientific Presentations and Publications Derived in Whole or in Part from the Kaplan-ISA project titled*: Biodiversity, species ranges, and gene flow in the abyssal Pacific nodule province,* and associated collaborations

Scientific Presentations

Galéron, J., Scolan, P., Fifis, A., Sibuet, M. 2006. Spatial variability of megafaunal assemblages in deep-sea polymetallic nodule fields in the NorthEast Pacific. Oral presentation. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Galéron, J., Menot, L., Sibuet, M. and the *Nodinaut* scientific team. 2006. Baseline study of benthic communities and of their habitats in deep-sea polymetallic nodule fields in the North-East Pacific : the *Nodinaut* cruise. Poster. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Glover, A.G., Paterson, G.P. Smith, C.R., Ebbe, B. The Abyssal Polychaete Inter-calibration Project (APIP): a CeDAMar project funded by the Census of Marine Life to undertake taxonomic work on abyssal polychaetes, 9th International Polychaete Conference, Portland, ME, USA, August 2007.

Howell, K.L., Billett, D.S.M., Smith, C.R., Tyler, P.A., Thiel, Hj., George, R.Y., and The Participants of the 11th International Deep-Sea Biology Symposium. 2006. Knowledge transfer and deep-ocean management: a perspective from the deep-sea research community. Oral presentation. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Lambshead, P.J.D., Lunt, D.H., Floyd, R.M., Elce, B., Smith, C.R., Rogers. A. 2006. Unexpected novel lineages of abyssal Metazoa. Oral presentation. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Mahatma, R., Martinez Arbizu, P. 2006. Meiofauna Communities of the Pacific Nodule Province. Oral presentation. 11th Deep Sea Biology symposium, Southampton (10-14 July 2006).

Meldal, B.H.M., Floyd, R., Bhadury, P., Blaxter, M.L., Smerdon, G.R., Austen, M.C., Rogers, A.D., Lambshead, P.J.D. 2005. Barcoding of marine nematodes. Meeting on Molecular Barcoding, Natural History Museum, London.

Menot, L., Galéron, J., Fifis, A., Sibuet, M. 2006. Influence of nodules on macro-infaunal communities in the abyssal Pacific. Poster. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Miljutina, M., Miljutin, D., Mahatma, R., Martinez Arbizu, P. 2006. Structure of nematode communities from a deep-sea polymetallic Nodule site in the Northeastern Pacific. Oral presentation. 11th Deep Sea Biology symposium, Southampton (10-14 July 2006).

Mincks, S.L., Smith, C.R. Morgan, C. 2006. Using biological parameters in a geological model of the Pacific manganese nodule province: Applications for biodiversity preservation. Oral presentation. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Nozawa, F., Ohkawara, N., Kitazato, H., Gooday, A.J., 2006. 'Live' benthic foraminifera from the abyssal equatorial Pacific nodule province. Poster presentation, 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Paterson, G.L., Glover, A, Menot, L., Dyal, P., Floyd, R., Rogers, A, Galéron, J, Sibuet, M., Smith C.R. 2006. Biodiversity of abyssal polychaetes. Oral presentation. 11th Deep-Sea Biology Symposium, Southampton (10-14 July 2006).

Paterson, G.L.J., Glover, A. Smith, C.R., Dyal, P., Rogers, A., Menot, L., Galéron, J. & Sibuet, M. Abyssal biodiversity: a worms eye view. 2006. Oral presentation. Scottish Association for Marine Science, Oban Scotland. September 2006. Invited seminar.

Paterson, G.L.J., Glover, A. Smith, C.R., Dyal, P.,Rogers, A., Menot, L., Galéron, J. & Sibuet, M. Abyssal biodiversity: a worms eye view. 2007. Oral presentation. Lecture course on Marine Biodiversity, University of Valparaiso, April 2007.

Schmidt, S., Menot, L., Galéron, J., Smith, C., Khripounoff, A. 2006. Bioturbation coefficients of deep-sea sediments from polymetallic nodule fields of the Clarion-Clipperton Fracture Zone. Poster. Assemblée Générale de l'European Geosciences Union (EGU), Vienne, Autriche, 2-7 April 2006.

Smith, C.R. 2006. The Ecology and Biodiversity of the Abyssal Pacific Seafloor and the Potential Impacts of Manganese Nodule Mining. Oral Presentation, Steering Committee Meeting, Census of Diversity of Abyssal Marine Life, Iraklion, Crete, October, 2006.

