# Ecological impacts of pollution on marine soft-sediment assemblages

**Joanne Banks**

Research Thesis submitted in partial fulfilment of the requirements for a Bachelor of Science with Honours

School of Zoology

University of Tasmania

2006

## Acknowledgements

Thank you to my supervisors Professor Craig Johnson and Catriona Macleod who made this project so enjoyable. Thanks to Ruth Eriksen, Christine Coughanowr and Mike Rushton of The Derwent Estuary Program (DPIWE) for financial, intellectual and physical assistance. Much appreciation goes to the general staff in the Department of Zoology and at TAFI for help with equipment, materials, boats etc. with a particular debt of gratitude to Richard Holmes who worked so hard with me in the field and was ever chipper in the face of mud. Huge amounts of gratitude go to my friends, turned volunteers….Jane Basset, Mark Davidson and Tim Tewson. Finally, and most importantly, to my ever patient partner Jan Huisken, who supported me through this very challenging year and kept me sane, thank you!

Table of Contents

List of Figures and Tables

# 1. Abstract

In this study, manipulative laboratory experiments were used to define the mechanisms responsible for observed relationships between heavy metal pollution and soft-sediment assemblages evident from field surveys. Heavily polluted and lightly polluted sites were selected based on previous surveys. Assemblages from the polluted site were 60% less taxonomically rich and 38% less diverse, with a less consistent community composition dominated by a small number of non-indigenous or cryptogenic species. Polychaetes were more abundant; however, 96% of the individuals belonged to a single tolerant species, which consistently dominated samples throughout the survey period. Fluctuations in the abundance of two r-selected, opportunistic species were responsible for a comparatively higher degree of temporal variability in community composition.

Manipulative experiments demonstrated the direct effects of pollution. Fauna from the reference site challenged with heavily polluted sediment became more like those normally found at the polluted site, supporting fewer families, derived from fewer taxa. Bivalves and polychaetes were reduced, while crustaceans generally did not survive within the experimental mesocosms irrespective of the extent of pollution they were subject to. Challenging faunal assemblages with sediments either containing the natural fauna or from which fauna were removed tested indirect effects of sediment contamination. Sediments with residents intact led to a greater decline in abundance of potentially establishing fauna, suggesting that some fauna may be excluded from the polluted site because of biotic interactions.

This study provides additional evidence that contamination of sediments by anthropogenic pollutants can have serious consequences for the ecology of benthic environments. Importantly, it shows that impacts on fauna may occur by multiple mechanisms.

## 2. Introduction

Acute and chronic exposure to anthropogenic contaminants occurs in coastal regions worldwide (Vitousek *et al.*, 1997; Lenihan *et al.*, 2003). Urban and industrial centres are often developed around estuaries (Lindegarth & Hoskin, 2001) and as a result, these estuaries are particularly prone to anthropogenic inputs. Because many anthropogenic activities release metals into the marine environment, e.g. industrial and mining wastes (Johnston *et al.*, 2002; Piola & Johnston, 2006), and because estuaries tend to be depositional environments dominated by soft-sediments (Hirst, 2004) to which metals readily adsorb, accumulation of metals in estuarine soft sediments is a common form of coastal pollution (Watzin & Roscigno, 1997; Lindegarth & Underwood, 2002; Wang *et al.*, 2002). Heavy metal pollution of marine environments is a worldwide problem, increasing with the escalating industrialisation of developing nations (Islam & Tanaka, 2004), and threatens the diversity and persistence of marine benthic assemblages (Lindegarth & Underwood, 2002).

Heavy metal contamination of sediments can affect soft-sediment fauna in a number of ways. In the most severe circumstances, contamination may cause direct mortality and lead to ongoing impacts on recolonisation and recruitment dynamics (Watzin & Roscigno, 1997). If metals directly impact community structure this can potentially modify the nature of interspecific interactions, further altering community structure

indirectly (Keough & Quinn, 1998; Adams, 2005). Infaunal assemblages are an important functional component of estuaries (Hirst, 2004). They play a critical role in modifying physical and chemical conditions at the sediment-water interface, and they provide important ecosystem services such as bioturbation of sediments, decomposition of organic matter and recycling of nutrients through feeding and burrowing activities, and as a result transfer energy to higher trophic levels (Gaston *et al.*, 1998; Hirst, 2004). Infaunal communities are fundamentally linked to sediment condition and as a consequence are particularly vulnerable to sediment contamination (Seitz, 1998; Trannum *et al.*, 2004b).

A clear understanding of the ecological effects of contaminants is essential to manage and minimise human impacts on the marine environment (Morrisey *et al.*, 1996). The formulation of pollution prevention and remediation strategies requires information on the types and levels of disturbance which will cause changes in the communities (Lindegarth & Underwood, 2002). However, establishing causal relationships between pollutant loading and changes in the system ecology is difficult since there are many complex ways in which stressors can disrupt the ecosystem function (Adams, 2005). Ecotoxicology methods tend to concentrate on toxicity tests using a single "model" species, and so have limited applicability to complex ecosystems. A single chosen species may not be representative of the species found in the area of interest and single species studies do not take into account the significance of interspecific biological interactions (Trannum *et al.*, 2004b). Studies which examine the changes to entire communities are more likely to provide useful information for understanding the consequences of pollution in ecological systems (Terlizzi *et al.*, 2005).

Many previous studies have shown correlations between metal contaminants and changes in the diversity, abundance, dominance and distribution of species within

marine soft-sediment sediments assemblages (e.g. Ward & Hutchings, 1996; Stark, 1998b; Lancellotti & Stotz, 2004). Although establishing a correlation between changes in community attributes and impact criteria is an essential first step towards identifying the effects of anthropogenic pollution, this cannot be interpreted as evidence of a causal relationship (Lindegarth & Underwood, 1999). Variability in community structure is often confounded by the inherent spatial and temporal variability of soft-sediment systems, or by the presence of other "natural" or manmade factors (Stark, 1998b; Lindegarth & Underwood, 1999). Classic approaches for impact assessment, such as the BACI method (Before-After-Control-Impact) are frequently not applicable as investigators typically lack the baseline data necessary to assess the type and severity of impacts (Underwood, 1991). These difficulties can only be overcome by using manipulative techniques to test hypotheses about causes and effects (Lindegarth & Underwood, 2002; Adams, 2005).

Manipulative field techniques have been used to explore the direct effects of metals on patterns of infaunal assemblages (Morrisey *et al.*, 1996; Stark, 1998a; Lindegarth & Underwood, 1999, 2002). Other studies have concentrated on the long-term effects of sediment contamination by monitoring recolonisation and recruitment patterns (Watzin & Roscigno, 1997; Stark *et al.*, 2003b; Stark *et al.*, 2004; Trannum *et al.*, 2004a). However, carrying out manipulative experiments in the field can be very expensive, and costs and other difficulties associated with field work usually mean that these kinds of experiments can only be relatively simple. Mesocosms studies provide a useful compromise between single species tests and field experiments (Fletcher *et al.*, 2001). Although, mesocosms can never completely mimic natural conditions, responses of benthic fauna in mesocosms can provide insights into the complex direct and indirect effects of contaminants on natural communities (Drake *et al.*, 1996; Milward *et al.*, 2004).

The Derwent Estuary in Tasmania, southeast Australia, has several major sources of industrial and urban contamination. Contrary to expectations, a recent study showed that benthic infaunal community composition was most strongly correlated with natural geomorphology and salinity gradients, rather than patterns of metal contamination (Macleod & Helidoniotis, 2006). However, at the most highly polluted location, a 'hot-spot' of contaminant accumulation next to an industrial zinc smelter, the fauna was distinctive and showed a marked reduction in both diversity and abundance compared to other locations in the estuary. The authors suggested that the highly elevated metal levels at this site appeared to be influencing the community composition (Macleod & Helidoniotis, 2006).

This study further explored the relationship between the benthic community assemblages and the extremely high range of sediment contamination in the Derwent and developed two lines of enquiry. The first stage of this study involved characterising the biota and sediment chemistry at two study sites within the middle and lower reaches of the estuary representative of high and low levels of heavy metal contamination. The pollution 'hot-spot' identified by Macleod and Helidoniotis (2006) and a lightly polluted reference site selected based on its similar geomorphology properties (see Methods). The second stage investigated the causal links between contamination levels and faunal assemblage patterns. Previous experiments have shown that benthic fauna can respond to contamination within a very short timeframe (Morrisey et al., 1996; Lindegarth & Underwood, 1999). Consequently, a series of manipulative experiments were devised to test the community response to changing environmental and ecological factors. The first of these tested whether fauna from the reference site changed when exposed to heavily polluted sediments, and whether the nature of any change moved the fauna toward the configuration normally found at the polluted site (after Morrisey et al., 1996; Stark,

1998a; Lindegarth & Underwood, 2002). Further experiments determined how much of the faunal response to contamination was attributable to the direct effects of toxicity and how much was attributable to changes in faunal composition affecting community dynamics through interspecific interactions.

# 3. Methods

This study considered the weight of evidence indicated by both field surveys and laboratory based experiments:

1) Field surveys were undertaken to characterise assemblages and sediment chemistry at heavily polluted and lightly polluted sites.
2) Manipulative laboratory experiments were conducted to define mechanisms responsible for observed differences in fauna.

## 3.1 Study sites

The sites chosen for this study were situated in the well-mixed, middle and lower reaches of the Derwent estuary, located near the city of Hobart (Fig. 1).

