

# Influence of an Antarctic waste dump on recruitment to nearshore marine soft-sediment assemblages

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**ABSTRACT:** Abandoned waste dumps in Antarctica pose an environmental hazard due to contaminant mobilisation in marine and terrestrial habitats. At Casey Station, east Antarctica, a shoreline waste dump has contaminated adjacent marine sediments with metals, hydrocarbons and organic carbon. This study experimentally assessed a model whereby contamination of marine sediment can lead to changes in recruitment and differences in soft-sediment assemblages. We tested the hypotheses that recruitment would be different at disturbed 'station' locations compared to controls and different in contaminated sediment compared to control sediment. We conducted 2 reciprocal sediment-transplant field experiments over 2 consecutive years in which defaunated sediments were deployed at disturbed locations and control locations and were recovered after 9 mo (March to November: winter), and also after 12 mo. The majority of fauna recruiting to the experiment were highly motile species with non-pelagic lecithotrophic larvae, such as gammarids, tanaids, isopods and gastropods. There were large differences in recruiting assemblages between all locations and there were significant differences in recruitment between disturbed and control locations. Assemblages in contaminated sediment were significantly different from those in control sediment. Differences in abundances of individual taxa between control and contaminated sediment were complex and difficult to interpret. Assemblages recruiting to the control locations were more variable than those recruiting to disturbed locations. This study provides evidence that contaminants in marine sediments adjacent to the waste dump at Casey Station may be having an environmental impact.

**KEY WORDS:** Environmental impact · Waste dump · Pollution · Soft-sediment macrofauna · Recruitment · Antarctica

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## INTRODUCTION

The problem of waste disposal at Antarctic research stations was historically dealt with by dumping rubbish into convenient terrestrial or marine locations adjacent to station facilities. Since the advent of the Protocol on Environmental Protection to the Antarctic Treaty in 1991 this practice has changed and in most cases waste is now returned to its country of origin. Most established Antarctic stations, however, have abandoned waste dumps of varying sizes containing a

large variety of materials (e.g. Snape et al. 1998a), including metals and hydrocarbons (Snape et al. 2001). The migration of these contaminants off dump sites, principally by melt water entrainment and tidal inundation (Snape et al. 2001), has potential for environmental impact. For example, at the Australian base, Casey Station, in east Antarctica, metals and hydrocarbons have accumulated in marine sediments in Brown Bay, adjacent to an abandoned dump site (Snape et al. 2001, Stark et al. 2003b). Soft-sediment assemblages in Brown Bay are different from those in nearby control

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areas (Stark 2000, Stark et al. 2003c) and there are correlations between high concentrations of metals and lower diversity and dominance of opportunistic type species (Stark et al. 2003b). Experimental evidence is required, however, to demonstrate that the presence of contamination in sediments causes the observed differences in soft-sediment assemblages at Brown Bay.

Field experiments that examine the recruitment or recolonisation of benthic assemblages are a useful tool for the study of environmental impacts (Watzin & Roscigno 1997, Glasby & Underwood 1998, Olsgard 1999). Recruitment is defined here in the sense of Renaud et al. (1999) to incorporate settlement of larvae, post-settlement events and immigration of juveniles and adults from surrounding habitats over the period of study. Marine environmental impacts may be first manifest as changes in recruitment, which also constitutes the only path by which many populations could recover (Fairweather 1991). Recruitment is an important structuring factor in hard-bottom benthic communities (Underwood & Denley 1984, Caley et al. 1996, Hunt & Scheibling 1997), but its role in structuring soft-bottom communities is less clear and has been shown to have a wide variety of influences (review by Olafsson et al. 1994).

Pollution has the potential to create significant impacts in benthic assemblages during recruitment of fauna, particularly in sediments where contaminants accumulate (Bonsdorff et al. 1990, Olsgard 1999). Settling larvae have been found to be sensitive to chemicals and toxins (Dubilier 1988), to display selective preferences for particular substrates (Woodin 1986, Butman 1987, Butman & Grassle 1992) and to discriminate between settlement sites on the basis of presence of other fauna, grain size, organic matter and chemical cues (Gray 1974, Ambrose 1984a,b, Woodin 1986, Dubilier 1988). Early life history stages, during settling and metamorphosis of invertebrate larvae, are the most critical in development (Obreski 1979, Watzin & Roscigno 1997, Olsgard 1999). Pollution of sediments may change settlement cues, resulting in avoidance by larvae or early mortality of recruits (Menzie 1984). Juvenile and adult recruits have also been shown to selectively discriminate between contaminated and control sediments in manipulative experiments (Lenihan et al. 1995).

Studies of environmental impacts in soft sediments are usually confined to sampling existing (i.e. already established) assemblages (e.g. Cross & Thomson 1987, Hyland et al. 1994). Existing assemblages may have been affected by past, short-term (pulse) disturbances or may continue to be affected by ongoing, long-term (press) disturbances (sensu Glasby & Underwood 1996). The establishment of new assemblages, however, is only affected by ongoing disturbances (Glasby &

Underwood 1996). Methods are necessary that can distinguish the history of a location (i.e. past disturbances) from the comparison of locations under present conditions (Glasby & Underwood 1996). Recruitment experiments can be used to determine if a disturbance is having an ongoing effect. Ongoing disturbances, such as a sewage outfall, are likely to have a press effect, but a disturbance such as the dumping of rubbish on an Antarctic shoreline may either cause a short-term pulse effect or if contamination is persistent, may have an ongoing press effect.

Based on the premise that differences in soft-sediment assemblages at Casey between potentially disturbed locations (the waste tip and the sewage outfall, hereafter referred to as 'station' locations) and control locations are being caused by contamination of sediment by anthropogenic pollutants, the aims of this study were to test the hypotheses that (1) recruitment is different between locations, (2) recruiting assemblages at station locations are generally different from those at control locations, (3) recruiting assemblages are different between contaminated sediment and control sediment, and (4) there are differences in variability of recruitment between control and station locations, and between control and contaminated sediments.

## MATERIALS AND METHODS

**Study locations and experimental design.** Casey Station is situated in the Windmill Islands in East Antarctica at 66° 17' S, 110° 32' E (Fig. 1). The shallow (<35 m) marine benthic environment consists of poorly sorted glacial till overlying bedrock, and is a mosaic of muddy/sand, gravel, cobbles and boulders. The benthic terrain is very uneven, with many small shelves and benches and small basins, and large areas of homogenous flat seabed are rare in nearshore areas. The experiments were conducted in Brown Bay, O'Brien Bay and Shannon Bay (Fig. 1).

**Brown Bay:** Rubbish was dumped along the shoreline of and into Brown Bay for over 21 yr and was discontinued in 1986 (Deprez et al. 1999). During the annual summer thaw, a melt stream runs through the dump site and into Brown Bay, entraining contaminated particulates and dissolved material from the dump. Brown Bay sediment is contaminated by heavy metals and hydrocarbons from the abandoned garbage dump (Snape et al. 1998b, 2001, Scouller et al. 2000).

**O'Brien Bay:** Two undisturbed control locations were situated in O'Brien Bay (Locations 4 and 5 in Fig. 1). The sediment is uncontaminated and many metals found at Brown Bay are below detection limits at O'Brien Bay (Snape et al. 2001, Stark et al. 2003b).

**Shannon Bay:** A second station location was also used, situated in front of the Casey Station sewage out-fall in Shannon Bay (Location 1 in Fig. 1). Sediments in the bay are contaminated, but to a lesser extent than in Brown Bay (Stark et al. 2003b).

**Expt 1, March to November 1997:** Sediments were deployed at 2 locations, Brown Bay and O'Brien Bay (Fig. 1). Within each location 2 trays of each sediment type (contaminated and control) were placed at each of 2 depths (shallow: ~15 m; deep: ~25 m), with 6 replicate pots per tray. The 2 depths at each location were separated by up to several hundred metres, potentially confounding effects of depth with spatial variation. Effects of depth at Brown Bay may be further confounded by the proximity of the shallow site to the waste dump (~100 m), while the deep site was ~300 m away. For statistical analyses, each combination of location and depth was treated separately (termed 'site'), and any depth effects were interpreted with caution. At each site the 4 trays were haphazardly placed by boat in an area ~20 m in diameter. The trays were left in place from March to November 1997, during which time the sea surface remained frozen; 1 tray of control sediment and 1 of contaminated sediment were lost from the O'Brien Bay-shallow site due to icebergs.

**Expt 2, March to November 1998 and to February 1999:** Expt 1 had indicated possible effects of depth, so the second experiment was confined to 12–15 m depth. Experimental units were deployed in February/March 1998 at 4 locations, 2 control locations (O'Brien Bay-2 and O'Brien Bay-3) and 2 station locations (Shannon Bay and Brown Bay) (Fig. 1). There were 2 sampling times, at approximately 9 and 12 mo after deployment. At each location there were 4 trays of control sediment and 4 trays of contaminated sediment. Each tray was approximately 3 to 5 m apart. We retrieved 2 trays of each sediment type in late November/early December 1998; the remaining 2 trays of each treatment were retrieved in February 1999 and, at this time, 1 control tray was missing from Brown Bay and both control trays were missing from O'Brien Bay-3.