Tittensor, D. Schlacher, T., Smith, C.R. 2006. Endemism in undersampled environments: real or artifact? Oral Presentation, Steering Committee Meeting, Census of Diversity of Abyssal Marine Life, Iraklion, Crete, October, 2006.

Workshop Organized

Abyssal Polychaete Inter-calibration Project (APIP) Workshop
8 Jan - 11 Jan 2007
Organized by: Dr Adrian Glover, Dr Gordon Paterson (Department of Zoology, Natural History Museum, UK), Dr Brigitte Hilbig, DZMB, Senckenberg Museum, Germany, Dr Craig Smith, University of Hawaii at Manoa
http://www.cedamar.org/

Publications

Bhadury P., M.C. Austen, D.T. Bilton, P. John D. Lambshead, A.D. Rogers, G.R. Smerdon. 2005. Combined morphological and molecular analysis of individual nematodes through short-term preservation in formalin. *Molecular Ecology Notes* 5, 965-968.

Cook, A.A., P. Bhadury, N.J. Debenham, B.H.M. Meldal, M.L. Blaxter, G.R. Smerdon, M.C. Austen, P.J.D. Lambshead, A.D. Rogers. 2005. Denaturing Gradient Gel Electrophoresis (DGGE) as a tool for the identification of marine nematodes. *Mar. Ecol. Prog. Ser.* 291, 103-113.

Floyd, R.M., A.D. Rogers, P.J.D. Lambshead, and C.R. Smith. 2005. Nematode-specific PCR primers for the 18S small subunit rRNA gene. *Molecular Ecology Notes* 5, 611-612.

Glover, A.G. and C.R. Smith. 2003. The deep seafloor ecosystem: current status and prospects for change by 2025. *Environmental Conservation*, 30(3), 1-23.

Gooday, A.J., O.E. Kamenskaya, and H. Kitazato. 2007. The enigmatic deep-sea, organic-walled foraminiferal genera *Chitinosiphon*, *Nodellum* and *Resigella* (Protista): a taxonomic re-evaluation. *Systematics and Biodiversity*, in press.

Gooday, A.J., H. Nomaki, H. Kitazato. 2007. Modern deep-sea benthic foraminifera: a brief review of their biodiversity and trophic diversity. Geological Society, in press.

Hannides, A. and C.R. Smith. 2003. The northeast abyssal Pacific plain. In: *Biogeochemistry of Marine Systems,* K.B. Black and G.B Shimmield, eds., CRC Press, Boca Raton, Florida, 208-237.

Howell, K.L., D.S.M. Billett, C.R. Smith, P.A. Tyler, H. Thiel, R.Y. George, and The Participants of the 11th International Deep-Sea Biology Symposium. Knowledge transfer and deep-ocean management: a perspective from the deep-sea research community. Submitted to *Conservation Biology*.

Khripounoff, A., J.C. Caprais, O. Crassous, J. Etoubleau. 2006. Geochemical and biological recovery of the disturbed seafloor in polymetallic nodule fields of the Clipperton-Clarion Fracture Zone (CCFZ) at 5000 m depth, *Limnology and Oceanography* 51 (5).

Lambshead, P.J.D., G. Boucher. 2003. Marine nematode deep-sea biodiversity – hyperdiverse or hype? *Journal of Biogeography* 30 (4), 475-485.

Lambshead, P.J.D., C. Brown, T. Ferrero, L.E. Hawkins, C.R. Smith and N.J. Mitchell. 2003. Biodiversity of nematode assemblages from the region of the Clarion-Clipperton Fracture Zone, an area of commercial mining interest. *BMC Ecology* 3,1.

Mincks, S.L., P.L. Dyall, G. Paterson, C.R. Smith, A.G. Glover. (submitted) A new species of *Aurospio* (Annelida, Spionidae) from Antarctica, with an analysis of its ecology, reproductive biology and evolutionary history. *Marine Ecology*.

Nozawa, F., H. Kitazato, M. Tsuchiya, and A.J. Gooday. 2006. 'Live' benthic foraminifera at an abyssal site in the equatorial Pacific nodule province: abundance, diversity and taxonomic composition. *Deep-Sea Research I.* 53 (8) 1406-1422.

Ohkawara, N., H. Kitazato, A.J. Gooday. in prep. A tiny new psammosphaerid foraminifer from the abyssal Equatorial Pacific.