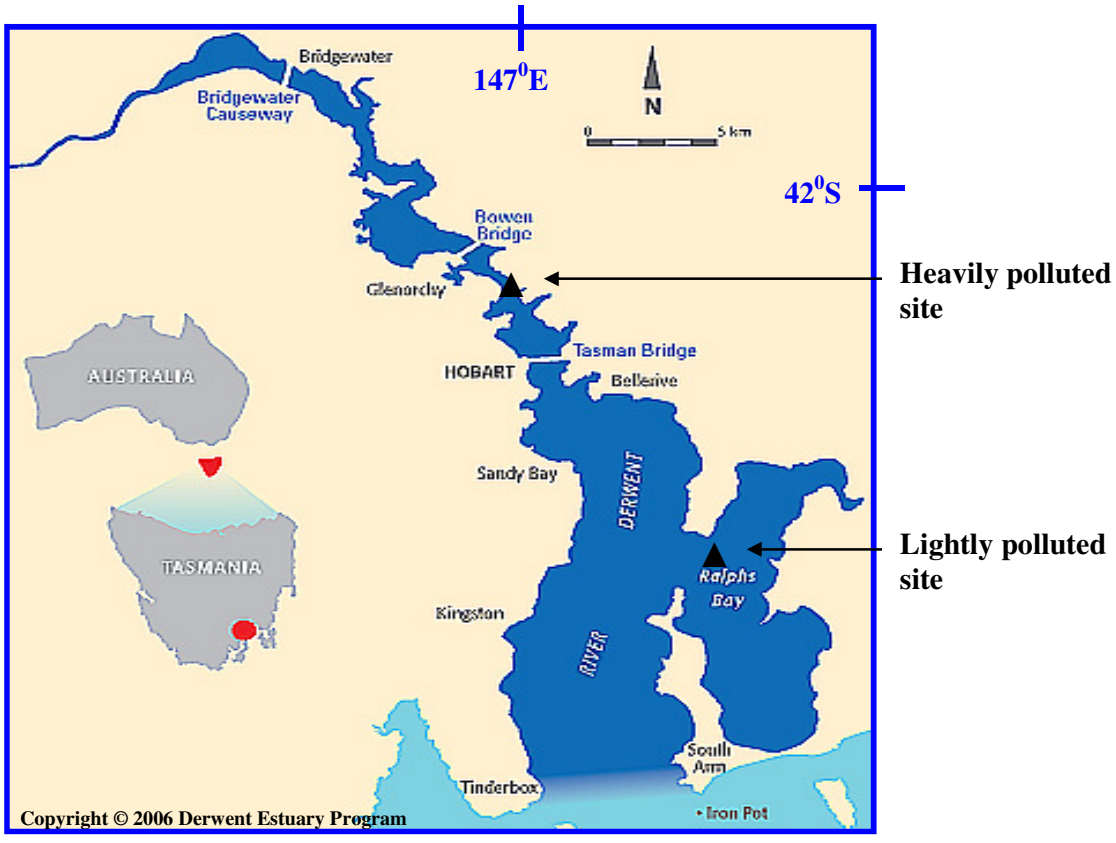


FIGURE 1. Location of study sites in the Derwent estuary, southeastern Tasmania.

Two sites were selected on the basis of previous surveys by Macleod & Helidoniotis (2006). One is located next to a large industrial zinc smelter and is heavily contaminated by heavy metals (hereafter referred to as the 'polluted site'). The other is a relatively lightly polluted site situated within the lower estuary at the mouth of Ralph's Bay (hereafter referred to as the 'reference site'). These sites are representative of the upper and lower levels of heavy metal contamination found within sediments in the Derwent Estuary. The reference site was chosen to match the sediment profile (<85% silt/clay), bottom salinity (>34 ppm) and depth (12 m) of the polluted site (DPIWE-DEP, 2003). Previous work demonstrated that the fauna at the reference site was representative of assemblages found throughout the middle and lower reaches of the estuary (Macleod & Helidoniotis, 2006).

## 3.2 Sample collection and processing

All fauna and sediment samples for surveys and laboratory work were collected using a Van Veen grab (surface area/0.0675 $m^2$, volume/ca 7.5 litres). Fauna were identified to the lowest practicable taxonomic level and enumerated. For crustaceans, molluscs and polychaete worms this was usually to family level. Although species identification was beyond the scope of this study, fauna were separated into nominal species groups and enumerated to enable species richness calculations to be made.

### *3.2.1 Surveys*

i Sampling times: Four surveys were conducted monthly, from October to January.

ii Fauna samples: Four replicate grabs were collected at each site during each visit. Grab contents were transferred into 1mm mesh nylon bags, sieved to remove the bulk of the sediment, and fixed in 4% formalin buffered in seawater. Prior to sorting and identification, samples were rinsed and preserved in 70% ethanol.

iii Sediment samples: Two replicate cores (250 mm length x 45 mm internal diameter) were collected at each site during the first visit. The core contents were transferred into sterilised glass jars for heavy metal analysis, which was undertaken by Analytical Services Tasmania (AST), using acid digestion and inductively coupled plasma emission spectroscopy.

### 3.2.2 *Collections for manipulative experiments*

Twenty-four samples of fauna and sediment were collected from each site for the manipulative experiments (see below). Grab contents were transferred carefully into sealable plastic boxes and transported back to the lab for further processing. The boxes were tightly packed to maintain integrity of the sediment profile during transit and covered with a double layer of shade cloth to control temperature. Animals destined for translocation to mesocosms (see below) were removed from the sediment in the laboratory by careful sieving through a 1mm mesh. At the end of the experiments the mesocosm contents were sieved through a 1 mm mesh and processed as per the survey samples.

## 3.3 Mesocosm design

The mesocosms in this study were 9-litre clear plastic boxes (270 x 180 x 180 mm) with tight fitting lids (Fig 2). The main section of each lid was removed and replaced with 1mm mesh to allow water to flow through the mesocosms. Water entered the box via 4 mm irrigation tubing fixed in a hole 20 mm from the top edge of the box, and exited via the mesh in the lid of the mesocosm. Individual valves on the inflow water supply enabled the flow rate to each mesocosm to be maintained at a consistent rate (1 $lhr^{-1}$). The water supply was pumped directly from the Derwent Estuary, providing a constant flow of oxygenated water at ambient temperature. Temperature control was further

effected by immersing mesocosms in continuously flowing water within a series of four large concrete tanks (Fig 2). The tanks were covered with shade cloth to reduce light levels. One grab (ca. 7.5 litres of sediment) was added to each mesocosm, leaving ca. 50 mm of over-lying water between the sediment surface and the lid.

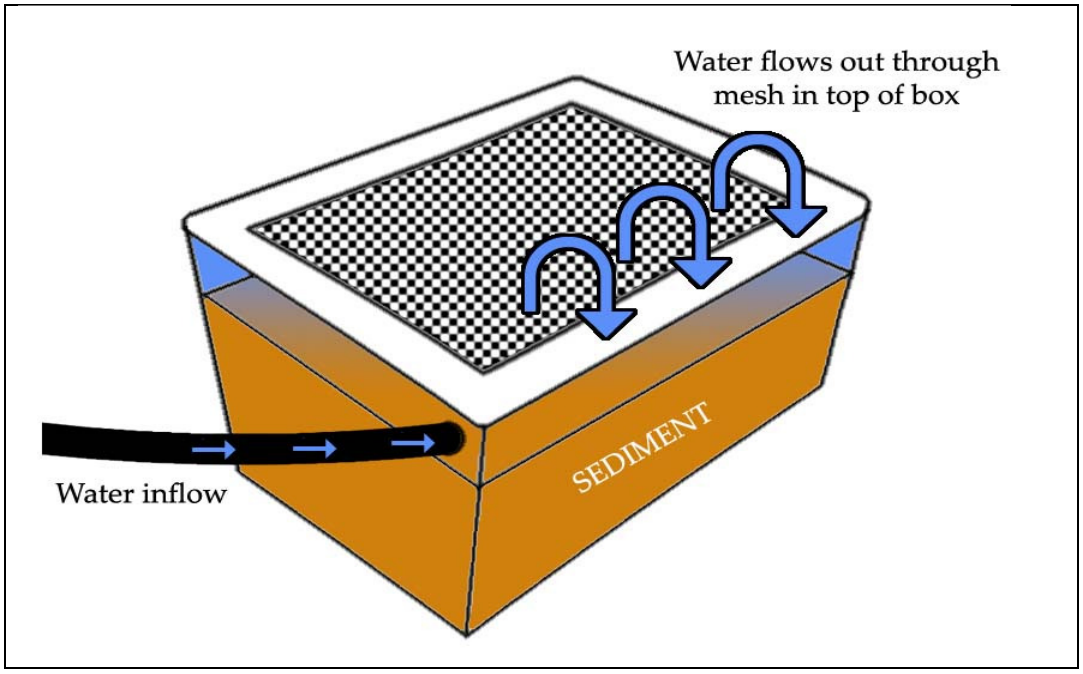


FIGURE 2. Design of mesocosm boxes illustrating water flow.

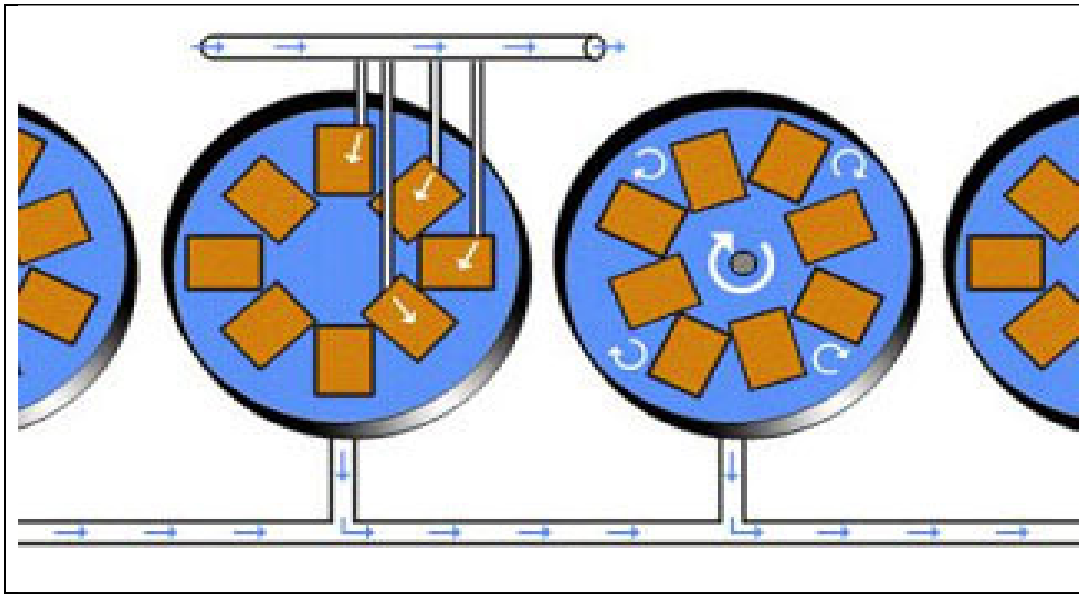

FIGURE 3. Experimental set-up – mesocosms boxes were immersed in continually flowing water within four large concrete tanks.