**Defaunated sediments and experimental units:** Sediment was collected from O'Brien Bay (control, uncontaminated sediment) and from Brown Bay (contaminated sediment) by van Veen grab and by divers using polyethylene buckets. Sediment was defaunated by freezing at  $-20^{\circ}\text{C}$  for a minimum of 48 h, defrosting to room temperature and sieving through a  $500\ \mu\text{m}$ -mesh screen with seawater taken from the same sites. Material  $>500\ \mu\text{m}$  was discarded to reduce effects of grain size differences between the 2 sediment types. The sediment was left to settle and overlying water was siphoned off, then homogenised and placed in plastic flowerpots, 12 cm diameter and 12 cm deep. Each pot had three  $8 \times 8\ \text{cm}$  holes cut into the sides and a 9 cm diameter hole in the bottom, covered by  $300\ \mu\text{m}$  mesh, to prevent hypoxic conditions in the sediment. Each experimental unit consisted of a plastic tray ( $60 \times 35 \times 10\ \text{cm}$ ) containing 6 pots attached with plastic cable ties. Each tray had a rope bridle to allow lowering and raising, and a retrieval rope with a subsurface buoy ~3 to 4 m below the surface to prevent freezing into the sea ice. The units were gently lowered to the bottom from boats, and on retrieval were gently raised to the surface by divers. Pots were not covered or sealed on retrieval, and minor quantities of surface sediments may have been lost, but this was considered an equal effect among all treatments.

When retrieved, 4 samples from each tray were sieved through a  $500\ \mu\text{m}$ -mesh and fixed in 7% formalin

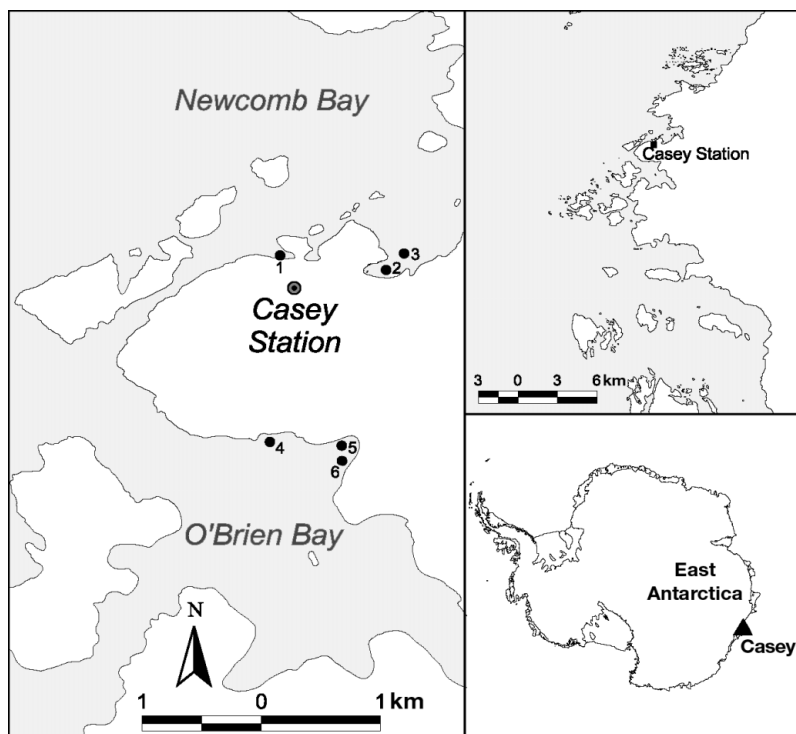


Fig. 1. Location of Casey Station and positions of experiments. 1: Shannon Bay (Expt 2); 2: Brown Bay shallow (Expt 1) and Brown Bay (Expt 2); 3: Brown Bay deep (Expt 1); 4: O'Brien Bay-3 (Expt 2); 5: O'Brien Bay shallow (Expt 1) and O'Brien Bay-2 (Expt 2); 6: O'Brien Bay deep (Expt 1)

with Biebrich scarlet stain; 2 samples from each tray were frozen for analysis of total organic carbon (TOC) and metals. Samples were sorted under a dissecting microscope and preserved in 70% ethanol. Fauna were identified to the highest and most convenient taxonomic resolution possible. Polychaetes were identified to family, crustaceans, bivalves and gastropods were generally identified to species. Echinoderms were identified to order, excepting the burrowing heart urchin of the genus *Abatus*. Other fauna included nemertean, sipunculids, priapulids, oligochaetes and nematodes.

**Statistical analyses.** Tests of the multivariate null hypotheses of no differences among *a priori* defined groups were done using non-parametric multivariate analysis of variance (NP-MANOVA, Anderson 2000, 2001, McArdle & Anderson 2001) and generalised discriminant analysis using the method CAP (canonical analysis of principal coordinates; Anderson 2002, Anderson & Robinson 2003, Anderson & Willis 2003). The *F*-ratio constructed in NP-MANOVA is analogous to Fisher's *F*-ratio and is constructed from sums of squared distances (in this case Bray-Curtis dissimilarities calculated from 4th-root-transformed species abundances) within and between groups (Anderson 2001). CAP has the advantage over NP-MANOVA of taking into account the correlation structure among variables (species), and provides a constrained ordination that maximises the differences among *a priori* groups (Anderson & Willis 2003). NP-MANOVA was done using a 2-factor orthogonal design with permutation of residuals under a reduced model (Anderson 2000, 2001). Constrained CAP ordinations were also compared to unconstrained non-metric multidimensional scaling (NMDS) ordinations. In addition ANOSIM was used to test for differences among unbalanced *a priori* defined groups using the PRIMER software package (Plymouth Marine Laboratory, UK). Similarity matrixes were calculated using the Bray-Curtis similarity measure on 4th-root-transformed abundances. Assemblage variability among groups was determined using the index of multivariate dispersion (IMD), which contrasts the average rank of similarities among one group (e.g. contaminated) to the average rank of similarities among another group (e.g. control) (Clarke & Warwick 1994). IMD is constrained to range from -1 to +1, and the endpoints of this range indicate that variation among samples from one group is greater than variation among samples from the other group. Taxa responsible for differences between groups were determined using similarity of percentages analysis (SIMPER; Clarke 1993).

Univariate analyses were done using analyses of variance (ANOVA) on abundances of taxa identified as being important contributors to differences between

groups (using SIMPER), and also on diversity indices. Data from Expt 1 were analysed by a 4-factor design with location, sediment type and depth as fixed, orthogonal factors and the 4th factor, tray, nested in the other 3 factors ( $n = 4$  replicate pots per tray). Additionally, a 2-factor ANOVA was done with location and sediment type (as fixed, orthogonal factors) on the total number of taxa in each tray (pooling replicate pots within trays and using  $n = 4$  trays of each sediment type at each location as replicates). Data from Expt 2 were analysed by a 4-factor design with 3 orthogonal factors: time, location, sediment type and the 4th factor, tray, were nested in the above 3 factors ( $n = 4$  replicate pots per tray). Cochran's *C*-test was used to test for homogeneity of variances. If variances were heterogeneous, data were transformed (Snedecor & Cochran 1980, Underwood 1981). Where heterogeneity of variances could not be removed by transformation and were significant at  $p = 0.05$ , a lower probability level of  $p = 0.01$  was used. Multiple comparisons among means were done using a Student-Newman-Keuls (SNK) test. The Shannon-Wiener diversity ( $H'$ ), species richness (Margalef's  $d$ ) and evenness (Pielou's  $J'$ ) were calculated using natural logarithms (Base  $e$ ). Several trays of sediment were lost during the experiments (due to icebergs and sea ice), and to compensate, the number of degrees of freedom of the residual was reduced in the ANOVAs and the averages of other appropriate treatments were substituted to balance the analyses (Underwood 1981, 1997).

To compare variability among treatments in Expt 1 the slope of the relationship between  $\log(\text{variance} + 1)$  and  $\log(\text{mean} + 1)$  (Warwick & Clarke 1993) was examined using the abundance of each taxon averaged over the 8 replicate pots in the 2 trays in each treatment. This measure therefore includes variability at 2 spatial scales, within trays and between trays.

