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Recruitment of Antarctic marine eukaryotes onto artificial surfaces

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Abstract Activities related to Antarctic research stations have caused significant local impacts on the marine environment, potentially affecting the recruitment of benthic invertebrates. Herein, we report the community structure of recruiting marine eukaryotes onto artificial substrata using molecular techniques. Slides were deployed at three sites adjacent to McMurdo Station, Scott Base, and Cape Armitage in McMurdo Sound. Denaturing gradient gel electrophoresis (DGGE) analysis revealed complex and diverse eukaryotic communities had established on artificial surfaces deployed at a range of site and depth regimes after 12 months. Analysis of similarity results detected significantly greater variability in community profiles among sites than within sites. The nonmetric multidimensional scaling plot constructed from DGGE banding patterns revealed different benthic communities had established at 12 and 18 m depths. Despite this, the variation in community composition was greater among sites than between depths, especially at Cape Armitage and Scott Base. Sequence analysis of excised DGGE bands revealed a predominance of arthropod and dinoflagellate sequences at Cape Armitage. In contrast, a wide diversity of phyla including cnidaria, bryozoa, protozoa, dinoflagellates, arthropods, platyhelminths, and annelids were present adjacent to the two research stations. The abundance of diatoms detected in Cape Armitage benthic assemblages exceeded the abundance of diatoms from McMurdo Station and Scott Base by almost two orders of magnitude. The discovery that distinct eukaryotic communities recruit at different sites and depths is probably due to complex interactions between multiple factors including water quality, larval supply, and light. The detection of sessile phyla on slides at each of the sites indicates that the pollution profiles present at each site is not an impediment to successful recruitment of these species.

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Introduction

The Antarctic marine environment remains relatively unexplored, and increasing human pressure in this region necessitates a clearer understanding of community dynamics in this sensitive ecosystem. The marine environment of Ross Island, McMurdo Sound, supports a rich and diverse benthic community and the overall structure is similar to sponge-characterised temperate reefs, but comprised of unique species assemblages (Dayton et al. 1974; Battershill 1989). The sessile macrofaunal benthos is populated by sponges, alcyonarians, actinarians, stoloniferans, hydrozoans, ascideans, and bryozoans, whereas the motile benthos is characterised by fish, echinoids, asteroids, nemerteans, isopods, pycnogonids, and molluscs (Dayton et al.

1970). The eastern side of McMurdo Sound is characterised by oscillating north–south currents and productive plankton blooms supporting an extremely dense infaunal assemblage (Dayton and Oliver 1977a, b). It is clear that the marine environment of McMurdo Sound is stable over time, especially in deeper strata. The potential for disturbance in Antarctic marine assemblages may therefore be particularly acute due to normally low levels of environmental stressors and extremely slow growth rates, particularly during crucial developmental stages (Battershill 1989; King and Riddle 2001).

The benthic communities of McMurdo Sound have been impacted by anthropogenic input from McMurdo Station (Lenihan et al. 1990; Lenihan and Oliver 1995). Historical pollution of Winter Quarters Bay in areas adjacent to the former US dump site and the ice dock used by supply ships includes a large quantity of debris and contamination of marine sediments with trace metals (Lenihan et al. 1990), hydrocarbons (Risebrough et al. 1990; Kennicutt et al. 1995; Crockett and White 2003), and butyltins (Negri et al. 2004). In addition, McMurdo Station discharges ~270,000-l sewage/day during summer, which has resulted in a large accumulation of particulate matter less than 100 m from the coast (Conlan et al. 2004). The extent of marine contamination at nearby Scott Base (NZ, summer population ~50, Fig. 1) is not well described. Sewage output at Scott Base reaches ~17,000 l/day during summer (Harris et al. 2001) and localised elevation of trace metals has been described near the sewage outfall (Anderson and Chague-Goff 1996). Cape Armitage is between McMurdo Station and Scott Base (Fig. 1) and supports a richly diverse benthic community (Dayton et al. 1970; Battershill 1989). Cape Armitage has low levels of impact compared with McMurdo Station (Risebrough et al. 1990; Lenihan 1992), however high but patchy concentrations of butyltins were recently described at this site (Negri et al. 2004).