## 3.4 Experimental design

The general experimental hypothesis in this study was that the observed differences in community composition of infaunal assemblages detected between the polluted and reference sites were due to both the direct effects of sediment toxicity and the indirect effects of shifts in interspecific interactions occurring in response to heavy metal pollution. A series of experiments was designed to isolate these effects. Experiments were run for 1 month as previous manipulative experiments have shown that benthic fauna responds to contamination within a few weeks (Lindegarth & Underwood, 1999) and Morrisey et al (1996) found no further changes in benthic assemblages after a month.

### *3.4.1 Experiment 1: Does sediment toxicity directly influence faunal assemblages?*

The following null hypotheses were tested in a single experiment:

**$H_0$ 1: Faunal assemblages from the reference site will suffer comparable mortality when transplanted to mesocosms containing lightly polluted or heavily polluted sediments**

**$H_0$ 2: Faunal assemblages from the polluted site will suffer comparable mortality when transplanted to mesocosms containing lightly polluted or heavily polluted sediments.**

To investigate the direct effects of sediment toxicity it was necessary to first remove any effects of resident animals prior to adding the test assemblages. To avoid disruption of the sediment structure and minimise any mobilisation of heavy metals, sediments were defaunated by freezing at –20 $^{o}$C for 72 hours (Trannum *et al.*, 2004b). After thawing, sediments were effectively allowed to acclimate within the experimental system for 1 week.

Unfortunately this technique for eradicating the effects of resident fauna introduced strong artefacts that confounded the outcome of experiment 1.

### *3.4.2 Experiment 2: Do sediments and resident assemblages collectively influence fauna?*

The designs for experiments 1 and 2 were similar, with the exception that in this experiment the translocated fauna were added to mesocosms containing test sediments including resident assemblages rather than sediments which had been treated by freezing. The following null hypotheses were tested in a single experiment:

**$H_0$ 3: Faunal assemblages from the reference site will suffer comparable mortality when transplanted to mesocosms containing sediment and communities from the reference or polluted sites.**

**$H_0$ 4: Faunal assemblages from the polluted site will suffer comparable mortality when transplanted to mesocosms containing sediment and communities from the reference or polluted sites.**

There were four replicates of four 'treatment' groups per site, viz.-

A. 'Natural' – characterised by fauna sieved from grab contents with no further treatment.
B. 'Mesocosm' – characterised by fauna transferred directly with grab contents into mesocosms and maintained for 28 days.
C. 'Light pollution' – characterised by adding fauna into mesocosms for 28 days containing lightly polluted sediment (containing resident communities) collected from the reference site.
D. 'Heavy pollution' – characterised by adding fauna into mesocosms for 28 days containing heavily polluted sediment (containing resident communities) collected from the polluted site.

Fig. 4 illustrates the specific comparisons used to test the capacity of additional fauna from the two sites to establish within mesocosms containing lightly polluted and heavily

polluted sediments with resident intact (C versus D). In addition to testing the main hypotheses, the design also included an assessment of the effects of maintaining fauna within the mesocosm apparatus (A versus B).

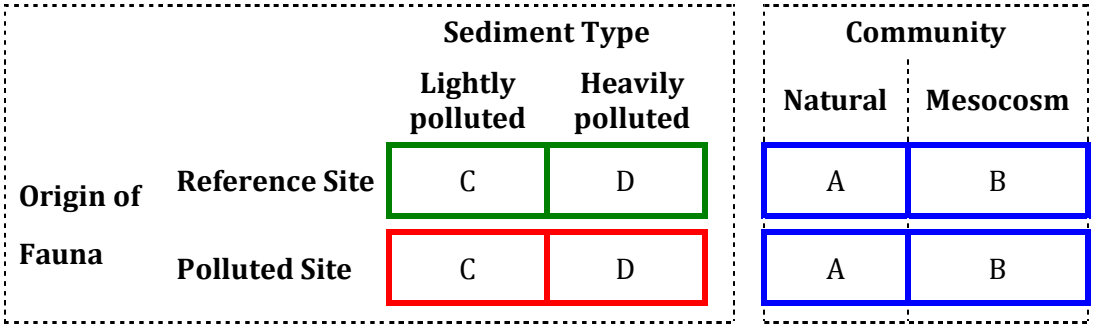


FIGURE 4. Schematic representation of the experimental design: The green boxes show the comparison of treatments for **$H_0$ 3**, assessing the differential response of fauna from the reference site to treatment within lightly polluted (C) and heavily polluted (D) sediments. The red boxes show the comparison of treatments for **$H_0$ 4**, assessing the differential response of fauna from the polluted site to treatment within lightly polluted (C) and heavily polluted (D) sediments. For both faunas, the 'natural community' (A) was compared with the community maintained for 28 days within the mesocosm apparatus (B) (blue boxes). (N=4).

### *3.4.3 Experiment 3: Separating the effects of sediment contamination and residual fauna on newly establishing individuals*

The following null hypotheses were tested in a single experiment:

**$H_0$ 5: Faunal assemblages from the reference site will suffer comparable mortality when transplanted to mesocosms containing heavily polluted sediment in the presence or absence of the resident community.**

**$H_0$ 6: Faunal assemblages from the polluted site will suffer comparable mortality when transplanted to mesocosms containing lightly polluted sediment in the presence or absence of the resident community.**

In addition to testing the main hypotheses, the design for experiment 3 also included a reassessment of the direct effects of sediment toxicity ($H_0$ 1 and $H_0$ 2) as experiment 1 failed. In experiment 3, sediments were defaunated by sieving. To enable any metals that

moved into solution (as a result of sieving) to recombine with the sediment, the sieve water was collected and returned to the mesocosms, after which the sediments were allowed to settle for 24 hours before translocating animals. To test whether the sieving process washed away contaminants, 2 sediment samples were taken from both the sieved and unsieved treatments for heavy metal analysis at the end of the experiment. Figure 5 shows that there was no significant difference in the concentration of contaminants between these treatments.

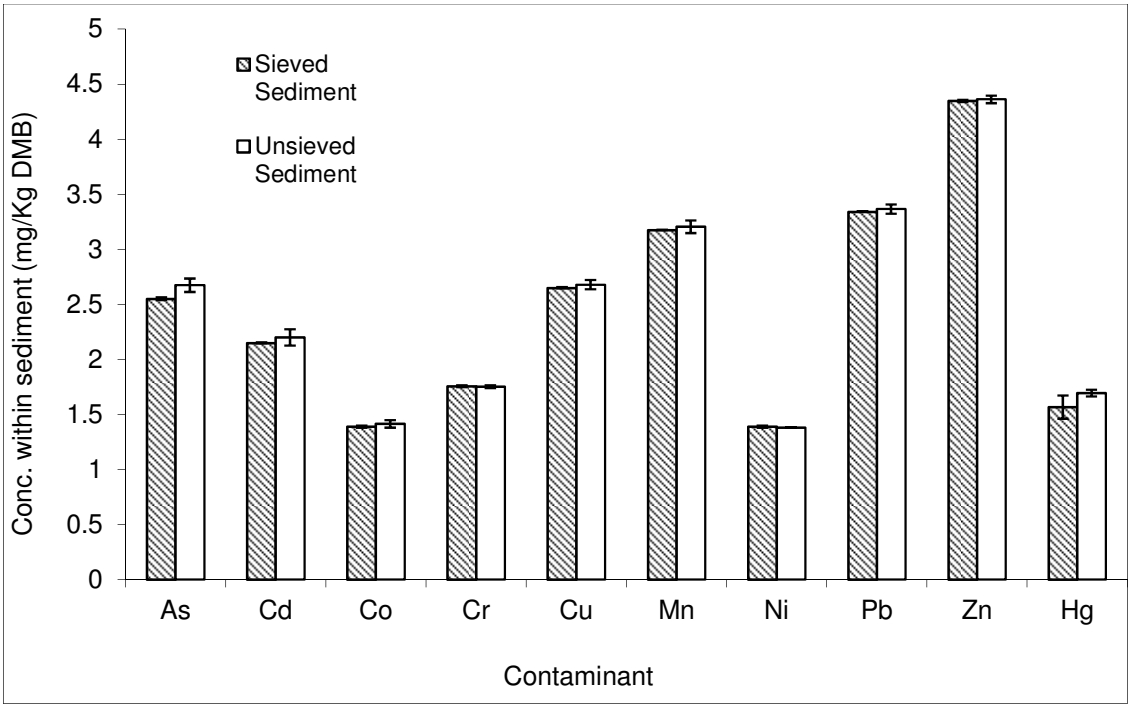


FIGURE 5. Heavy metal concentrations (log mg $kg^{-1}$) within sieved and unsieved sediments sampled at the end of experiment 3 (Mean $\pm$S.E.; n=2).

There were three 'treatment' groups per site, viz.-

A. 'Light pollution' – characterised by adding fauna into mesocosms for 28 days containing lightly polluted sediment (collected from the references site) that earlier had been sieved to remove resident communities.