**Geochemical analysis of samples.** Samples for geochemical analysis were frozen, stored and transported to Australia at  $-20^{\circ}\text{C}$ . Sediments for heavy-metal analysis were thawed for 12 h then oven-dried for 48 h at  $60^{\circ}\text{C}$ . Metals were extracted from 5 g of whole sediment using 50 ml 1M HCl, stirring continuously for 30 min at  $-25^{\circ}\text{C}$ . The extractant was filtered through a 0.45  $\mu\text{m}$  CN filter and analysed by inductively coupled plasma-mass spectrometry (ICP-MS) for copper, lead, zinc, cadmium, manganese, nickel, arsenic, chromium, iron and antimony. Sediments for total organic carbon (TOC) analysis were oven-dried, concentrated HCl was added to each sample to remove inorganic carbon, and 50 mg per sample of sediment was analysed by a Dohrmann 190 combustion infrared total organic carbon analyser using Method USEPA9060. Each sample was analysed 2 to 3 times and averaged to obtain the final measurement.

## RESULTS

### Sediments

Concentrations of copper, lead, zinc, silver and TOC were significantly greater in the contaminated sediment from Brown Bay than the control sediment from O'Brien Bay in pre-deployment sediments and after 9 mo in both experiments (Fig. 2) Concentrations of arsenic were slightly higher in contaminated sediment, and differences between treatments for cadmium were variable (Fig. 2). The highest concentrations of metals were in the contaminated sediments retrieved from Brown Bay, indicating further input of metals to those

sediments (Fig. 2). The physical properties of the sediments from the 2 locations are similar: Brown Bay sediments have been classified as coarse-skewed, medium-to-coarse silts, and those from O'Brien Bay as coarse-skewed, coarse silts to very fine sands (Stark et al. 2003b).

### Assemblage composition

A total of 61 taxa had recruited to sediments in Expt 1 and 83 taxa in Expt 2 by 12 mo (75 by 9 mo). The comparative densities of the major taxonomic groups, averaged over each treatment, are shown in Fig. 3.

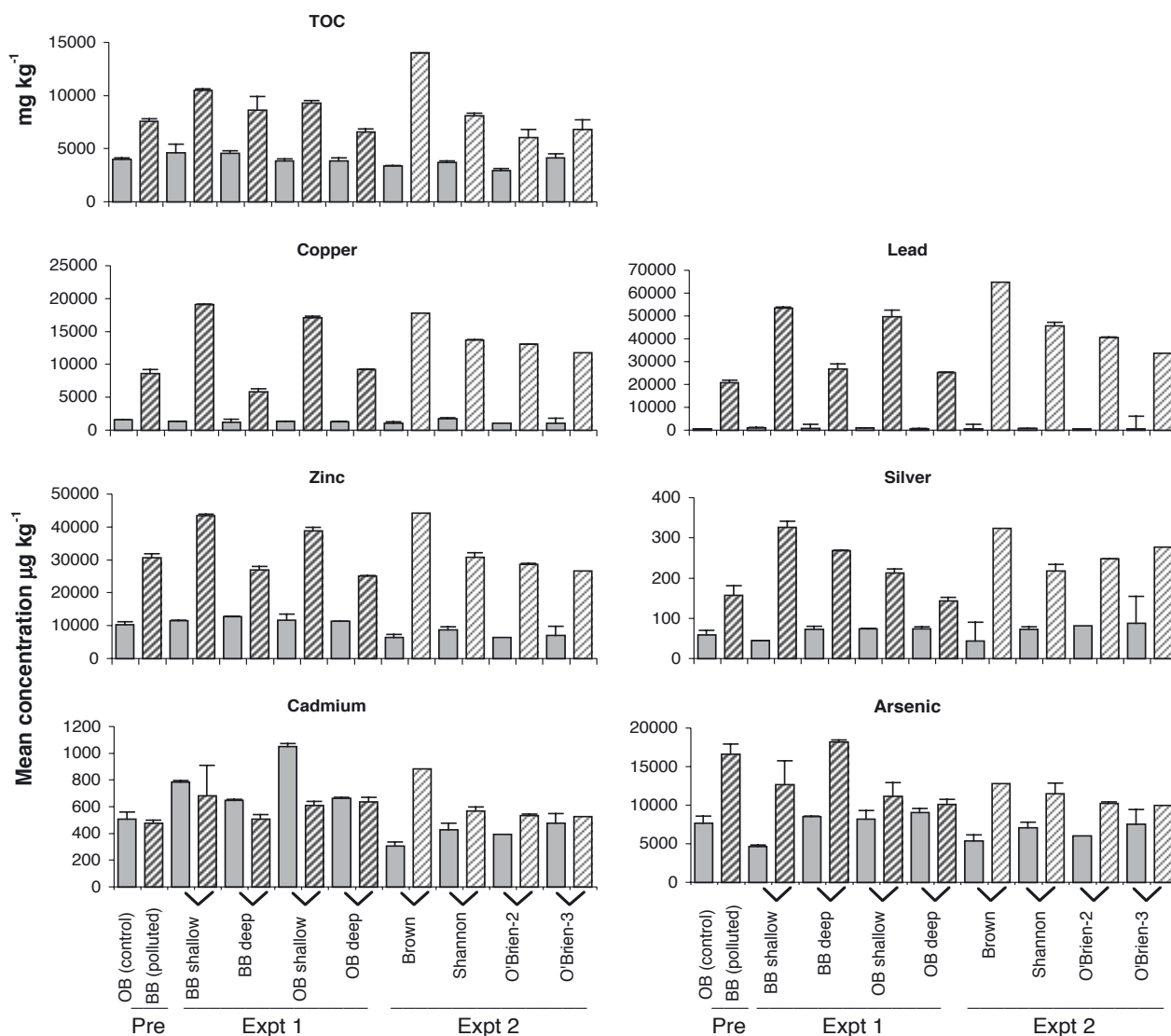


Fig. 2. Expts 1 and 2. Average concentration of total organic carbon (TOC) and heavy metals in each treatment at end of Expt 1 and at 9 mo in Expt 2, n = 2. Shaded bars: control sediments; hatched bars: contaminated sediments; OB: O'Brien Bay, BB: Brown Bay; Pre: concentrations in pre-deployment sediments



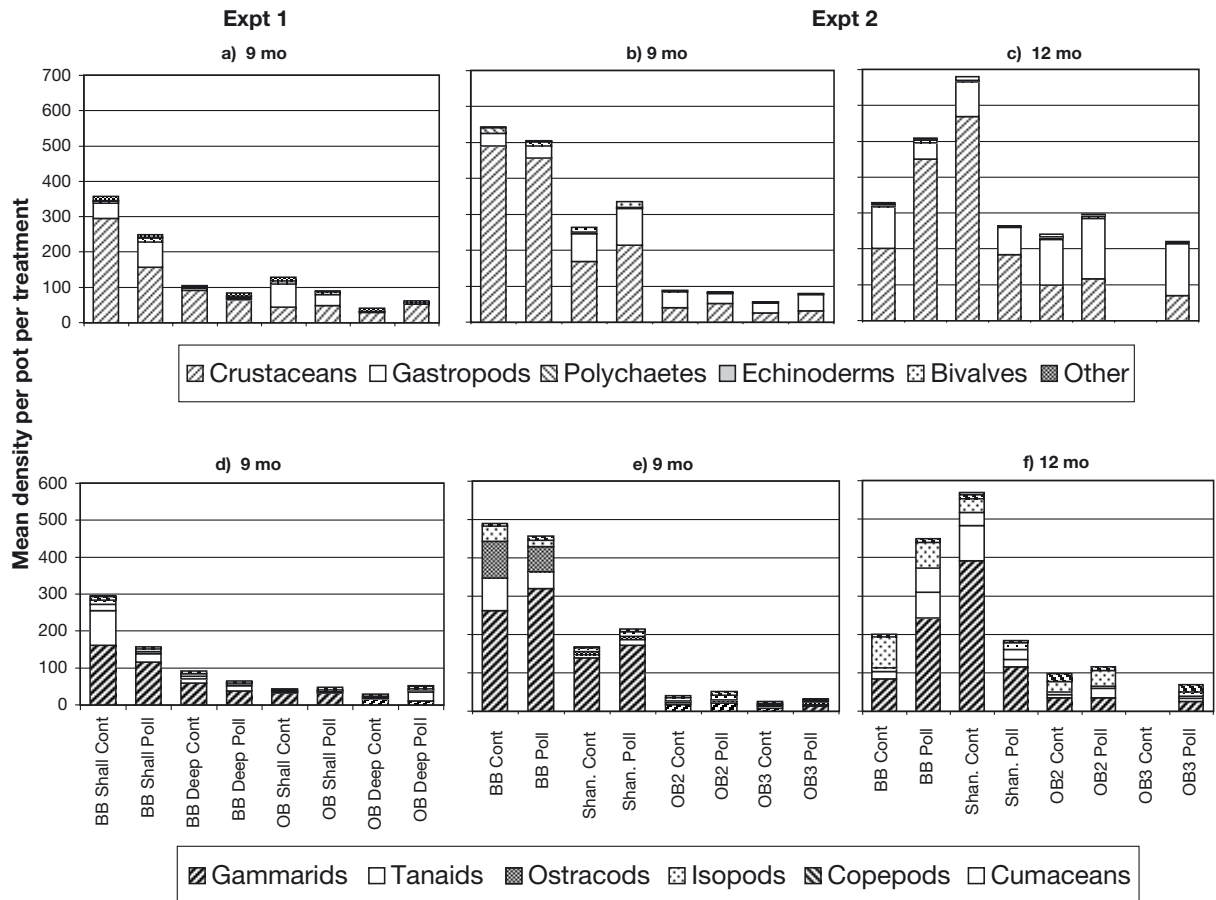


Fig. 3. Expts 1 and 2. (a–c) Average proportions of major taxa in each treatment. Other: nemerteans, nematodes, oligochaetes. (d–f) Average proportion of major crustacean taxa in each treatment at each location. BB: Brown Bay; OB: O'Brien Bay; Shan: Shannon Bay; Shall: shallow; Cont: control; Poll: polluted