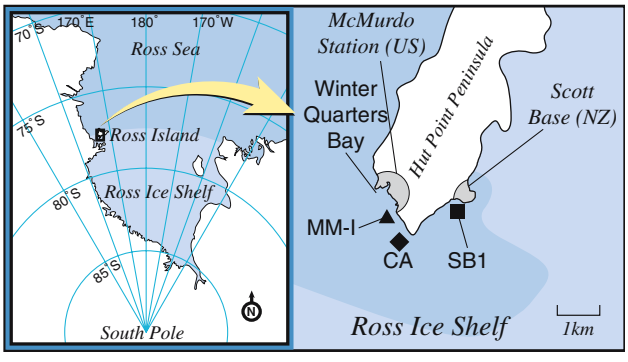

Fig. 1 Location of Ross Island, Antarctica, and individual sampling sites for recruitment analyses

Previous research has demonstrated that the high level of pollution in Winter Quarters Bay caused a reduced abundance of benthic macro-organisms and a shift in community composition to a profile dominated by motile species, in particular, highly opportunistic polychaete worms (Lenihan et al. 1990). Community recovery from high levels of chemical contamination in this region is likely to be slow, especially considering the slow rate of degradation of most chemicals in the Antarctic environment. The ability of organisms to find suitable settlement substrata is a key component in the recovery process and any modifications that affect chemical or tactile cues will influence recovery rates.

In this study, we deployed glass slides as standard settlement surfaces in nearshore benthic habitats of McMurdo Sound, Antarctica, to assess the complexity of eukaryotic communities that developed after 12 months. Molecular techniques were used to describe differences in the associated invertebrate populations that had recruited onto the slides at two depths across a pollution gradient.

Materials and methods

Community establishment

Glass microscope slides were sterilised and placed in square PVC frames (56 per grid) allowing the top surface of the slides to be exposed to the seawater. Duplicate frames were deployed at 12 and 18 m at three sites in McMurdo Sound, Antarctica (Fig. 1, Table 1). The 12-m depth represents a zone that is periodically scoured by ice and is dominated by motile invertebrates. The 18-m depth represents a stable benthic habitat rarely scoured and characterised by sponges. Site 1 (MM-I) is located approx. 100 m from the sewerage outfall at McMurdo Station. Site 2 (SB-1) is located approx. 50 m from the sewerage outfall at Scott Base. Site 3 (CA) is located between MM-I and SB-1 and supports high benthic biodiversity (Battershill 1990). Slides were deployed in October 2001 and retrieved in October 2002. Although light measurements were not taken over the course of the year, the fast ice did not break out and this, combined with continual snow cover throughout the summer of 2001/2002, is likely to have minimised light penetration to the sites. The slide frames were free of anchor ice when assessed in February and October 2002. The frames were collected and transported to the Scott Base

Table 1 Site descriptions

Site	Latitude/longitude	Site description	Mean sediment contamination (mg kg^{-1})	
CA	77°51.55′/ 166°40.74′	Cape Armitage: medium currents, gentle slope, sponge spicule mat, small rocks, sand and mud; no visible anthropogenic debris; high species diversity and a stable reef community; characterised by sponges, crustaceans, soft corals, echinoderms, and dense beds of the bivalve *Laternula elliptica*	THC	4.2
			PAH	0.077
			PCB	< 0.005
			Butyltins	1340
			Cd	0.15
			Pb	3.7
			As	1.9
SB-1	77°51.05′/ 166°45.54′	Pram Point Scott Base: within 200 m of Scott Base sewage outfall; high currents, steep scree slope, rocks, and mud; some anthropogenic debris evident; medium benthic diversity dominated by echinoderms, crustacea, coelenterates, sponges, and polychaete worms; shallow depths often affected by ice-scouring	THC	12
			PAH	0.18
			PCB	0.020
			Butyltins	28
			Cd	0.05
			Pb	5.4
			As	1.1
MM-I	77°51.02′/ 166°39.56′	McMurdo Station RO intake jetty: within 100 m of sewage outfall and 500 m of shipping berth; low current, steep slope, rocks, and mud; anthropogenic debris evident; medium benthic diversity, dominated by polychaete worms and crustaceans with opportunistic life histories; bivalves, sponges, cnidarians, bryozoans, and other benthic species also present	THC	40
			PAH	0.28
			PCB	0.031
			Butyltins	149
			Cd	0.23
			Pb	9.1
			As	4.5

Sediments were sampled from within 15 m of each of the sites and analysed for TOC, PCBs, PAHs (Negri et al. 2006), and butyltins (Negri et al. 2004)

laboratory in seawater (1°C) within 30 min. To determine community composition of recruiting eukaryotes, 30 slides from each frame were randomly selected and frozen at –80°C for DNA extraction and 26 slides were fixed in 4% paraformaldehyde for fluorescence microscopy.

Community analysis

DNA extraction

DNA was extracted from individual slides by scraping the biofilms into Eppendorf tubes using sterile scalpels and 0.5 ml of grinding buffer per sample (2 ml of 1 M Tris, 4 ml of 0.5 M EDTA, 2 ml of 10% SDS, 400 µl of 5 M NaCl, and 11.6 ml of distilled water). Tubes were repeatedly immersed in liquid nitrogen and ground with plastic pestles. Samples were then incubated at 65°C for 60 min prior to addition of 187 µl of 5 M potassium acetate. Samples were incubated on ice for 30 min and centrifuged at 8,000 rpm for 15 min. The supernatants were transferred to fresh tubes and DNA was precipitated with 0.8 vol of isopropanol. DNA was further purified by electrophoresis in a 1.2% (w/v) low melting point agarose gel. DNA fragments larger than 2 kb were excised and recovered from the agarose using a gel extraction kit (Qiagen).