B. 'Heavy pollution' – characterised by adding fauna into mesocosms for 28 days containing heavily polluted sediment (collected from the polluted site) that earlier had been sieved to remove resident communities.

C. 'Resident fauna' – characterised by adding fauna into mesocosms for 28 days containing unsieved sediments. Fauna from the reference site was transferred into unsieved sediment from the polluted site and vice versa.

Fig. 6 illustrates the specific comparisons used to test the capacity of fauna from the two sites to establish within mesocosms containing lightly polluted and heavily polluted sediments with and without resident fauna.

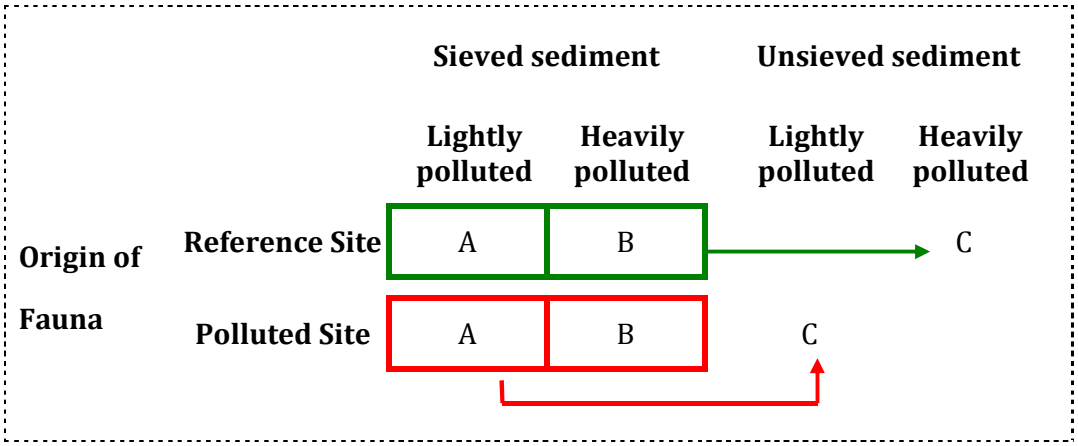


FIGURE 6. Schematic representation of the experimental design: The green boxes show the comparison of treatments for **$H_0$ 1** and the red for **$H_0$ 2**. The green arrow indicates the comparison of treatments for **$H_0$ 5** and the red for **$H_0$ 6**. Full details in text.

## 3.5 Data analysis

### *3.5.1 Univariate analysis*

The assumptions of Analysis of Variance (ANOVA) were examined prior to analysis. In cases of heteroscedastic data, the appropriate transformation to stabilise variances were determined from the relationship between group standard deviations and means,

(Draper & Smith, 1981) and are expressed in terms of the untransformed variate *Y*. All analyses were undertaken using the SAS statistical package v.6.12.

Examining between-site variation in assemblage composition

Shannon diversity was calculated for each month. Mean faunal density and species richness were compared among sites (fixed effect) and sampling dates (random effect) using model III ANOVA. Shannon diversity was compared between sites by single factor Model I ANOVA, with data pooled across samples within months.

Between-site variation in richness at the levels of phyla, class, family and species were examined by single factor Model I ANOVA. For these analyses, data from all months were pooled (n=16). Where significant interaction effects were noted, post-hoc paired comparisons were carried out using a REGWQ (Ryan-Elinot-Gabriel-Welsch) multiple range test.

Analysing the effects of laboratory manipulations

Changes in total faunal abundance, species richness and Shannon diversity were assessed using model I ANOVA. The subset of taxa identified by the SIMPER routine (see next section) as contributing most to the dissimilarity between treatment groups were also compared among treatments using ANOVA.

### *3.5.2 Multivariate analysis of experimental data*

Multivariate analyses offer powerful techniques for detecting differences between complex assemblages (Lindegarth & Hoskin, 2001). The structure of assemblages and changes in community composition were evaluated using the SIMPER routine in PRIMER 5 (Clarke & Warwick, 2001). SIMPER decomposes the average Bray Curtis similarity within sites and dissimilarity between sites into contributions from each variable and ranks their individual contribution. Both the frequency of occurrence and abundance of

each taxa are taken into account. Only the higher-contributing taxa that cumulatively accounted for 90% of the total average (dis)similarity were included in the analyses. Analyses were undertaken on square root transformed data at the level of family.

Multivariate patterns were visualised using non-metric multi-dimensional scaling (nMDS) ordinations obtained from Bray-Curtis dissimilarities.

Permutational multivariate analysis of variance (PERMANOVA, Anderson 2004) based on Bray-Curtis dissimilarities was used to test the significance of difference in multivariate assemblages among sites and treatments. Each term in the analysis was tested using 9999 random permutations. The two p-values reported in the results section, refer to the permutation P-value (P-perm), obtained using permutation of samples, and the Monte Carlo P-value (PMC), which is theoretically expected under limitless numbers of permutations for that particular term in that particular dataset. These two should be very similar for large numbers of permutations; differences arise if there are too few possible permutations to obtain reasonable power, in which case using the Monte Carlo P-value is recommended. Due to the small sample sizes and limited permutations (35) the Monte Carlo values are favoured in all post-hoc tests.

# 4. Results

## 4.1 Temporal survey

### *4.1.1 Heavy metal contamination*

Heavy metal contamination in sediments sampled from the polluted site was up to two orders of magnitude greater than in sediments from the reference site (Table 1). Although sediments at the reference site contained heavy metals, levels were appreciably lower than the polluted site and consequently the site was considered to be comparatively lightly polluted for this study. Contamination levels at the reference site were comparable with other areas within the middle and lower reaches of the Derwent Estuary (Green & Coughanowr, 2003). Sediments from the polluted site markedly exceeded high trigger values set by the Australia and New Zealand Environment and Conservation Council (ANZECC, 2000). ANZECC follows US National Ocean and Atmospheric Administration (NOAA) sediment quality guidelines linking contamination with ecological effects; 'high trigger' points indicating levels of contamination for which there is a 50% probability of biotic change (Macleod & Helidoniotis, 2006).

TABLE 1. Heavy metal concentrations (mg $kg^{-1}$ sediment) at study sites with ANZECC high trigger values for each contaminant. (Mean +S.E.; n=2)

| | Reference site | | Polluted Site | | ANZECC |
|---|---|---|---|---|---|
| Metal | Mean | S.E. | Mean | S.E. | High Trigger |
| Zinc | 467 | 21.5 | 55 300 | 14 600 | 410 |
| Lead | 191 | 7.5 | 10 665 | 3 035 | 220 |
| Copper | 29 | 1 | 2 880 | 1 230 | 270 |
| Arsenic | 8 | 0 | 2 400 | 160 | 70 |
| Cadmium | 2 | 0 | 606 | 174 | 10 |
| Mercury | 3 | 0.35 | 160 | 10 | 1 |

### *4.1.2 Assemblage characteristics*

Differences in the mean density of fauna (total number of animals per grab) between the reference and polluted sites throughout the sampling period, and between months at either site were not significant (Fig. 7a and Table 2). In marked contrast species richness at the polluted site was considerably lower than at the reference site (Fig 7b and Table 2). Two-way ANOVA of species richness indicated a significant interaction between site and sampling month (Table 2). REGWQ pairwise contrasts showed that species richness was significantly different between sites and significantly lower at the polluted site in October compared with other sample months at that site. Differences between months at the reference site were not significant ($P>0.05$).

Shannon diversity indices were calculated for each site from data pooled across samples within months (Fig7c.). Species diversity at the polluted site was considerably lower than at the reference site (Table 3). Diversity was highest at the reference site in mid-summer (December) and lowest at the polluted site in spring (October).

The disparity in species richness and diversity of the assemblages between sites is evident from inspection of the rank-abundance curves (Fig. 8). Fauna from the polluted site generates a curve with a steep slope and attenuated tail, indicating high abundance of relatively few species and a low overall evenness. In contrast, the reference site has a shallower slope and a long tail to the right, indicating a large number of species and high level of evenness.