Crustaceans comprised the majority of fauna in both experiments. In Expt 2 gastropods comprised a larger proportion than in Expt 1, particularly by 12 mo, when there was a general decrease in the dominance of crustaceans and an increase in the proportion of gastropods (Fig. 3). The greatest temporal change was in the control sediment at Brown Bay, where crustaceans decreased from 90 to 60% of the total, but the fauna in the contaminated sediment changed very little (Fig. 3b,c). Control sediment at Shannon Bay also displayed greater temporal change than contaminated sediment. Crustaceans were more dominant at the station locations than control locations. Gastropods comprised a greater proportion of recruitment in shallow sites than in deep sites (Fig. 3a).

The proportions of the major groups of crustaceans in Expt 1 were similar at most locations except O'Brien Bay deep, where there were proportionally fewer gammarids in the contaminated treatment (Fig. 3d). There were a larger proportion of copepods and cumaceans at O'Brien Bay (Fig. 3). In Expt 2, gammarids were dominant at the station locations and the control loca-

tions had a more even distribution of crustacean taxa (Fig. 3e,f). However this dominance at station locations had lessened by 12 mo and isopods had increased in abundance and comprised a greater proportion of the crustacean assemblage (Fig. 3f).

In Expt 1, mean densities in recruiting assemblages ranged from  $3606 \text{ m}^{-2}$  (mean number of individuals  $\text{m}^{-2}$ ) in control sediment at O'Brien Bay deep to  $31527 \text{ m}^{-2}$  in control sediment at Brown Bay shallow (using a  $0.5 \text{ mm}$  screen). Densities were higher in the second experiment with a minimum of  $4933 \text{ m}^{-2}$  in control sediment at O'Brien Bay-3 and a maximum of  $48019 \text{ m}^{-2}$  in control sediment at Brown Bay after 9 mo. By 12 mo, mean abundances at most locations had increased by 2 to 4 times, with densities in contaminated sediment at O'Brien Bay-3 of  $19391 \text{ m}^{-2}$  (from  $7002 \text{ m}^{-2}$ ) and in control sediment at Shannon Bay of  $60188 \text{ m}^{-2}$  (from  $23241 \text{ m}^{-2}$ ). The densities observed in a survey of surrounding sediments, using cores taken by divers, (on a  $1 \text{ mm}$  screen) ranged from  $10900 \text{ m}^{-2}$  at O'Brien Bay-3 to  $40400 \text{ m}^{-2}$  at Brown Bay (Stark et al. 2003a). Examination of abundances in surrounding sediment in



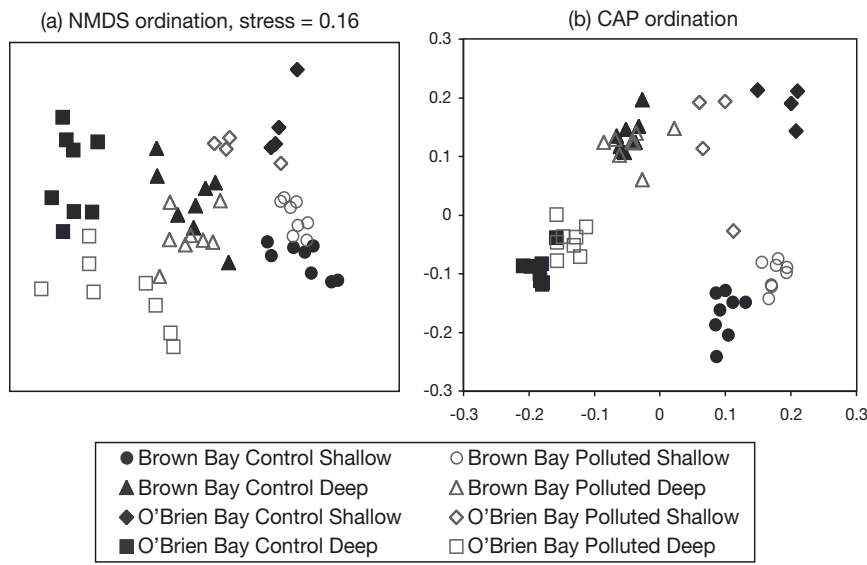


Fig. 4. Expt 1. Ordinations of assemblages in transplanted sediments. (a) NMDS ordination of all samples, data 4th-root-transformed; (b) CAP ordination of all samples

*Skenella paludionoides*, the tanaid genus *Nototanais*, nemertean, and the isopod *Austrosignum cf. grande*, all of which were more abundant in Brown Bay and contributed ~39% to the dissimilarity between the 2 locations. The taxa that were more abundant at O'Brien Bay were nematodes, cumaceans, the ostracod *Scleroconcha* sp. I, 2 gammarid species (*Paroediceroides sinuatus* and *Monoculedes* sp. VIIA) and 3 families of polychaetes (nephtyids, polynoids and syllids).

**Effect of contaminated sediment.** NP-MANOVA indicated significant differences between assemblages in control and contaminated sediment and *a posteriori* comparisons indicated these were greatest at O'Brien Bay (Table 1). Differences between

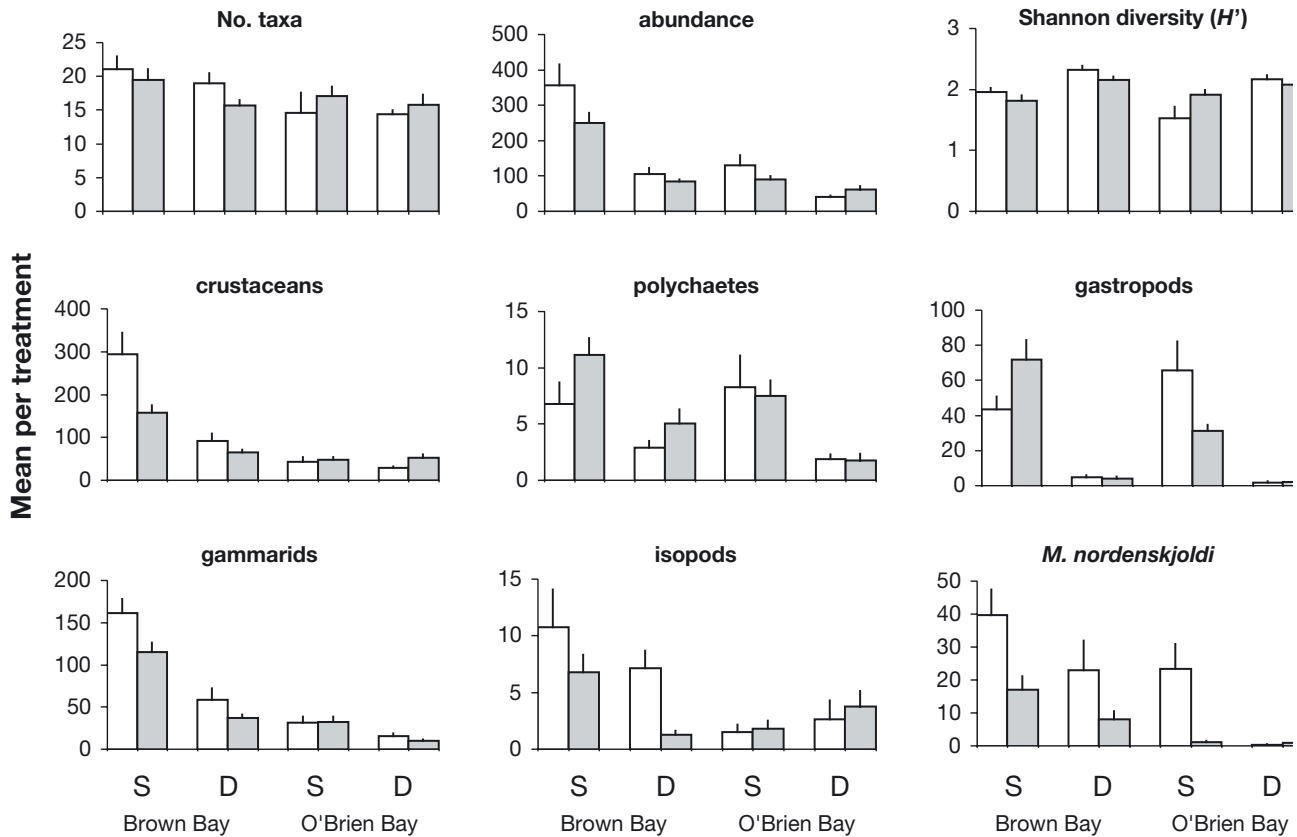


Fig. 5. Expt 1. Mean (+SE) abundance of various taxa and diversity within each treatment. n = 8, except at O'Brien Bay shallow location where n = 4. S: shallow site; D: deep site; open bars: control sediment; shaded bars: contaminated sediment; *M. nordenskjoldi*: *Methalimedon nordenskjoldi*



assemblages in the 2 sediment types within each site are apparent in both unconstrained nMDS and constrained CAP ordinations (Fig. 4). The species contributing to differences between control and contaminated sediment treatments (from SIMPER analysis) were variable between locations and depths, but consisted mainly of crustaceans (gammarids, tanaids and isopods) and, in particular, the gammarids *Methalimedon nordenskjoldi* and *Schraderia gracilis*, which were generally more abundant in control sediment.