Diatom cloning and restriction fragment length polymorphism analysis

For analysis of the diatom community, the universal 16S rRNA gene primer (359f: 5′-GGGGAATYTTCCGCAATGGG-3′) and equimolar amounts of the cyanobacteria/diatom-specific primers (781r (a): 5′-GACTACTGGGGTATCTAATCCCATT-3′ and 781 r (b): 5′-GACTACAGGGGTATCTAATCCCTTT-3′) were used (Nübel et al. 1997). Our results show that these ‘cyanobacteria/diatom-specific’ primers are not highly specific and actually co-amplify some bacterial strains. PCR was performed on DNA extracted from randomly selected triplicate slides per site. PCR products were visualised on a 1% agarose gel, bands were excised, replicate samples for each site were combined, and gel purified using Qiagen Minelute kit. Purified PCR products were then cloned with a TOPO TA cloning kit according to the manufacturer’s instructions (Invitrogen). Plasmids were checked for inserts by PCR. A negative control cloning reaction was performed in which no DNA was added.

The DNA inserts from 120 clones per site library were PCR-amplified, and the products digested with the restriction enzymes *Hae*III and *Hha*I (Invitrogen) for 3 h at 37°C and electrophoresed in a 3% ultra-high-resolution agarose gel (Progen) at 60 V for 4 h.

Ethidium-bromide-stained gels were visualised using a Fluorimager (Biorad). Clones were analysed using Fragment NT analysis application Version 1.1a and sorted into operational taxonomic units according to restriction patterns. A 100-bp DNA ladder (New England Biolabs) was used as a molecular weight marker and fragments smaller than 100 bp were not included in the analysis. Operational taxonomic units were de-replicated and representative clone inserts from each of the eight unique operational taxonomic units were sequenced using M13 vector primers and the PRISM Ready Reaction Kit (PE Applied Biosystems) and ABI 310 and 373 automated sequencers.

Denaturing gradient gel electrophoresis

The 18S rRNA gene from DNA extracted from eight new replicate slides per depth at each site were amplified by PCR with eukaryotic primers: NS1f: 5′-GTAGTCATATGCTTGTCTC-3′ and NS2r: 5′-GGCTGCTGGCACCAGACTTGC-3′ (White et al. 1990). The reverse primer was modified to incorporate a 40-bp GC clamp (Muyzer et al. 1993). Denaturing gradient gel electrophoresis (DGGE) gel conditions were previously optimised for this primer set (Webster et al. 2004a). Three consecutive sets of PCR reactions were performed as described by Ferris et al. (1996) under identical thermal cycling conditions and using a master mix of PCR reagents to avoid any PCR variability between samples. Products from the triplicate PCR reactions per replicate were combined and 15 μl applied to duplicate 40% (w/v) polyacrylamide (37:5:1) gels containing a 35–70% denaturing gradient of formamide and urea. Gels were electrophoresed at 60°C for 17 h in 1× TAE buffer at 50 V using the Ingeny D-Code system. Gels were stained with 1× Sybr Gold for 30 min, visualised under UV illumination, and photographed. To avoid inter-gel variability in band migration, all samples were run on duplicate gels and only bands which migrated to the same endpoint (as determined by distance from marker bands) in duplicate gels were excised and sequenced. Individual bands were excised using a 10-μl pipette tip, the DNA eluted in 20-μl Milli-Q purified H_2O, and 5-μl of this template was re-amplified by PCR. PCR products were sequenced using the forward primer and the PRISM Ready Reaction Kit (PE Applied Biosystems) and ABI 310 and 373 automated sequencers.

Phylogenetics

All sequences were compared to available databases using the Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1997). Chimeric sequences were identified using the program CHECK_CHIMERA (Maidak et al. 1999). This facilitated determination of approximate phylogenetic affiliations (phyla level) for sequences retrieved from DGGE analysis. For clone library sequences, evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated as described by Jukes and Cantor (1969). Partial sequences were compiled and manually aligned in ARB software package (http://www.arb-home.de; Ludwig et al. 1998). Initially, trees were calculated with full 16S rRNA gene sequences for all close relatives of target sequences using the neighbour-joining, Fitch–Margoliash, and maximum parsimony methods in ARB. Partial target sequences were subsequently imported to the tree without changing branch topology using the ARB parsimony-interactive method. The robustness of inferred tree topologies was evaluated after 1,000 bootstrap re-samplings of the neighbour-joining data in the Phylip, Seqboot program.