Rogers, A.D., P.J.D. Lambshead. 2004. Molecular studies of nematode diversity; past, present and future. In: Cook, R. & Hunt, D.J. (Eds). Proceedings of the Fourth International Congress of Nematology, 8 - 13 June 2002, Tenerife, Spain. Nematology Monographs and Perspectives Volume 2. Brill Academic Publishing.

Smith, C.R., F. De Leo, A. Bernardino, A. Sweetman. P. Martinez Arbizu. (In review) Abyssal biodiversity, ecosystem function and climate change. *Trends in ecology and evolution.*

Smith, C.R., A.W.J. Demopoulos. 2003. Ecology of the deep Pacific Ocean floor. In: *Ecosystems of the World Volume 28: Ecosystems of the Deep Ocean*, P.A. Tyler, ed., Elsevier, Amsterdam, pp. 179 – 218.

Smith, C.R., J. Drazen, S.L. Mincks. 2007. Deep-sea biodiversity and biogeography: perspectives from the abyss. *International Seabed Authority Seamounts Workshop Report*, in press.

Smith, C.R., L.A. Levin, A. Koslow, P.A. Tyler and A.G. Glover. 2007. The near future of deep seafloor ecosystems. In: *Aquatic Ecosystems: trends and global prospects,* N. Polunin, ed., Cambridge University Press, in press.

Veillette, J., J. Sarrazin, A.J. Gooday, J. Galéron, J.C. Caprais, A. Vangriesheim, J. Etoubleau. K. Juniper. Ferromanganese nodule fauna in the equatorial North Pacific Ocean: species richness, faunal cover and spatial distribution. Submitted to *Deep Sea Research.*

Veillette, J., S.K. Juniper, J. Sarrazin and A.J. Gooday Influence of surface texture and microhabitat heterogeneity in structuring nodule faunal communities. Submitted to *Deep Sea Research.*

APPENDIX 2

Molecular Protocols used for Polychaetes

1. DNA extracted using Qiagen DNeasy kit

Reagent	Volume	Final Concentration
Sigma water	24.5 µl	
10x Qiagen Buffer	5.0 µl	1x
2 mM dNTPs	5.0 µl	200 µM of each dNTP
Qiagen Q solution (5x)	10.0 µl	1x
25 mM Magnesium	3.0 µl	3.0 mM
10 µM primer	0.5 µl	0.1 µM
10 µM primer	0.5 µl	0.1 µM
Qiagen Taq (5 U/µl)	0.5 µl	2.5 U
DNA	1.0 µl	15-80 ng

2. PCR profile for both 18S and 16S genes:
Annealing Temperature: 18S - 50˚C
16S - 48˚C

3. 18S gene:

1. Primary PCR with 18SE-f/18P-r; used 1ul of template DNA
2. Clean up primary PCR with Micrcon 100 filter units
3. Set up heminested PCR using 1ul of the cleaned primary PCR as template

Primer combinations:
278 - 18E/18Q-r; 18N-f/18P-r
288 - 18E/18Q-r; 18N-f/18P-r
342- 18E/1324r; 18O-f/18P-r

4. 16S gene

1. 1ul of template DNA for PCR
2. Primers 16SAR/16SAB

Routine PCR amplification was tried using the Folmer et al. (1994) universal primers for the mitochondrial gene CO1 and universal primers for cytB and 16S genes, with annealing at 48°C. Small subunit ribosomal RNA genes were amplified using a two-step heminested approach. Primary ampilfications of full length SSU rRNA genes used published PCR conditions (50°C annealing) and the primers 18SA/1SB primers (Wiklund et al., 2005) or 18E/18P (Halanych et al., 1998). The primary amplifications were spin dialysed to remove the first set of primers using Microcon-100 concentrators (Amicon) against sterile water (450ul). One microliter of the retentate was used for each of two (per sample) secondary nested amplifications containing one of the following primer pairs: 18SA/1,324R; 620F/18SB; 18E/1,324R; 620F/18P.

The low volume of template DNA recovered from the abyssal samples led to the use of a heminested approach for studies of the 18S gene. This increases the ability to recover DNA from degraded specimens by using two PCR reactions and 2 overlapping primer pairs to sequence the whole SSU gene.

Polychaete abundance, biodiversity and species ranges have been analysed from the main database using standard statistical packages. For DNA sequences, alignments were carried out using ClustalX and trees constructed using Clustal (neighbour-joining) and MrBayes (Bayesian inference).