While differences among assemblages in control and contaminated sediment were clear, patterns of differences for individual taxa were complex and difficult to interpret. Sediment effects on individual taxa were mostly confined to 1 location or depth within a location. The most significant overall effect was for the gammarid *Methalimedon nordenskjoldi*, which was more abundant in control sediment (Fig. 5). At Brown Bay the total number of taxa recorded in control sediment was greater than in contaminated sediment at both sites (shallow: control = 44, contaminated = 39; deep: control = 36, contaminated = 33). Cumaceans were present in all control sediment in very low densities but the only contaminated sediment in which they were present was from the O'Brien Bay deep site. Polychaetes were more abundant in contaminated sediment at Brown Bay (Fig. 5). When the number of taxa were pooled in each tray and analysed by 2-factor ANOVA (location and sediment type), the total number of gammarid and isopod taxa were greater in control sediment than in contaminated sediment (ANOVA, gammarid taxa:  $F = 9.00$ ,  $p = 0.01$ ; isopod taxa:  $F = 5.12$ ,  $p = 0.04$ ; SNK  $p < 0.05$ ).

**Effect of depth.** The effect of depth is also apparent in the NMDS and CAP ordinations, with all deep samples on the left of the ordination and the shallow on the right (Fig. 4). Recruitment was greater at shallow sites than at deep sites, but this was influenced overall by greater recruitment at the Brown Bay shallow site than at all others. Abundances of some taxa, including total

individuals, gastropods and polychaetes were significantly greater at the shallow sites (Table 2, Fig. 5). The deep sites had significantly greater Shannon diversity ( $H'$ ), species richness ( $d$ ) and evenness ( $J'$ ) (Table 2, Fig. 5). Differences in abundances between depths were variable and often only at 1 site or in 1 sediment type.

**Variability.** The level of assemblage variability between samples as measured by the multivariate relative dispersion (RD) indicated that assemblages in O'Brien Bay (RD = 1.45) were more variable than in Brown Bay (RD = 0.75); deep assemblages (RD = 1.15) were more variable than shallow (RD = 0.72); and assemblages in control sediment (RD = 1.10) were more variable than those in contaminated sediment (RD = 0.90). Differences in assemblage variability related to sediment type were further confirmed by examination of the slope of the relationship between  $\log(\text{variance} + 1)$  and  $\log(\text{mean} + 1)$  for each taxon. There was greater variability within trays of control sediment than trays of contaminated sediment. The slope coefficient ( $b$ ) was less for fauna in contaminated sediments than in control sediments for all locations except O'Brien Bay deep (Brown Bay: shallow, control  $b = 1.75$ , contaminated  $b = 1.48$ ; deep, control  $b = 1.57$ , contaminated  $b = 1.42$ ; O'Brien Bay: shallow, control  $b = 1.64$ , contaminated  $b = 1.36$ ; deep, control  $b = 1.48$ , contaminated  $b = 1.63$ ). Differences among slopes were not analysed further because of potential correlations among species within each sample (Chapman et al. 1995).

## Experiment 2

Expt 2 confirmed significant differences in recruiting assemblages between locations and between control and contaminated sediment, with a significant interaction between location and sediment type (Table 3). The multivariate interaction again only related to varying differences between control and

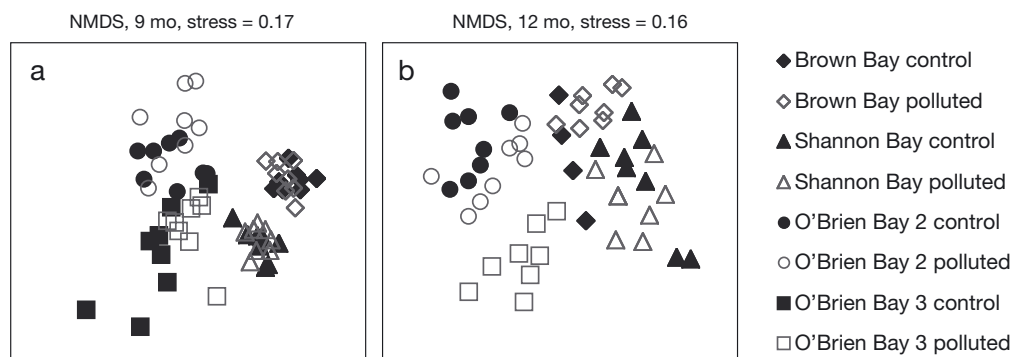


Fig. 6. Expt 2. NMDS ordinations of assemblages recruiting to each location at 9 and 12 mo

contaminated sediment within different locations. The NMDS ordinations at 9 mo and at 12 mo revealed the 2 *a priori* defined groups, with the control locations in 1 group on the left of the ordinations and the station locations on the right (Fig. 6a,b).

These 2 groups were significantly different (ANOSIM:  $R = 0.69$ ,  $p < 0.001$ ).

**Effect of location.** As in Expt 1, location had the strongest effect on assemblage patterns and patterns of abundance of individual taxa. Assemblages were sig-

Table 3. Expt 2. NP-MANOVA on Bray-Curtis distances for assemblages colonising control and contaminated sediment at 4 locations (based on 4th-root-transformed abundances). na = not analysed due to missing treatment

Source	9 mo				12 mo			
	df	MS	<i>F</i>	p	df	MS	<i>F</i>	p
Location (Loc)	3	29688.95	37.19	0.0001	2	14336.17	17.46	0.0001
Sediment (Sed)	1	1345.04	1.69	0.046	1	2007.64	2.44	0.019
Loc × Sed	3	2448.14	3.07	0.0001	2	6154.39	7.49	0.0001
Residual	56	798.26			38	821.16		
Total	63				43			
<b>Pairwise <i>a posteriori</i> comparisons between each location</b>								
		9 mo			12 mo			
		<i>t</i>	p	avg. dissimilarity	<i>t</i>	p	avg. dissimilarity	
Brown vs Shannon		5.47	0.0002	60.06	2.44	0.0002	54.81	
Brown vs O'Brien-2		6.67	0.0002	83.35	3.89	0.0001	60.25	
Brown vs O'Brien-3		6.96	0.0002	83.40				
Shannon vs O'Brien-2		6.27	0.0002	77.77	4.42	0.0001	66.36	
Shannon vs O'Brien-3		6.13	0.0002	74.05				
O'Brien-2 vs O'Brien-3		2.53	0.0002	53.43				
<b>Pairwise <i>a posteriori</i> comparisons between control and contaminated sediment at each location</b>								
		9 mo		12 mo				
		<i>t</i>	p	<i>t</i>	p			
Brown Bay		1.58	0.007	3.06	0.0002			
Shannon Bay		1.50	0.067	2.11	0.009			
O'Brien Bay-2		1.59	0.002	2.02	0.008			
O'Brien Bay-3		1.85	0.016	na	na			

Table 4. Expt 2. Summary of significant results from 4-factor ANOVAs and SNK tests. \*significant at  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , T1: 9 mo; T2: 12 mo