Data analyses

Multivariate relationships between the presence and absence of bands on DGGE gels were assessed to compare the similarity of benthic assemblages between sites using Kruskal's nonmetric multidimensional scaling (nMDS) (Cox and Cox 1994). A binary matrix was constructed and converted to a distance matrix using the R statistical package (http://www.R-project.org). These data were analysed using non-metric nMDS and analysis of similarities (ANOSIM) (Clarke 1993). An optimal nMDS solution was obtained by minimising stress values. The relationships among samples are represented in a plot of the first two dimensions of the nMDS solution.

Results

Eukaryotic community analysis by DGGE

DGGE analysis of slides deployed in Antarctic waters revealed complex and diverse eukaryotic communities inhabited the surfaces at each of the three sites (a total of 49 distinct bands were observed and used for nMDS analysis; Fig. 2). ANOSIM results detected significantly greater variability in community profiles among sites than within ($p < 0.001$). The nMDS plot constructed from DGGE banding patterns revealed little variability between replicate slides for McMurdo Station 12-m depth and higher variability among replicate assemblages that had established at other sites and

depths (Fig. 3). Community composition always overlapped to a small extent across each depth within a site. The assemblage profile observed at 18 m at McMurdo Station had commonality with all other sites.

Eukaryotic community composition by 18S rDNA sequence analysis

18S rDNA sequence information was obtained for representatives of each DGGE band at each site and depth. This data was interrogated to phyla level, and a comparison between site and depth revealed distinct differences (Fig. 4). The lowest representation at phyla level was evident at Cape Armitage, which was dominated by arthropods and dinoflagellates. On the basis of the NCBI Blast results, 100, 88, and 80% of the arthropod sequences corresponded to copepod species at Cape Armitage, McMurdo Station, and Scott Base, respectively (data not shown), with at least ten distinct copepod species represented in total. The only variability observed between depths at Cape Armitage was the presence of bryozoa in 12-m assemblages and protozoa on 18-m slides. Arthropods comprised a much lower proportion of the sequenced bands from both McMurdo Station and Scott Base communities. Slides from Scott Base contained the highest number of different phyla in the 18-m assemblages, which had representatives of the arthropoda, protozoa, dinophyta, bryozoa, platyhelminthes, porifera, nematoda, and nemertea. The Scott Base 12-m assemblage differed from that at 18 m by the absence of protozoa, dinophyta, and platyhelminthes and by the presence of annelids and cnidaria. McMurdo Station communities contained a relatively large proportion of protozoa and platyhelminth worms compared with the other sites and had a similar community composition at both depths. The McMurdo Station 12-m communities differed from those at 18 m by the presence of bryozoa and annelids and by the absence of cnidaria.

Diatom density

A large variability in the total abundance of diatoms was evident in assemblages from the three Antarctic sites (Table 2). At both McMurdo Station and Scott Base, less than two diatoms were observed by fluorescence microscopy per 100-μm^2 slide area. In contrast, at Cape Armitage an average of 44–99 diatoms were observed per 100 μm^2 on 12 and 18 m surfaces, respectively.

Diatom phylogeny

Sequencing of clones generated using the primer 781r revealed low diversity (Fig. 5). Clone 1 was dominant in libraries from all the three sites and had highest phylogenetic homology to an uncultured cyanobacterium retrieved during a study into the diversity of prokaryotes in salt marshes. Clone 2 was present in libraries from Cape Armitage and Scott Base and also

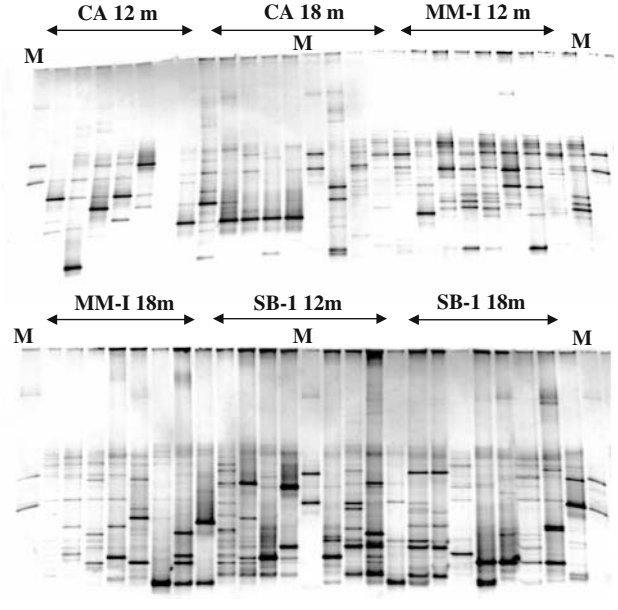

Fig. 2 *DGGE* profiles of 18S rDNA-defined eukaryote populations from replicate samples ($n = 8$) at *CA* (Cape Armitage), *MM-I* (McMurdo), and *SB-1* (Scott Base) at 12- and 18-m depths