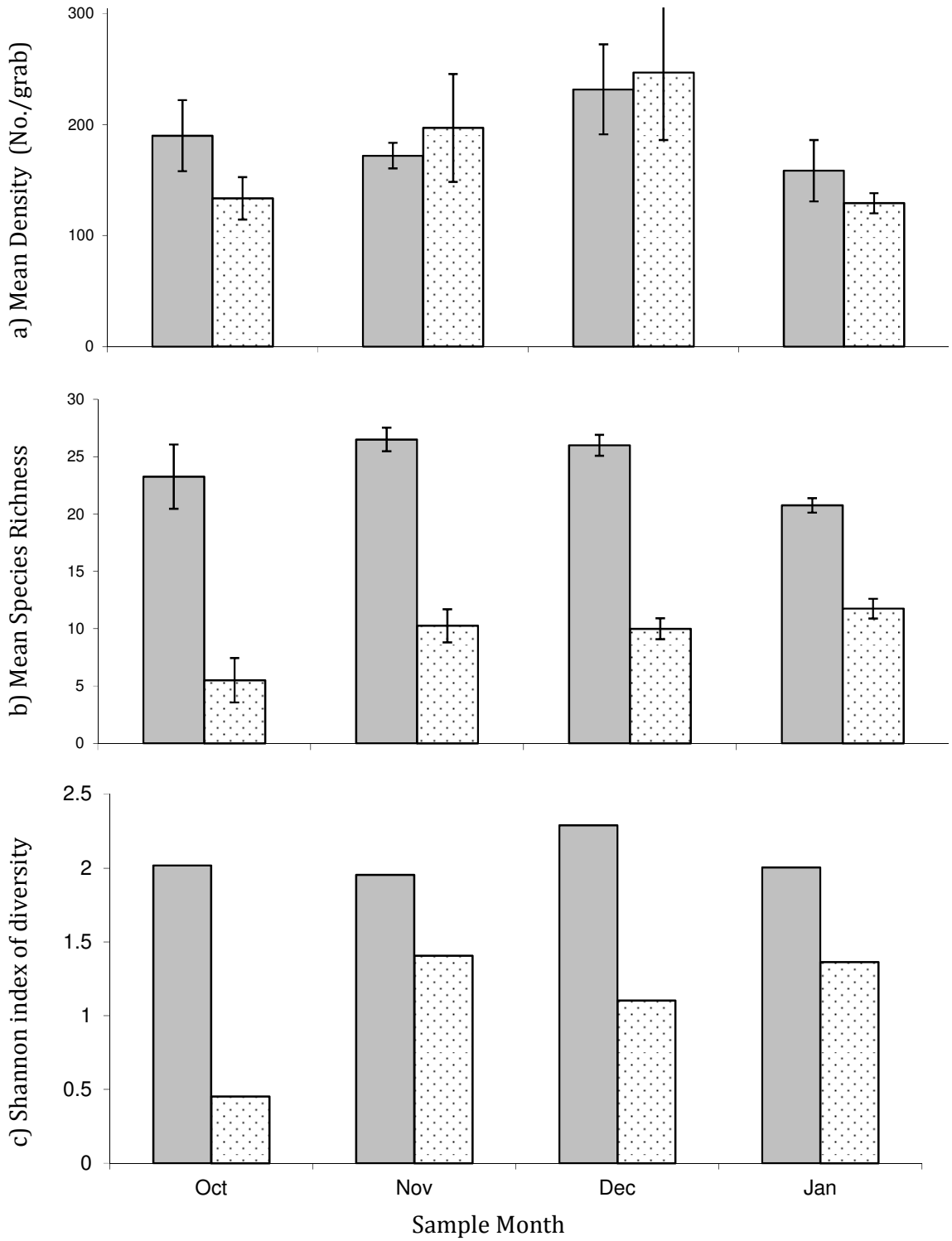


FIGURE 7. Properties of benthic assemblages sampled monthly between October 2005 and January 2006 at the reference site (shaded) and polluted site (dotted), (+S.E., n=4, (a) Mean density, (b) mean species richness, and (c) Shannon index of diversity (data pooled across samples).

TABLE 2. Two-way analysis of variance of the mean density and species richness of fauna sampled monthly from October 2005 to January 2006 at the reference site and polluted site (n=4)

| Source | df | MS | F | p |
|---|---|---|---|---|
| Density | | | | |
| Site | 1 | 0.1229 | 0.88 | 0.3586 |
| Month | 3 | 0.3263 | 2.33 | 0.1002 |
| Site x Month | 3 | 0.0585 | 0.42 | 0.7423 |
| Species Richness | | | | |
| Site | 1 | 1740.5 | 197.97 | 0.0001 |
| Month | 3 | 26.9167 | 3.06 | 0.0474 |
| Site x Month | 3 | 30.5833 | 3.48 | 0.0315 |

Transformation: density = log (Y+1), no transformation was required for species richness data.

TABLE 3. One-way analysis of variance of Shannon diversity index of assemblages between sites (data pooled across sample months, n=4)

| Source | df | MS | F | p |
|---|---|---|---|---|
| Site | 1 | 1.942716 | 17.98233 | 0.0054 |

No transformation required.

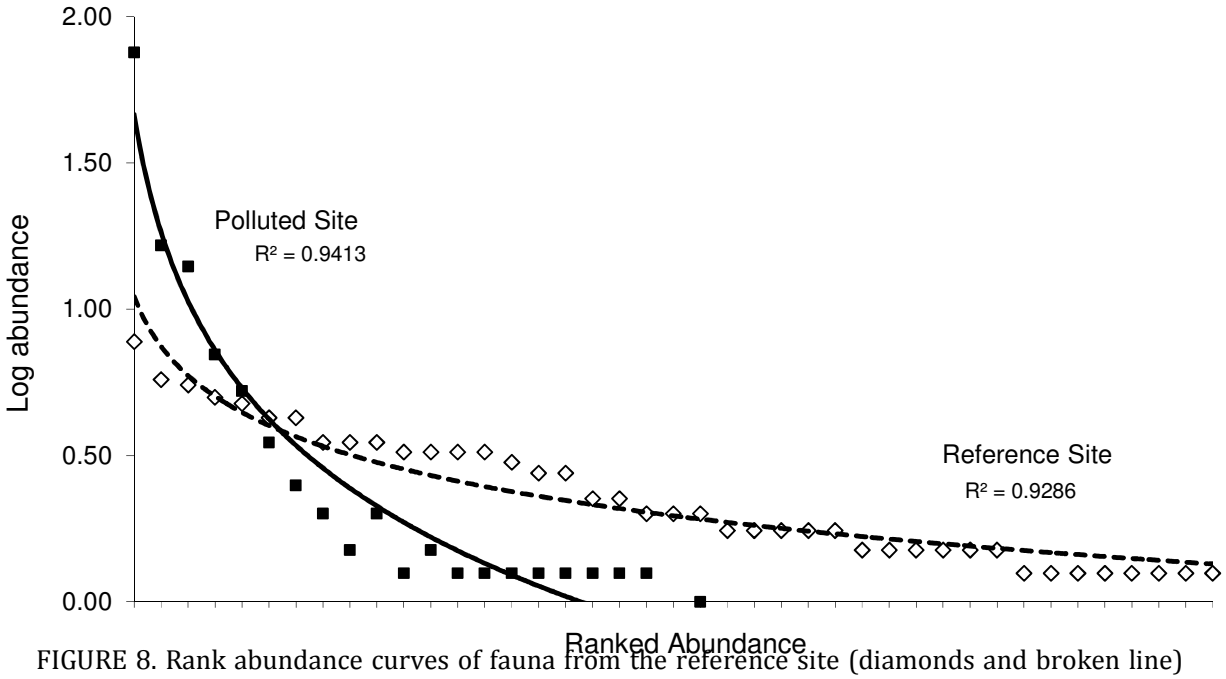


FIGURE 8. Rank abundance curves of fauna from the reference site (diamonds and broken line) and polluted site (black squares and solid line). Ranks signify proportional contribution to the whole community in order of decreasing abundance. The data was pooled from 4 months samples (n=16) and log transformed. $R^2$ values signify a good fit of the curves to the data points.

Assemblages from the reference site were taxonomically more complex than assemblages from the polluted site (Fig. 9). One-way ANOVAs showed that richness was significantly different between these sites at all of the taxonomic levels assessed ($P<0.001$) (Table 4). Two-way analysis was ruled out as the crossed factors would lack independence – all species also being contained within the taxonomic level of family, class, phyla and so on.

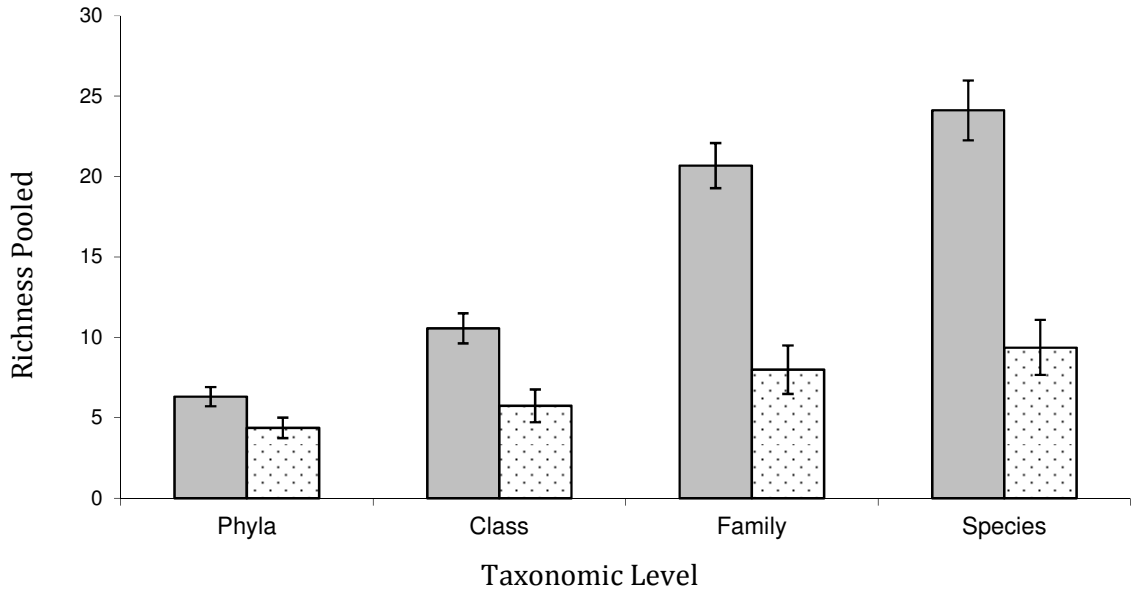


FIGURE 9. Mean taxonomic richness of assemblages sampled at the reference site (shaded) and polluted site at four taxonomic levels. Mean values were calculated from data pooled across the 4 months of samples. (Mean +S.E., n=16).

TABLE 4. One-way analyses of variance of taxonomic richness of assemblages sampled from the reference and polluted sites. Data was pooled across 4 months of samples (n=16).

| Source | df | MS | F | p |
|---|---|---|---|---|
| Phylum | 1 | 1.73489 | 18.04 | 0.0001 |
| Class | 1 | 7.3263 | 40.42 | <0.0001 |
| Family | 1 | 30.0615 | 110.04 | <0.0001 |
| Species | 1 | 34.7854 | 103.15 | <0.0001 |

Transformation: $Y^{-0.48}$.

### *4.1.3 Community composition*

Non-metric multidimensional scaling ordination indicated that community composition differed markedly between sites and that communities at the polluted site were more variable during the sampling period than those at the reference site (Fig. 10). These differences between the sites were highly significant (PERMANOVA, $F=62.35$, $P<0.001$).