	Time	Location	Sediment	Tray	Time × Loc	Time × Sed	Loc × Sed	Time × Loc × Sed
Total taxa	T2 > T1***	BB > SB > OB2 > OB3***	ns	ns	ns	ns	ns	ns
Total individuals	T2 > T1***	BB > SB = OB2 > OB3***	P > C*	***	***	ns	**	*
Diversity ( <i>H'</i> )	ns	BB = OB2 > SB = OB3***	ns	***	**	ns	ns	ns
Richness ( <i>d</i> )	T2 > T1**	BB = OB2 > SB = OB3***	ns	ns	ns	ns	ns	ns
Evenness ( <i>J'</i> )	ns	OB2 = BB = OB3 > SB***	ns	***	***	ns	*	ns
Crustaceans	T2 > T1**	BB > SB > OB2 > OB3***	P > C*	***	**	ns	*	*
Gammarids	ns	BB = SB > OB2 > OB3***	ns	*	*	ns	*	*
<i>Orchomenella franklini</i>	T1 > T2*	SB > BB > OB3 > OB2***	ns	***	ns	ns	ns	*
<i>Methalimedon nordenskjoeldi</i>	ns	BB > SB > OB3 = OB2***	ns	***	**	ns	ns	ns
Isopods	T2 > T1***	BB > OB2 = SB > OB3***	ns	ns	ns	ns	***	ns
Ostracods	ns	BB > SB > OB3 = OB2***	ns	***	***	ns	ns	ns
Tanaids	ns	BB > SB > OB2 = OB3***	ns	***	ns	ns	ns	ns
Polychaetes	ns	BB > OB2 > SB = OB3***	ns	ns	***	*	*	ns
Capitellids	ns	BB > SB = OB2 = OB3***	ns	ns	ns	ns	ns	ns
Gastropods	T2 > T1***	SB = OB2 > BB = OB3**	ns	***	**	ns	***	*
<i>Onuba gelida</i>	ns	OB2 > OB3 = BB > SB***	C > P*	**	ns	ns	ns	ns

nificantly different from each other at all locations, with the greatest similarity between the 2 locations within O'Brien Bay (Table 3). Multivariate patterns of differences between locations were similar at 9 and 12 mo, but assemblages at Brown Bay and Shannon Bay were slightly more variable at 12 mo, as apparent in the greater spread of samples from these locations in the 12 mo NMDS ordination (Fig. 6). The mean abundance of most taxa were significantly greater at the station locations than at the control locations at 9 mo but by 12

mo differences had decreased (Table 4, Fig. 7). Diversity, evenness and richness did not show any pattern relating to the station locations; however abundances of some individual taxa did, e.g. capitellid polychaetes and crustaceans, mainly gammarids, e.g. *Orchomenella franklini*, *Methalimedon nordenskjoldi* and *O. pinguides* were significantly more abundant at station locations, Brown Bay and Shannon Bay (Fig. 7). The gastropod *Onoba gelida* was more abundant at the control locations O'Brien Bay-2 and -3 (Table 4, Fig. 7).

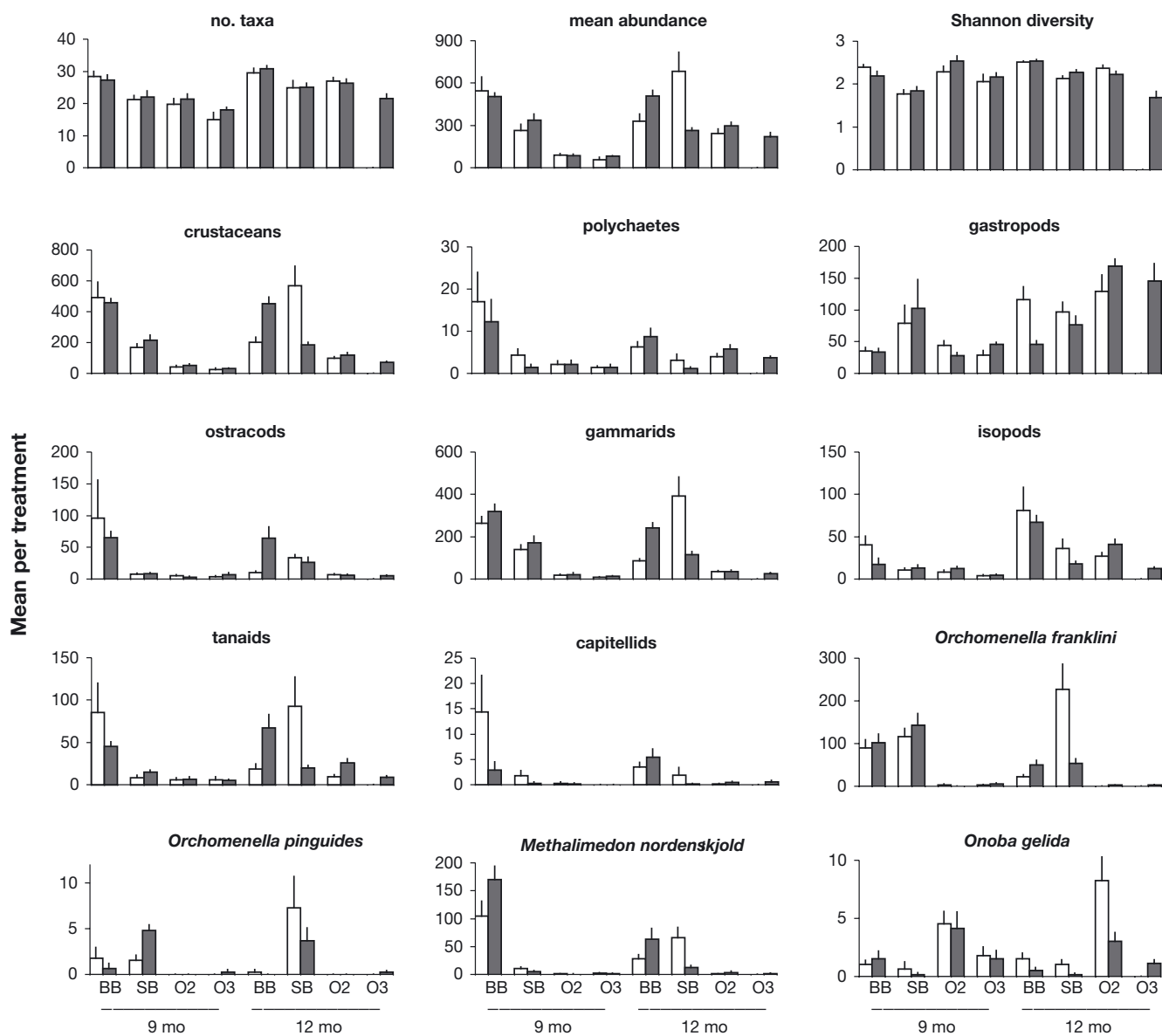


Fig. 7. Expt 2. Mean (+SE) abundance of various taxa and diversity per treatment at 9 and 12 mo. n = 8, except for control sediment at Brown Bay and O'Brien Bay-3 at 12 mo where n = 4. BB: Brown Bay; SB: Shannon Bay; O2: O'Brien Bay-2; O3: O'Brien Bay-3. Open bars: control sediment; shaded bars: contaminated sediment. Data missing from control 12 mo at O'Brien Bay-3 due to loss of trays through icebergs

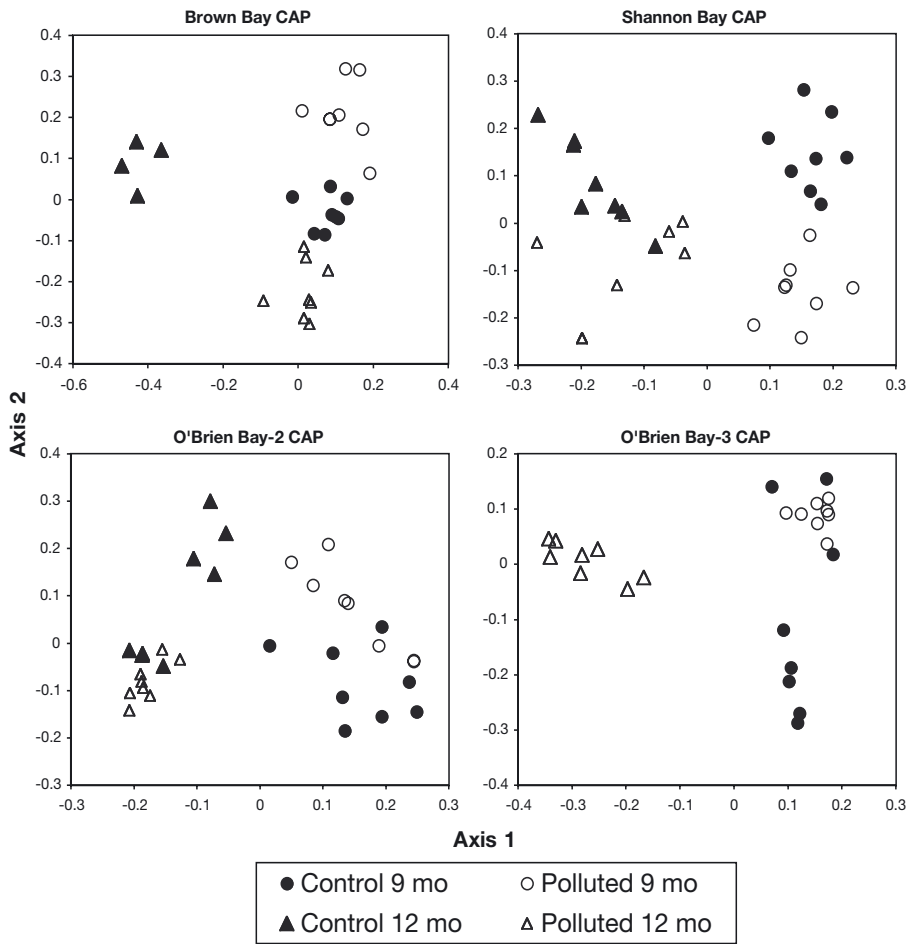


Fig. 8. Expt 2. CAP ordinations of assemblages recruiting based on Bray-Curtis dissimilarities of 4th-root-transformed abundance

**Effect of sediment contamination.** NP-MANOVA indicated significant differences between control and contaminated sediment at 9 and 12 mo (Table 3). *A posteriori* comparisons of sediment type revealed as-

semblages in control and contaminated sediment to be significantly different at all locations at 9 mo (except Shannon Bay) and 12 mo and that differences were greater by 12 mo (Table 3). Separate CAP ordinations of each location in Expt 2 reveal clear differences between assemblages in control and contaminated sediment at both times (Fig. 8).