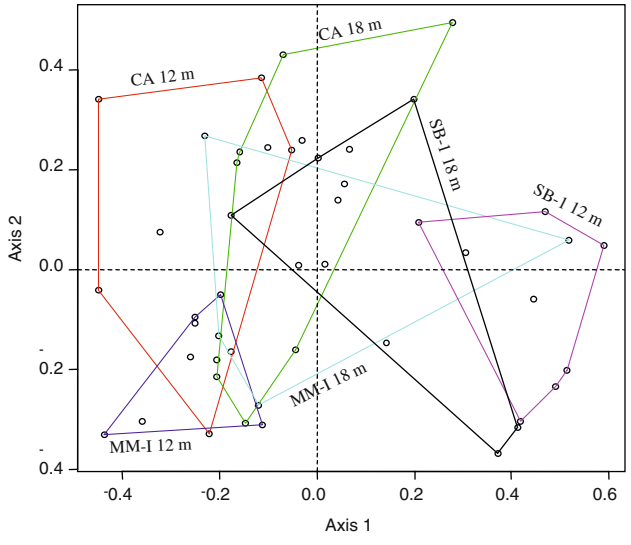

Fig. 3 *nMDS* plot representation of the *DGGE* banding profiles from *CA* (Cape Armitage), *MM-I* (McMurdo Station), and *SB-1* (Scott Base) from 12 and 18-m depths. All replicates from each site are within the boundaries illustrated. Data obtained by distance matrix analyses of *DGGE* fingerprints

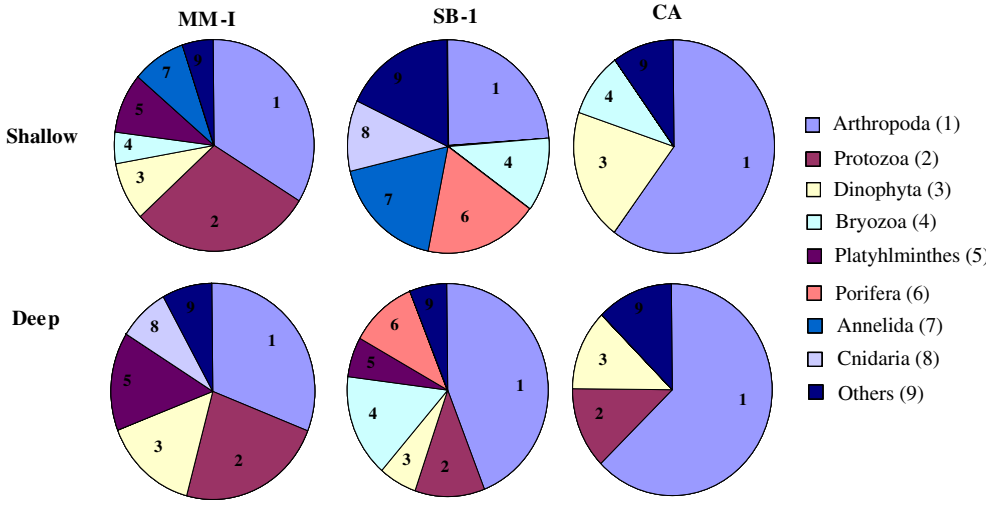

Fig. 4 Pie charts showing the relative proportion of different eukaryotic phyla as determined by sequencing of excised *DGGE* bands. Pie charts were constructed for slides from *CA* (Cape Armitage), *MM-I* (McMurdo Station), and *SB-1* (Scott Base) at 12- and 18-m depths

had the same uncultured cyanobacterium as its closest known relative. Clone 3 was present in libraries from Cape Armitage and Scott Base and had highest homology to an uncultured phototroph from the Arabian Sea. Clones 5–8 had highest homology to each other and were most closely affiliated with bacteria from hot springs within Yellowstone National Park (AF445743) and a uranium-contaminated aquifer (AY532580). On the basis of size and morphology under fluorescence microscopy, at least six different diatom morphotypes (A–F) were observed in Cape Armitage assemblages (Fig. 6).

Discussion

Variability in the community composition of marine recruits was detected across all the three sites and at two depths. The lowest number of phyla was evident at Cape Armitage and the highest number of phyla was detected at the sites adjacent to the research stations (McMurdo Station and Scott Base). There was no difference in the number of phyla represented at each depth within a site; however, some differences in the composition of phyla were evident. nMDS results revealed Cape Armitage assemblages had the highest similarity in community profiles between depths and McMurdo Station 12-m assemblages had the lowest variability between replicates. The greatest similarity between assemblages occurred among the 18-m slides at all sites. This suggests that environmental conditions at deeper sites may be less impacted and/or more stable.