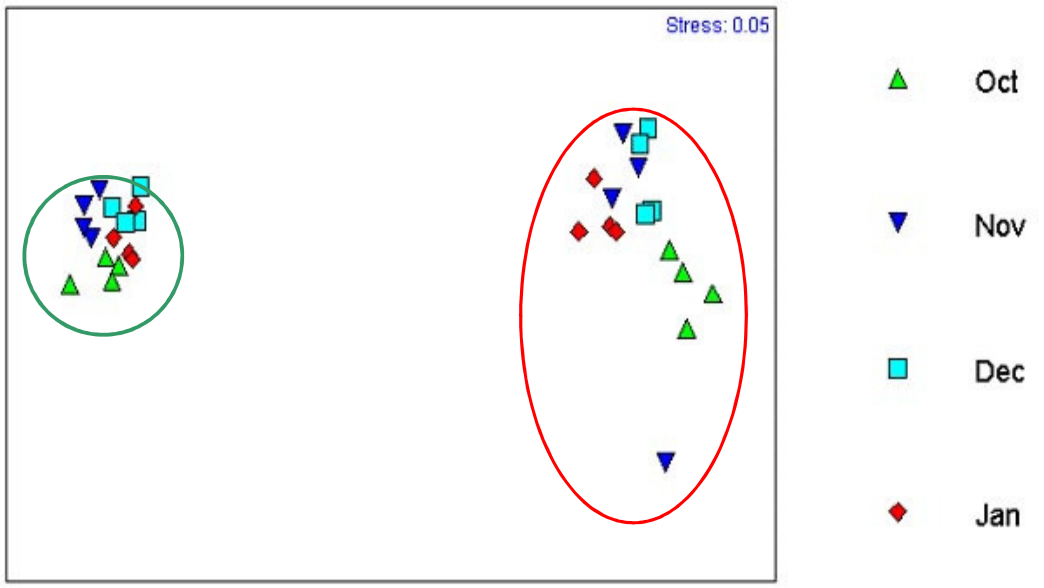


FIGURE 10. nMDS ordination of multivariate assemblages collected monthly at the reference site (green circle) and the polluted site (red ellipse) between October 2005 and January 2006. Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

The SIMPER protocol was used to identify the most important taxa for characterising assemblages and differentiating between sites. Table 5 lists the most influential taxa in discriminating between sites (90% cut-off level), along with their mean density and proportional contribution to similarity within assemblages. The average dissimilarity in assemblages between sites was 82%. Four families (cirratulids, maldanids, capitellids and corophids) accounted for 48% of this dissimilarity, with each of these families represented by only one or two species.

TABLE 5. SIMPER analysis partitioning the between-site dissimilarity attributable to individual taxa and the relative contribution of these taxa to within-site similarity. The mean density of taxa was calculated by converting animals/grab into individuals $m^{-2}$, by adjusting for the grab surface area of 0.0675 m2 (data was pooled across sample months, n=16). The taxa listed explain 90% of the dissimilarity between sites.

| | Between Site | Reference Assemblages | | | Polluted Assemblages | | |
|---|---|---|---|---|---|---|---|
| | Dissimilarity % | Mean Density | S.E. | Similarity % | Mean Density | S.E. | Similarity % |
| **Polychaetes** | 49.32 | | | 48.40 | | | 62.71 |
| Maldanidae | 17.62 | 1477 | 70 | 31.38 | | | |
| Cirratulidae | 17.10 | 15 | 5 | | 1679 | 129 | 61.91 |
| Capitellidae | 5.15 | 223 | 73 | 6.44 | 13 | 9 | 0.11 |
| Terebellidae | 2.61 | 46 | 9 | 3.21 | | | |
| Spionidae | 1.82 | 31 | 9 | 2.55 | 5 | 2 | 0.30 |
| Sabellidae | 1.65 | 23 | 5 | 1.84 | 49 | 18 | 0.39 |
| Sigalionidae | 1.33 | 15 | 4 | 1.32 | | | |
| Polynoidae | 1.06 | 9 | 2 | 1.04 | | | |
| Trichobranchidae | 0.98 | 11 | 4 | 0.62 | | | |
| **Molluscs** | 6.63 | | | 8.22 | | | |
| ***Bivalves*** | 5.16 | | | 7.04 | | | |
| Nuculanidae | 3.46 | 65 | 9 | 5.25 | | | |
| Semelidae | 1.70 | 22 | 6 | 1.79 | | | |
| ***Gastropoda*** | 1.47 | | | 1.18 | | | |
| Nassaridae | 1.47 | 21 | 8 | 1.18 | | | |
| **Crustaceans** | 24.08 | | | 28.66 | | | 25.78 |
| Tanaidacea | 4.90 | 281 | 69 | 9.63 | 33 | 10 | 3.48 |
| Cumacea | 3.08 | 62 | 15 | 3.77 | | | |
| Callianassidae | 2.45 | 32 | 5 | 3.73 | | | |
| ***Amphipoda*** | 12.71 | | | 9.53 | | | 21.32 |
| Corophiidae | 7.94 | 19 | 7 | | 553 | 176 | 17.15 |
| Ampeliscidae | 2.97 | 127 | 21 | 7.14 | 33 | 6 | 4.17 |
| Isaeidae | 1.80 | 25 | 5 | 2.39 | 2 | 1 | |
| **Other taxa** | 9.99 | | | 12.26 | | | 10.64 |
| Nemertea | 2.87 | 106 | 19 | 6.23 | 29 | 9 | 3.11 |
| Ophiuroidea | 1.52 | 16 | 3 | 1.88 | | | |
| Sipunculidae | 1.31 | 16 | 4 | 1.13 | | | |

Assemblages from both sites were moderately consistent throughout the sampling period, with an average similarity of 67% between all reference samples and 61% between all polluted samples (n = 16). The lower similarity among polluted samples was also evident in the ordination plot (Fig. 10). Assemblages from the reference site were characterised by a diverse taxa with fifteen families accounting for 90% of the similarity, compared with only four families demonstrating similar dominance within assemblages from the polluted site.

Polychaetes were the most useful group for discriminating between sediment conditions accounting for 49% of the dissimilarity (Table 5). Five polychaete families (maldanids, capitellids, terebellids, spionids and sabellids) contributed 45% of the total average similarity within assemblages from the reference site. Maldanids were highly characteristic (31% similarity) and very abundant with an average density of 1477 $m^{-2}$, comprising 80% of all polychaetes and 54% of total faunal abundance. The capitellid, *Mediomastus australiensis*, was the next most abundant taxon with a mean density of 223 $m^{-2}$. The remaining polychaete families had mean densities less than 50 $m^{-2}$. Polychaetes were more abundant (15%) and less diverse (56%) at the polluted site. Cirratulids were even more abundant at the polluted site than maldanids were at the reference site, with an average density of 1671 $m^{-2}$, accounting for 96% of all polychaetes, 65% of total faunal abundance and 62% of total similarity. One species, *Cirriformia filigera,* of cryptogenic origin, accounted for 74% of this cirratulid abundance. The only other polychaete observed in large numbers was the introduced sabellid, *Euchone limnicola*, which in late summer reached densities of 50 $m^{-2}$ but was largely absent at other times. *E. limnicola* was found at lower densities of 23 $m^{-2}$ at the reference site throughout the sampling period.

Table 5 shows that crustaceans were the next most important group in discriminating between sites (26%) Callianassids and cumaceans were abundant at the reference site and absent at the polluted site. Tanaids were present at the polluted site, but their abundance and diversity was reduced (85% fewer and only 2 species compared with 6 at the reference site). Decapod crustaceans were largely absent from the polluted site. Two species of crabs (*Hexapus granuliferus* and *Halicarcinus ovatus*) and two species of squat lobster (*Munida haswelli* and an unidentified species) were sampled relatively frequently at the reference site but did not occur at the polluted site (these taxa were not influential in discriminating between sites at the 90% cut-off level). Amphipods were both more abundant and rich at the polluted site than the reference site, with an average dissimilarity of 13%. However, of the seven amphipod families detected at the polluted site the introduced amphipod, *Corophium ascherusicum,* accounted for 93% of the total amphipod abundance. *C. ascherusicum*, was the second most abundant species at the polluted site overall and showed a distinct peak in abundance during November and December (84%).

Molluscs were not well represented within the polluted sediments. The only bivalve detected was the introduced species *Corbula gibba* and it was present in relatively low densities. Corbula was also found at low densities at the reference site, along with four other bivalve families (cardiids, mytilids, nuculanids and semelids). Nassarid gastropods were relatively common at the reference site but were absent from the polluted site, although the samples contained a large number of empty shells.

The main source of temporal variability within the polluted site (Fig 8) was also explored using SIMPER. The three species that best discriminated the polluted conditions over time were: *C. ascherusicum,* a corophid amphipod; *E. limnicola,* a sabellid polychaete, and *Aphelochaeta* sp, a cirratulid polychaete. Abundances of each of these

species overlaid on the ordination plot of the total community data from the polluted site show sequential 'boom-bust' dynamics with *C. ascherusicum* dominating in mid summer and *E. limnicola* in late summer (Fig. 10a, and b). A similar treatment of *C. filigera* (Fig 10c) shows clearly that this species was consistently present at high densities throughout the sampling period, contributing up to 97% of the total faunal abundance (mean, 54%).