Differences in the abundances of individual taxa between the 2 sediment types were very complex and difficult to interpret due to many significant interaction terms between sediment type and either or both time and location (Table 4, Fig. 7). For example, there were large but contrasting differences in the abundance of crustaceans in control and contaminated sediment at Brown Bay and Shannon Bay at 12 mo (Fig. 7). Where effects of contaminated sediment are evident they are often restricted to 1 time or 1 location within that time (Fig. 7).

Significant time × location × sediment interactions are summarised in Table 5. These interactions show that at station locations, assemblages in control sediment changed more over time than those in contaminated sediment. There were a greater number of differences between 9 and 12 mo at Brown Bay and Shannon Bay for control sediment than for contaminated sediment (Table 5). There was roughly an equal number of changes through time for control and contami-

Table 5. Expt 2. Results of SNK tests for Time × (Location × Sediment) interactions. Differences between times in each sediment type at each location. T1: 9 mo; T2: 12 mo; C: control; P: polluted; ns: not significant

Taxa	Brown Bay		Shannon Bay		O'Brien Bay-2		O'Brien Bay-3	
	C	P	C	P	C	P	C	P
Total individuals	ns	ns	T2 > T1	ns	T2 > T1	T2 > T1	-	T2 > T1
Crustaceans	T1 > T2	ns	T2 > T1	ns	T2 > T1	T2 > T1	-	T2 > T1
Gammarids	T1 > T2	ns	T2 > T1	ns	T2 > T1	ns	-	ns
<i>Orchomenella franklini</i>	T1 > T2	ns	ns	T1 > T2	ns	ns	-	ns
<i>Heterophoxus videns</i>	T1 > T2	T2 > T1	T2 > T1	T2 > T1	ns	ns	-	ns
<i>Orchomenella</i> sp. IIA	ns	ns	T2 > T1	ns	ns	ns	-	ns
<i>Scleroconcha</i> sp. I	T1 > T2	ns	T2 > T1	T2 > T1	ns	ns	-	T1 > T2
Gastropods	T2 > T1	ns	ns	ns	T2 > T1	T2 > T1	-	T2 > T1
<i>Onuba turqueti</i>	ns	ns	ns	ns	ns	T2 > T1	-	T1 > T2
<i>Skenella paludionoides</i>	T2 > T1	ns	ns	ns	T2 > T1	T2 > T1	-	T2 > T1

nated sediment at O'Brien Bay-2 (Table 5—the control treatment was lost from O'Brien Bay-3 at 12 mo).

**Temporal effects.** Assemblages changed significantly from 9 to 12 mo at Brown Bay (ANOSIM:  $R = 0.51$ ,  $p < 0.001$ ), Shannon Bay ( $R = 0.57$ ,  $p < 0.001$ ) and O'Brien Bay-2 ( $R = 0.42$ ,  $p < 0.001$ ). CAP ordinations of each location show separation of samples from 9 and 12 mo (Fig. 8). For individual taxa, differences between 9 and 12 mo were common, and overall there was a general increase in the number of taxa, the number of individuals and in the abundance of many major taxa, e.g. crustaceans, gastropods (Table 4, Fig. 7) and echinoderms. Several crustacean taxa decreased in abundance from 9 to 12 mo at Brown Bay (Table 5).

The most abundant species at 9 mo was the gammarid *Orchomenella franklini*, which appears to be an opportunistic, colonising species at station locations (highly motile, brooder with direct developing young), where it recruited in large densities and also occurs in the *in situ* assemblages in large densities (Stark 2000, Stark et al. 2003a). Abundances of *O. franklini* had decreased by 12 mo, but it was still one of the most dominant species at station locations and occurred in very low densities at control locations (Fig. 7).

**Variability.** Comparisons of variability of recruiting assemblages between locations showed the station locations to be less variable than the control locations at both sampling times, although by 12 months the difference in variability between the control and station locations was less than at 9 mo (Table 6a). Assemblages at the station locations were slightly more variable at 12 mo than at 9 mo and the control locations were less variable at 12 mo than at 9 mo (Table 6b). Examination of individual locations at 9 and 12 mo confirmed this pattern, with O'Brien Bay-2 and O'Brien-3 showing the greatest change in variability between times and Brown Bay the least (Table 6c). Within each location, however, there was no evidence of any difference in variability between control and contaminated sediment.

Table 6. Index of multivariate dispersion (IMD) values comparing variability between groups. Negative value indicates less variation in first group of comparison

a: Station locations vs control locations: 9 mo	-0.55
Station locations vs control locations: 12 mo	-0.07
b: Control locations: 9 mo vs 12 mo	0.50
Station locations: 9 mo vs 12 mo	-0.07
c: Brown Bay: 9 mo vs 12 mo	-0.40
Shannon Bay: 9 mo vs 12 mo	-0.51
O'Brien Bay-2: 9 mo vs 12 mo	0.70
O'Brien Bay-3: 9 mo vs 12 mo	0.60

## DISCUSSION

These experiments provided causal evidence for the model that differences between assemblages near the Casey waste dump and those in control areas are being caused by contamination from the waste dump. The hypotheses that recruitment and recolonisation of marine sediments would be different among locations and different between control and contaminated sediment were supported. Importantly, there was evidence of general recruitment differences between the station locations (near the waste dump and the sewage outfall) and control locations. However, as both station locations were within Newcomb Bay and both the control locations were within O'Brien Bay, some of the differences among recruiting assemblages may have been due to spatial variation caused by unmeasured differences between the 2 bays, such as currents or sea ice. It is not possible to differentiate between these 2 competing explanations (disturbance from contamination or spatial variation) without an additional control site outside O'Brien Bay. Previous surveys at Casey, however, sampled control locations outside these 2 bays and found them to be more similar to O'Brien Bay than to Newcomb Bay and that differences were not simply due to different bays (Stark 2000, Stark et al. 2003a). The importance of making comparisons of apparently polluted areas with several controls has been well demonstrated (Fairweather 1988, Underwood 1992, 1993, 1994); however, deployment of these experiments at an undisturbed control location outside O'Brien Bay was not possible because there were no other suitable locations within a safe working distance of the station for diving operations, which were necessary to retrieve the sediments.

Differences between locations were greater than those due to effects of contaminated sediment. Assemblages recruiting to Brown Bay (the location of the waste dump) were the most different from all the other locations, but bore most similarity to Shannon Bay (location of the sewage outfall). These station locations had initially greater recruitment (greater number of individuals after 9 mo) than the controls, and had a greater dominance of highly motile crustaceans, mainly gammarid amphipods of 3 species (*Orchomenella franklini*, *Heterophoxus videns* and *Methalimdon nordenskjoldi*). Brown Bay also had a significantly greater abundance of polychaetes. Large abundances of highly motile species of crustaceans and polychaetes have been found to be characteristic of contaminated and disturbed locations worldwide (Pearson & Rosenberg 1978, Raman & Ganapati 1983, Weston 1990, Stark 1998). Lenihan & Oliver (1995), however, detected a pattern of community response in relation to a pollution gradient in Antarctica at McMurdo Sta-

tion, of abundances and diversity of infauna increasing with increasing distance from a pollution source (a bay heavily contaminated by hydrocarbons and metals from a dump), with opportunistic polychaetes dominating the most contaminated areas and dense assemblages of motile crustaceans and polychaetes in moderately contaminated areas. In comparison, at Casey we found that abundances are greater at polluted locations, but pollution levels are lower and assemblages appear to be similar to those found at moderately polluted areas at McMurdo (Stark et al. 2003a). Pearson & Rosenberg (1978) described a response of infauna along an increasing gradient of pollution whereby moderate to high levels of pollution can increase overall abundances of infauna, mainly through large numbers of opportunistic species.