Primary biofilms are comprised of adsorbed macromolecules and attached bacteria enmeshed in a matrix of extracellular polymers (Mihm et al. 1981) followed by the appearance of eukaryotic phototrophs such as diatoms, dinoflagellates, cyanobacteria, and filamentous algae (Watnick and Kolter 2000). Despite lengthy periods of darkness under the sea ice, diatoms were detected in biofilms from all sites and depths, whereas dinoflagellates were detected at all sites and depths except the shallow Scott Base site. These results indicate that mature biofilms had established on the slides over the 12-month deployment which may have facilitated the subsequent recruitment of invertebrates (Leitz and Wagner 1993; Wieczorek and Todd 1998; Webster et al. 2004b), and/or attracted motile grazers.

A high abundance of diatoms was detected at Cape Armitage compared with McMurdo Station and Scott Base (Table 2). Although the light levels at all sites were low throughout the slide deployment, it is likely that small variations in illumination between sites may have occurred, potentially leading to differences in diatom abundances between sites. Previous work has shown that changes in irradiance after sea ice breakout can significantly influence the density of polar algae (Mcminn et al. 2004) and other research suggested that a mixture of photosynthetic response, trace metals, vertical mixing, and other factors affected the spatial distribution of diatoms in the Ross Sea (Van Hilst and Smith 2002). The elevated diatom numbers at Cape Armitage may have out-competed other recruits for

Table 2 Density of diatoms as observed by fluorescence microscopy

	Average diatoms (per 100 μm^2)		
	MM-I	CA	SB-I
Shallow (12 m)	1.8 ± 0.4	44 ± 1.5	0.02 ± 0.00
Deep (18 m)	0.6 ± 0.3	99 ± 2.9	0.12 ± 0.00

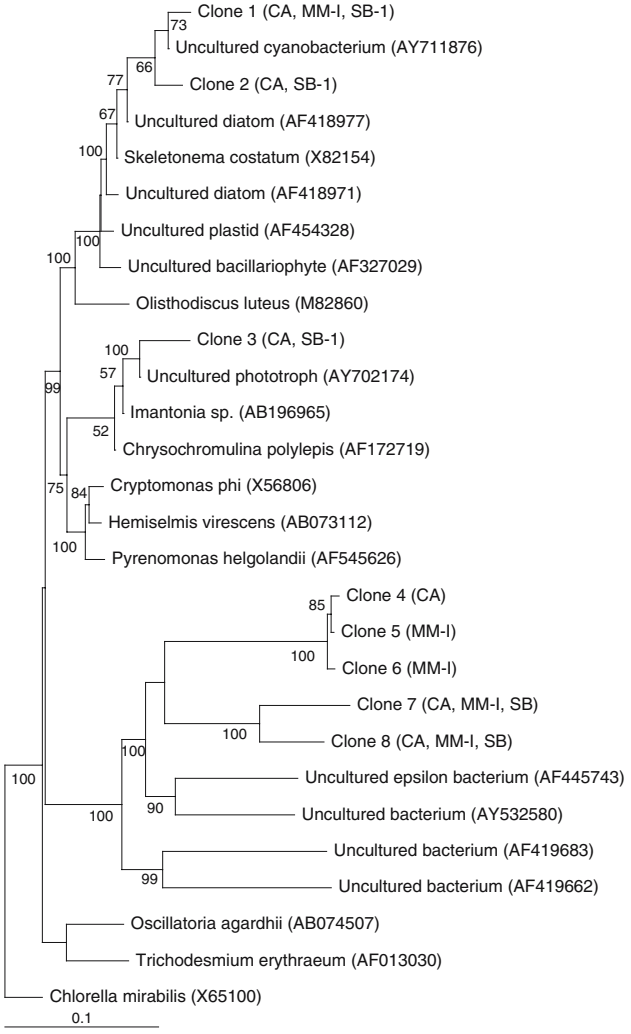

Fig. 5 Neighbour-joining phylogenetic tree from analysis of 16S rDNA gene sequence from eukaryotic clones 1–8, generated with diatom-specific primers. The numbers at the nodes are percentages indicating the levels of bootstrap support, based on a neighbour-joining analysis of 1,000 re-sampled data sets. Only values > 50% are shown. Scale bar represents 0.1 substitutions per nucleotide position

available space, explaining the reduced diversity at this site. The higher diatom densities may also explain the high proportion of grazing arthropods which dominated Cape Armitage slides (approx. 60% of sequences at both depths). A study in the South Shetland Islands showed considerable temporal fluctuations in the abundance and species composition of phytoplankton and suggested that the variability was attributable to grazing by salps, copepods, amphipods, and euphasids (Villafañe et al. 1995). Visual examination of slides suggests that further molecular and morphological analyses may have revealed additional diatom diversity. This study utilised 'diatom-specific' primers described by Nübel et al. (1997) and examined 360 clones by restriction digest analysis. However, it appears that since the publication of these diatom-specific primers, additional bacterial diversity has been discovered which co-amplifies with these primers. Hence, many of the DNA templates amplified in this study may have been of bacterial origin.