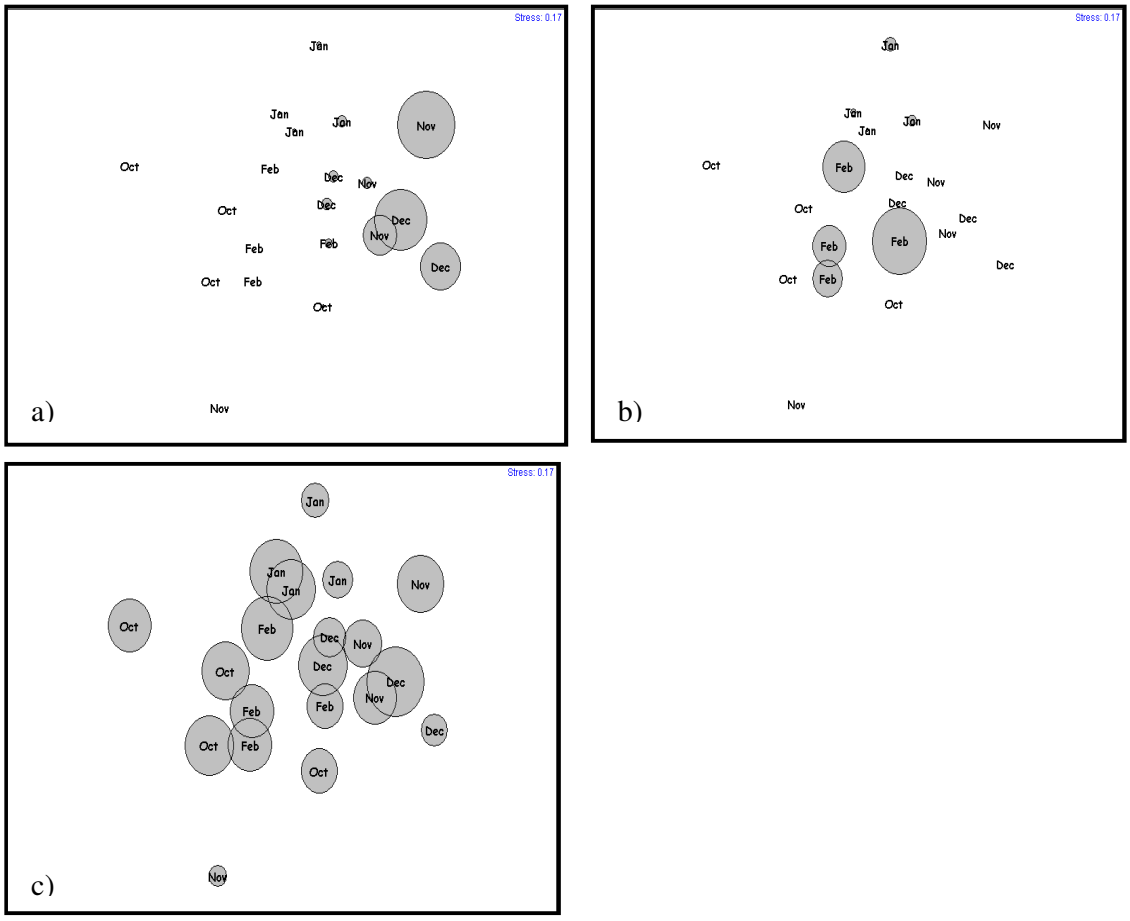


FIGURE 11.Bubble plots of (a) *Corophium ascherisicum*, (b) *Euchone limnicola*, and (d) *Cirriformia filigera* overlaid on an ordination plot of fauna samples collected monthly at polluted sites between October 2005 and January 2006.

## 4.2 Experimental manipulations

Field observations showed that the polluted site supported a comparatively simple fauna. Manipulative experiments were then performed to assess the mechanisms structuring those assemblages.

### *4.2.1 Experiment 1: Does sediment toxicity directly influence faunal assemblages?*

Unfortunately, this experiment was unsuccessful because initial freezing of sediments to eliminate fauna caused a marked increase in organic matter from decomposing animals. The large organic loads resulted in hypoxic conditions within all sediments and translocated fauna suffered massive mortality irrespective of treatments.

### *4.2.2 Experiment 2: Do sediments and resident assemblages collectively influence fauna?*

Experiment 2 tested the collective effects of sediment contamination and resident fauna on the survival of translocated fauna. There were four replications of four 'treatment' groups per site, viz.-

A. 'Natural' – characterised by fauna sieved from grab contents with no further treatment.
B. 'Mesocosm' – characterised by fauna transferred directly with grab contents into mesocosms and maintained for 28 days.
C. 'Light pollution' – characterised by adding fauna into mesocosms for 28 days containing lightly polluted sediment (containing resident communities) collected from the reference site.
D. 'Heavy pollution' – characterised by adding fauna into mesocosms for 28 days containing heavily polluted sediment (containing resident communities) collected from the polluted site.

<u>Survival within mesocosms</u>

The designs for experiments 1 & 2 included an assessment of how well the fauna survived incubation for one month within laboratory based mesocosm, hereafter referred to as the 'mesocosm effect'. This was indicated by comparing fauna supported in mesocosms one month after initial establishment (the 'mesocosm community') with the

fauna initially added to the mesocosm, indicated by the faunal composition in fresh grab samples (the 'natural community'). Although experiment 1 failed in testing the main treatment effects (see 4.2.1), the test for a 'mesocosm effects' was successful as the mesocosm treatments did not require defaunating and were therefore not frozen. There were significant mesocosm effects on the community composition of assemblages from both sites in both experiments (Table 6).

TABLE 6. Tests for 'mesocosm effect' on fauna from both sites during two laboratory experiments using permutational multivariate ANOVA (n=4).

| Source | df | MS | F | P |
|---|---|---|---|---|
| *Experiment 1* | | | | |
| Mesocosm effect on fauna from reference site | 1 | 3222 | 4.8543 | 0.0304 |
| Residual | 6 | 663 | | |
| Mesocosm effect on fauna from polluted site | 1 | 2819 | 4.9990 | 0.0283 |
| Residual | 6 | 564 | | |
| *Experiment 2* | | | | |
| Mesocosm effect on fauna from reference site | 1 | 2187 | 3.4520 | 0.0285 |
| Residual | 6 | 633 | | |
| Mesocosm effect on fauna from polluted site | 1 | 1795 | 2.3859 | 0.0582 |
| Residual | 6 | 752 | | |

Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

Mesocosm assemblages were 46% dissimilar to natural communities (Table 7; SIMPER analysis shows the individual taxa responsible for the dissimilarity). It is clear that maintaining assemblages from the reference site within the mesocosm apparatus resulted in losses of some taxa, particularly crustaceans and polychaetes, and accordingly

all experimental treatments were performed on a reduced community. After incubation of one month, sediments from the reference site still supported 37 families and 50 species (in the mesocosms) compared with 41 families and 63 species (in the 'natural' communities).

Fauna from the polluted site was slightly less affected. In this instance, only three species accounted for 58% of the dissimilarity between the mesocosm and natural assemblages. The gammarid amphipod, *Corophium ascheriscicum* was strongly affected, with abundances reduced by 91% in the mesocosm after incubation of one month. Numbers of phoronid worms declined by 80% and the abundance of the polychaete *Cirriformia filigera*, the dominant species in the impacted communities, was reduced by 20%. There was no significant reduction in the number of species or family richness.

Overall, it was concluded that despite the obvious effect of the mesocosms on community structure, the resultant communities were sufficiently complex and representative to enable testing for treatment effects.

TABLE 7. Mean abundance of individual taxa identified by SIMPER as contributing most to the dissimilarity between the natural and mesocosm communities derived from fauna collected at the reference site, after a 1-month incubation. The table shows the percentage differences in abundance and the contribution to dissimilarity between treatment groups that each taxon makes. (N=4).

*Groups – 'Natural' & 'Mesocosm' communities. Average dissimilarity = 47%*

| Family | 'Natural' Community Mean Abundance | 'Mesocosm' Community Mean Abundance | Abundance difference (%) | Contrib. to dissim. (%) |
|---|---|---|---|---|
| **Polychaetes** | | | | |
| Maldanidae | 107.75 | 62.63 | 42 | 8 |
| Capitellidae | 5.75 | 1.38 | 76 | 5 |
| Spionidae | 1.75 | 0.5 | 71 | 3 |
| Trichobranchidae | 1.5 | 0.75 | 50 | 3 |
| Terebellidae | 1.63 | 1.25 | 23 | 3 |
| Polynoidae | 0.63 | 0.25 | 60 | 2 |
| Sigalionidae | 1.25 | 0.88 | 30 | 2 |
| **Crustaceans** | | | | |
| Tanaidacea | 9.88 | 1.13 | 89 | 7 |
| Cumacea | 4.5 | 0.25 | 94 | 4 |
| Ampeliscidae | 6 | 1.63 | 73 | 4 |
| Isaeidae | 1.5 | 0.13 | 91 | 3 |
| Callianassidae | 0.88 | 0.63 | 28 | 2 |
| Hymensomatidae | 0.88 | 0.13 | 85 | 2 |
| Hexapodidae | 0.63 | 0.5 | 21 | 2 |
| Galatheidae | 0.5 | 0.13 | 74 | 2 |
| **Others** | | | | |
| Nemertea | 5 | 2 | 60 | 4 |
| Nuculanidae | 5.5 | 3.88 | 29 | 4 |
| Nassaridae | 2.63 | 0.38 | 86 | 3 |
| Sipuncula | 1.13 | 0.88 | 22 | 2 |

Manipulating fauna collected from the reference site to test H0 3

The global effect of treatment on fauna from the reference site was highly significant (Table 8; P (perm) <0.001). The following orthogonal planned contrasts were made:

- B versus C, to monitor the procedural effect of sieving fauna out of grab samples prior to transferring into mesocosms

- C versus D, to test the capacity of additional fauna from the reference site to establish within mesocosms containing lightly polluted and heavily polluted sediments with their resident fauna intact

While there appears to be some effect of sieving and reintroducing fauna (in addition to the 'mesocosm effect' already detailed, see above), this effect is relatively small (Table 7; contrast B versus C, PMC>0.05, 39% dissimilarity). This is apparent in the nMDS ordination, which shows a small separation between the 'Mesocosm' and 'Light Pollution' treatments (Fig. 12; overlapping green and light blue ellipses). There was a significant difference between assemblages maintained for a month within mesocosms containing heavily polluted sediments, with resident fauna, and those maintained within lightly polluted sediments, with resident fauna, (Table 8; contrast C versus D, PMC<0.05, 44% dissimilarity). This is apparent in the ordination as a separation between the green and red ellipses. Thus, heavily polluted sediments and/or their resident assemblages had a greater effect on the capacity of additional fauna from the reference site to establish than lightly polluted sediments and/or their resident assemblages.