Further evidence that differences at the station locations are due to disturbance is demonstrated by the effects of sediment contamination on recruitment. Concentrations of some metals were up to 2 orders of magnitude greater in the contaminated sediment, sourced from Brown Bay. Differences were more evident for whole assemblages than for individual taxa, which displayed complex and variable patterns of abundance in relation to contaminated sediment. Several studies have demonstrated negative effects of heavy metals on recruitment in soft sediments (Long et al. 1995, Watzin & Roscigno 1997, Olsgard 1999). Metals have been found to influence the distribution of bivalves via effects on recruitment (McGreer 1982, Bryan & Gibbs 1983), to reduce recruiting abundances (Watzin & Roscigno 1997, Olsgard 1999), and also to increase recruiting abundances in some instances, for example gastropods (Watzin & Roscigno 1997). Metals have also been demonstrated to affect established assemblages (as opposed to recolonisation); for example, crustaceans (mainly gammarids and cumaceans) were the fauna most affected in an experiment examining the effects of copper on *in situ* soft-sediment assemblages (Morrissey et al. 1996). Hall & Frid (1995) found that copper reduced the abundances of 4 dominant taxa, and when copper inputs ceased, recovery of the fauna took up to 1 yr with patterns varying between taxa. The response of fauna to pollutants is moderated by factors affecting bioavailability of contaminants such as grain size (Pesch 1979), presence of sulphides (Di Toro et al. 1990), current flow and hypoxia (Diaz & Rosenberg 1995). Grain size was very similar between the 2 sediment treatments, but the contaminated sediment had higher total organic carbon (TOC) levels and this may have influenced the differences between them. Recent work by Lenihan et al. (2003) has shown different responses to different contaminants at the level of phyla, with enhanced abundances of annelids in response to organic pollution and

decreased abundances of crustaceans and echinoderms in metal-contaminated sediments. As the contaminants in our experiment included metals and TOC, it was not possible to determine which had more influence, and they may have had opposing effects, enhancing recruitment of some taxa and negatively influencing others.

A parallel study at Casey found that sediment artificially contaminated by hydrocarbons had greater effects on recruitment than sediment treated with a complex of metals (Stark et al. 2003c). Hydrocarbon contamination in sediments in Brown Bay (although not measured in the present study) is as much as  $\sim 1000 \text{ mg kg}^{-1}$  total petroleum hydrocarbons (Stark et al. 2003b, J. Stark unpubl. data). Demonstrated effects of hydrocarbon contamination in sediment on recruitment of benthic infauna include reduction in species diversity and altered distribution of individuals among species (Bakke et al. 1988, Berge 1990, Bonsdorff et al. 1990). Oil in sediments represents a chemical and physical disturbance that results in primary toxic effects and secondary changes in sediment properties such as organic enrichment, and has been shown to increase densities of meiofauna at low concentrations and reduce densities of macrofauna at increasing concentrations (Spies et al. 1988). Effects may be more pronounced for juveniles and larvae and may affect amphipod embryos in the marsupial stages, leading to reduced juvenile survival (Lindén 1976, Elmgren et al. 1983). Bonsdorff et al. (1990) found negative effects of the water-soluble fraction of North Sea crude oil on population densities of an amphipod, especially the juveniles, but no effect on polychaete recruitment. Following oil spills, recovery of benthic infaunal assemblages has taken many years (Dauvin & Gentil 1990, Gilfillan et al. 1991, Lee & Page 1997). It is not possible to determine from this study whether metal, oil or organic carbon contamination of the sediments is responsible for differences between controls and contaminated treatments. These experiments demonstrate that the differences in soft-sediment assemblages observed between locations at Casey are influenced by the modification of recruitment patterns caused by the presence of anthropogenic contamination of the sediments.

These experiments also supported the hypothesis of differences in assemblage variability between control and station locations, which may reflect an environmental impact. Assemblages recruiting to station locations were less spatially variable than controls. Abundances of some taxa in trays of uncontaminated control sediment at station locations were more temporally variable than in trays of contaminated sediment. Environmental impacts have the potential not only to affect the mean abundance of populations or species diver-



sity, but may also alter temporal and spatial variability of populations and assemblages (Underwood 1991, 1992, 1993, 1994, Warwick & Clarke 1993). These findings concur with previous studies at Casey, which demonstrated less spatial variability in soft-sediment assemblages at polluted locations than at control locations (Stark 2000, Stark et al. 2003a).

Densities of fauna recruiting to the trays were comparable to those in surrounding sediments over 9 to 12 mo. Lenihan & Oliver (1995) found recruitment to be much slower around McMurdo Station, taking several years to approach densities found in nearby sediments. Recolonisation of defaunated sediments in our experiment appears to be dependent on surrounding assemblages, as they were dominated by the same motile crustaceans recruiting to the experiment, particularly peracarids of the orders Amphipoda, Isopoda and Tanaidacea (Stark 2000, Stark et al. 2003a). Most Antarctic peracarid crustaceans have non-pelagic lecithotrophic development, with brooding and release of fully developed dispersive juveniles throughout the year (Arntz et al. 1994), although some do restrict breeding to particular times of the year (Pearse et al. 1991). Protected, non-pelagic lecithotrophic development of larvae is generally very common in Antarctic benthos (Picken 1980, White 1984). At McMurdo Station, peracarid crustaceans were the major colonists after 1 and 3 yr at a moderately disturbed jetty site, but at a severely polluted location recruitment was dominated by opportunistic polychaetes (Lenihan & Oliver 1995). Lenihan & Oliver (1995) similarly concluded that recruitment of disturbed sediments at McMurdo was highly dependent on surrounding populations of motile invertebrates, and many colonists were adult and juvenile immigrants. This has implications for the recovery of impacted assemblages in Antarctica. If remediation of terrestrial waste dumps occurs, as is currently being undertaken at Casey Station, and there is no further input of contaminants to these station locations, there still remains extensive contamination present in the adjacent marine sediments. Hydrocarbons are utilised as an organic food source and degraded by bacterial populations, but metals are more persistent in sediments and could continue to influence recruitment processes in a press effect (*sensu* Glasby & Underwood 1996). Furthermore, as recruitment is apparently dominated by local pools of dispersive juveniles and adults, affected assemblages may change, to become more like control assemblages very slowly. Thus local pools of colonisers may be more influential than sediment contamination. Currents have been shown to be very weak around Casey Station, and are largely induced by severe winds when waters are ice-free (Tate et al. 2000). During the winter period of these experiments, surface waters remained

ice-covered and current velocities would have been very low. As a consequence, transport and immigration of larvae over long distances is unlikely to have occurred, and recruitment in this experiment is likely to have been controlled by micro-scale processes such as habitat selection and local immigration/dispersal. However, Antarctic benthic invertebrates have been shown to have longer developmental times than in other parts of the world (King & Riddle 2001), and this could increase their dispersive potential, but also increase their period of embryonic or juvenile exposure to contaminants, a period when they are most sensitive.

Recruitment mechanisms in this experiment included settlement of larvae from the water column and resuspension or active locomotion of fauna from surrounding benthic habitats. Larval settlement onto soft sediments is largely controlled by habitat selection (micro-scale) and passive deposition by hydrodynamic processes (large-scale) (Butman 1987). Surface-active fauna, such as gastropods and polynoid polychaetes, may have been able to crawl into the trays, which may explain the greater proportion of gastropods in the experimental sediments (3 to 52% of individuals) than in the surrounding sediment (<5%: Stark 2000, Stark et al. 2003c). Alternatively the experimental units may have provided enhanced conditions for gastropods, e.g. food or a refuge from predation. Lateral burrowing and immigration from below the surrounding sediment surfaces were eliminated by the tray design, which put the surface of the defaunated sediments approximately 10 cm above the surrounding substrate. Thus, brooding species without the facility for swimming dispersal or surface activity, such as burrowing polychaetes, may have been partially excluded. Polychaetes were relatively uncommon in this experiment, and were only present in low densities in the surrounding benthic communities. In contrast, many recruitment experiments in soft sediments have found that polychaetes are the dominant colonising fauna, independent of depth and habitat (Mattsson & Notini 1985, Berge 1990, Snelgrove et al. 1994, Olsgard 1999). Further hydrodynamic effects from setting the trays on top of the sediment, as opposed to burying them flush with the sediment surface, are likely to be very minimal because of the very low current velocities in Antarctic inshore waters as a result of almost year-round ice cover. There was no evidence of scouring or deposition around the trays or in the pots within them.

Manipulating soft-sediment assemblages provides a meaningful way of quantifying and predicting responses to contaminants under realistic conditions (Olsgard 1999). This study indicated that monitoring recruitment into soft sediments has potential for environmental monitoring situations where changes in

sediment chemistry may be expected as a result of contaminants entering the marine environment. Sources of variability that would need to be accounted for, were the technique to be used for routine monitoring, include spatial variability (both small-scale, between trays, and large-scale, between locations), depth and temporal variation. Similar methods are currently being used at Casey to monitor the remediation of the waste dump.

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