The community composition of shallow-water benthos in McMurdo Sound has been previously investigated using traditional techniques and macrobenthic communities assigned to three distinct zones (Dayton et al. 1970). Physical stressors (such as ice-scouring and anchor-ice lift up) were found to be primarily responsible for the vertical zonation of dominant sessile organisms with biological interactions also exhibiting varying degrees of significance. The 0–15-m zone was characterised by organic barrenness with few motile foraging species, the 15–30-m zone was comprised almost exclusively of coelenterates and clumps of sponges in the lower regions (except at Cape Armitage where currents reduced anchor ice formation and sponge species occurred around 20-m depth), and the 33-m and below zone which was characterised by a thick spicule mat supporting many sponge species, actinarians, hydroids, polychaetes, bryozoans, and a large density of molluscs (Dayton et al. 1970). This study, however, found that active recruitment of sedentary phyla such as bryozoa were common on shallow water slides from all sites, sponge recruits were detected on shallow and deep surfaces at Scott Base, and soft corals were

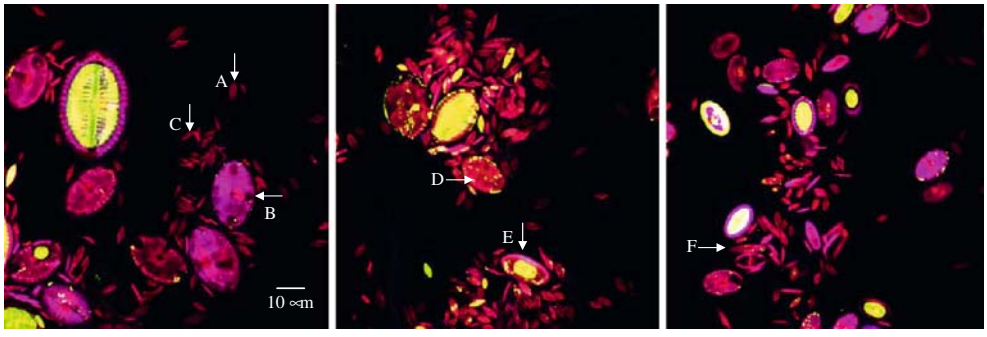

Fig. 6 Fluorescence micrographs of the density and diversity (cell types A–F) of diatom cells detected on slides from Cape Armitage (*CA*). Scale bar = 10 μm

detected on shallow slides at Scott Base and deep slides at Cape Armitage. Of the motile invertebrate phyla, only arthropods were detected at both depths at all three sites. Annelids were present on shallow slides from McMurdo Station and Scott Base but absent from Cape Armitage, platyhelminthes were present at both depths from McMurdo Station and Scott Base but absent from Cape Armitage, and protozoa were present on slides from all deep sites and on the shallow slides from McMurdo Station.

Dayton and co-workers examined distribution and abundance of macroorganisms in situ, whereas this study utilised molecular techniques to examine microscopic recruitment. Discrepancies between the two studies possibly reflect the different modes of analysis as both strategies are subject to biases. For example, visual census can overlook microscopic recruits or they may be very difficult to characterise using traditional taxonomic methods. On the other hand, the molecular techniques may be biased due to differences in DNA extraction efficiency, PCR amplification, and separation of bands on acrylamide gels (Suzuki and Giovannoni 1996; Ferrari and Hollibaugh 1999; Powell et al. 2003, 2005) and this may be why DGGE analysis overlooked some eukaryotic groups (such as diatoms). Several studies have highlighted the potential limitations of this technique (Jackson and Churchill 1999; Fromin et al. 2002; Powell et al. 2003; Gillan 2004). Despite these limitations, sequencing of excised DGGE bands provides considerable phylogenetic resolution of community structure and facilitates detection of specific shifts in community composition between samples. The submission of new sequences to public databases also provides other researchers with rapid and reliable data on species distribution globally. Future research effort should include a detailed comparison of traditional taxonomic and molecular techniques for describing benthic recruitment and also aim to determine the survival rates of recruited organisms at different sites and depths.

The contamination levels in sediments adjacent to the slide deployments were measured as part of previous studies (Negri et al. 2004, 2006) and can be used to provide some indication of pollution pressures in the vicinity of each site (see summary in Table 1). Although none of the sites exhibited very high contamination levels that could be detected within 50 m of the McMurdo Station outfall and historic dump (Lenihan 1992; Conlan et al. 2004; Negri et al. 2006), the sites represent a broader impact footprint of each station and a site impacted by shipping. McMurdo Station sediments were higher in THCs, PAHs, PCBs, and the trace metals Cd, Pb, and As than the other two sites and contained appreciable concentrations of butyltins. Scott Base sediments exhibited moderate levels of THCs, PAHs, PCBs, and Pb and the lowest concentrations of butyltins, Cd, and As of all the three sites. Cape Armitage sediments were the least contaminated apart from very high levels of butyltins from the ablation of antifouling paint of ships (Negri et al. 2004). The variability in contaminants across these sites may be directly affecting ecosystem structure and function, but the complexity of contamination profiles at each site and the uncertain relationship between sediment contamination and water quality precludes meaningful conclusions on the influence of pollution on recruitment. For instance, fluorescence microscopy revealed that benthic communities at Cape Armitage harboured an abundance of diatoms over an order of magnitude higher than what was observed at the other sites. Although Cape Armitage exhibited low levels of organic and trace metal contamination, its butyltin concentrations are particularly high (Negri et al. 2004). Other likely variables that may have influenced the high abundance of diatoms at Cape Armitage include subtle differences in light availability and differences in currents and larval supply. To examine the influence of specific pollutants on recruitment, future studies should include experimental manipulations that involve transplantation of surfaces preconditioned along a steeper and more consistent pollution gradient. Such a study would also need to separate the effects of primary biofilm development from pollution on the abundance of diatoms and settlement of eukaryotes.

Studies which examined whether contamination of marine sediment with metals, hydrocarbons, and organic carbon could lead to changes in recruitment of Antarctic soft-sediment assemblages found significant differences in recruitment between control and contaminated locations (Stark et al. 2003a, b, 2004; Cunningham et al. 2005). The composition of benthic diatom communities in Brown Bay, adjacent to the tip in the Thala Valley, correlated with concentrations of tin, copper, lead, and iron (Cunningham et al. 2005). The assemblage structure in other organisms separated according to concentrations of the metals Cd, Cu, Pb, Sn, and Zn (Stark et al. 2003a). In addition, impacted locations had initially greater recruitment than control sites and were dominated by highly motile crustaceans and polychaetes (Stark et al. 2004). Surveys of Winter Quarters Bay, McMurdo Sound, indicated that the abundance and diversity of macro-infauna decreased along an increasing pollution gradient (Lenihan and Oliver 1995). Opportunistic polychaetes dominated the most highly impacted sites with increased abundances of both highly motile crustaceans and polychaetes at

semi-impacted sites. In contrast, Pearson and Rosenberg (1978) detected an increase in abundance of macro-infauna along an increasing pollution gradient largely due to an increase in the presence of opportunistic species. In this study, motile annelid and platyhelminthes sequences were only detected at McMurdo Station and Scott Base sites and were not observed at Cape Armitage, which is at least 1 km from either of the bases. However, the largest number of arthropod sequences was detected at Cape Armitage possibly due to elevated diatom abundances. It is also possible that elevated concentrations of TBT at Cape Armitage (Negri et al. 2004) may have reduced recruitment of sessile invertebrates at this site. Tributyltin is a potent toxicant, capable of affecting a wide diversity of marine invertebrates (Fent 1996). The phyla level response to anthropogenic input can be highly variable and a study by Lenihan et al. (2003) showed an increased abundance of annelids in response to organic pollution and an increased abundance of echinoderms and crustacea in response to trace metal contamination.

The use of sterile artificial substrata eliminated some confounding site-specific variables such as sediment grain size and differences in existing microbial communities. However, potential site-specific variability in environmental factors such as light, currents, larval supply, and variability in successional rates may still have contributed to observed patterns of community composition. Also, the artificial surfaces may have underestimated the level of contamination faced by recruiting organisms as the surfaces were raised (~1 cm) above, and isolated to a degree from the surrounding sediments. This may have facilitated settlement in an environment that is not normally optimal for survival. The effect of glass chemistry and topology on the formation of microbial biofilms and subsequent eukaryotic recruitment is also an unknown factor. Detection of the sessile phyla bryozoa and cnidaria on slides at McMurdo Station and Scott Base and the high percentage of porifera sequences detected at Scott Base confirm that if suitable uncontaminated substrates are available, these organisms can recruit into environments with medium levels of historical pollution impact. Unfortunately, the use of DGGE precludes analysis of the relative biomass of each taxa among sites and depths. This is the first analysis of eukaryotic recruitment using molecular techniques and provides a comprehensive investigation of the microscopic eukaryotic components, potentially overlooked in visual surveys. The consistently distinct recruitment patterns between sites and depths indicate that molecular techniques can complement visual survey techniques as part of routine environmental monitoring.

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