TABLE 8. Permutational multivariate ANOVA to test the significance of differences in multivariate assemblages collected from the reference site responding to four treatments: A. 'Natural', B. 'Mesocosm', C. 'Light pollution' and D. 'Heavy pollution' (n=4). Global effect and a-priori planned contrasts were made between B and C, and C and D.

| Source | df | MS | Permutations | F | P(perm) |
|---|---|---|---|---|---|
| Treatments (A, B, C, D) | 3 | 2406 | 9999 | 4.6487 | 0.0001 |
| Residual | 12 | 517 | | | |
| Contrasts | t | Dissimilarit | Permutations | P(perm | P(MC) |
| 1. B versus C | 1.527 | 39% | 35 | 0.0281 | 0.0795 |
| 2. C versus D | 1.802 | 44% | 35 | 0.0292 | 0.0228 |

Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

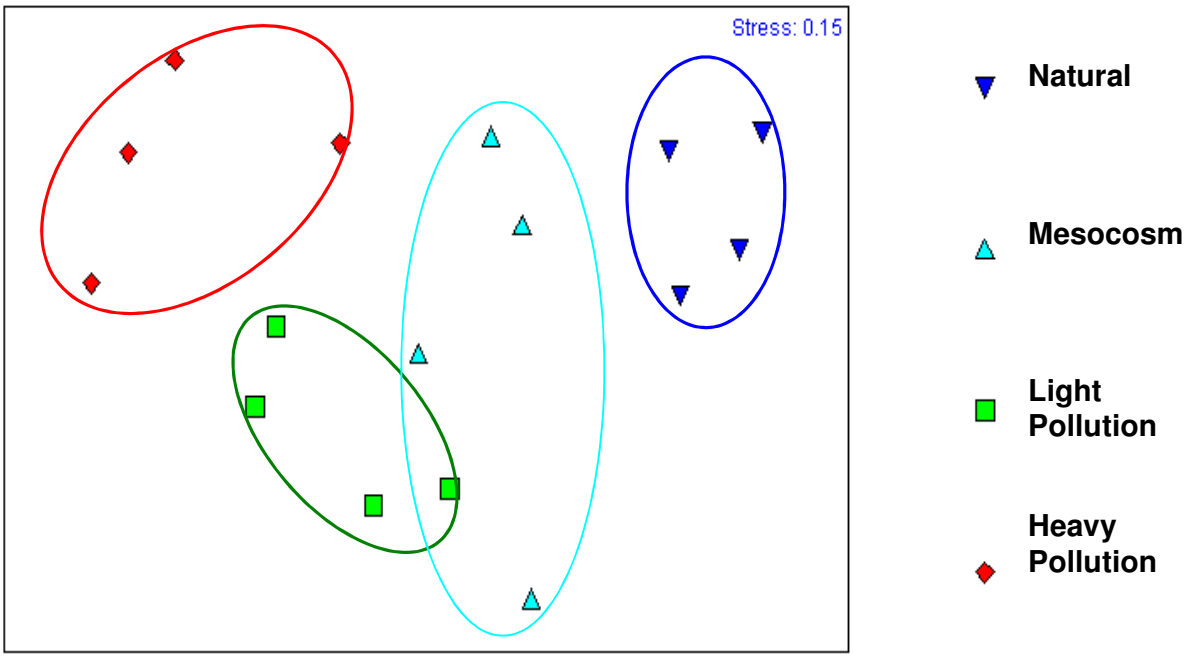


FIGURE 12. nMDS ordination of multivariate assemblages collected from the reference site responding to the four treatments – A) 'Natural', B) 'Mesocosm', C) 'Light pollution' and D) 'Heavy pollution' (n=4). Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

Manipulating fauna collected from the polluted site to test H0 4

The global effect of treatment on fauna from the polluted site was not significant (Table 9; P (perm) >0.05). The ordination plot shows a general scatter of samples, with no clear distinction between assemblages from the different treatments (Fig 13). Thus, the levels of sediment pollution and/or resident assemblages have not been shown to collectively influence the capacity of additional fauna from the polluted site to establish.

TABLE 9. Permutational multivariate ANOVA to test the significance of differences in multivariate assemblages collected from the polluted site responding to the four treatments – 'Natural', 'Mesocosm', 'Light pollution' and 'Heavy pollution' (n=4).

| Source | df | MS | Permutations | F | P(perm) |
|---|---|---|---|---|---|
| Treatment | 3 | 703.9999 | 9999 | 1.3136 | 0.2519 |
| Residual | 12 | 535.9229 | | | |

Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

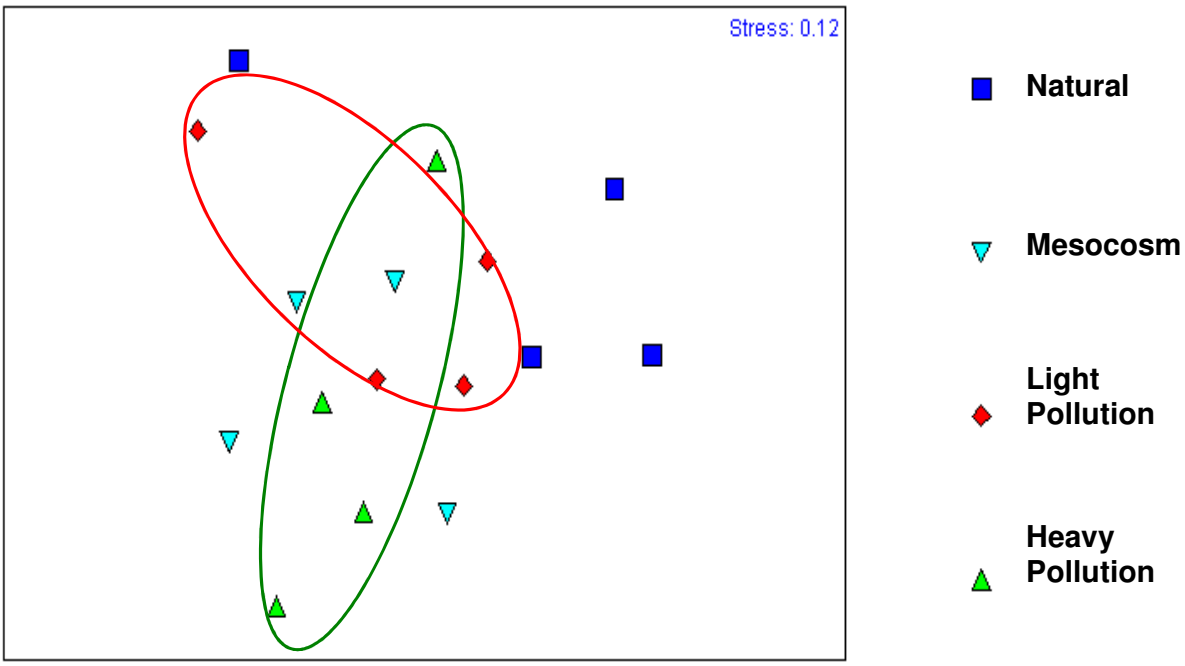


FIGURE 13. nMDS ordination of multivariate assemblages collected from the polluted site responding to the four treatments – A) 'Natural', B) 'Mesocosm', C) 'Light pollution' and D) 'Heavy pollution' (n=4). Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

### *4.2.3 Experiment 3: Separating the effects of sediment contamination and residual fauna on newly establishing individuals*

Experiment 3 isolated the effects of resident faunas from the direct effects of sediment contamination on the survival of translocated fauna. There were three 'treatment' groups per site, viz.-

A. 'Light pollution' – characterised by adding fauna into mesocosms for 28 days containing lightly polluted sediment (from the references site) that earlier had been sieved to remove resident communities.

B. 'Heavy pollution' – characterised by adding fauna into mesocosms for 28 days containing heavily polluted sediment (from the polluted site) that earlier had been sieved to remove resident communities.

C. 'Resident fauna' – characterised by adding fauna into mesocosms for 28 days containing unsieved sediments. Fauna from the reference site was transferred into unsieved sediment from the polluted site and vice versa.

Manipulating fauna collected from the reference site to test $H_0$ 5

The global treatment effect was highly significant (Table 10), while the direct effect of sediment pollution examined in particular *a-priori* pairwise contrasts was not significant (A versus B; PMC>0.05), and the effects of the resident fauna only marginally significant depending on which P value is accepted (B versus C; PMC>0.05, Pperm<0.05). The *a-priori* pairwise contrasts using permutational analysis lacked power due to the small sample sizes and so there was a strong likelihood of Type-II error. The ordination plot shows clear separation of the multivariate assemblages from the different treatment groups (Fig. 14). After one month, fauna collected at the reference site and transferred into mesocosms containing sieved heavily polluted sediment was 26% less abundant and

30% less taxonomically rich than fauna incubated within sieved lightly polluted sediment (Table 11).

TABLE 10. Permutational multivariate ANOVA to test the significance of differences in multivariate assemblages collected from the reference site responding to the three treatments – A) 'Light pollution', B) 'Heavy pollution' and C)'Resident fauna' (n=4).

| Source | df | MS | Permutations | F | P (perm) |
|---|---|---|---|---|---|
| Treatments (A, B, C) | 3 | 2411 | 5775 | 6.6791 | 0.001 |
| Residual | 12 | 361 | | | |
| **Contrasts** | **t** | **Dissimilarity** | **Permutations** | **P (perm)** | **P (MC)** |
| (A versus B) | 1.31 | 29% | 35 | 0.1137 | 0.1883 |
| (B versus C) | 1.59 | 33% | 35 | 0.0292 | 0.0860 |

Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

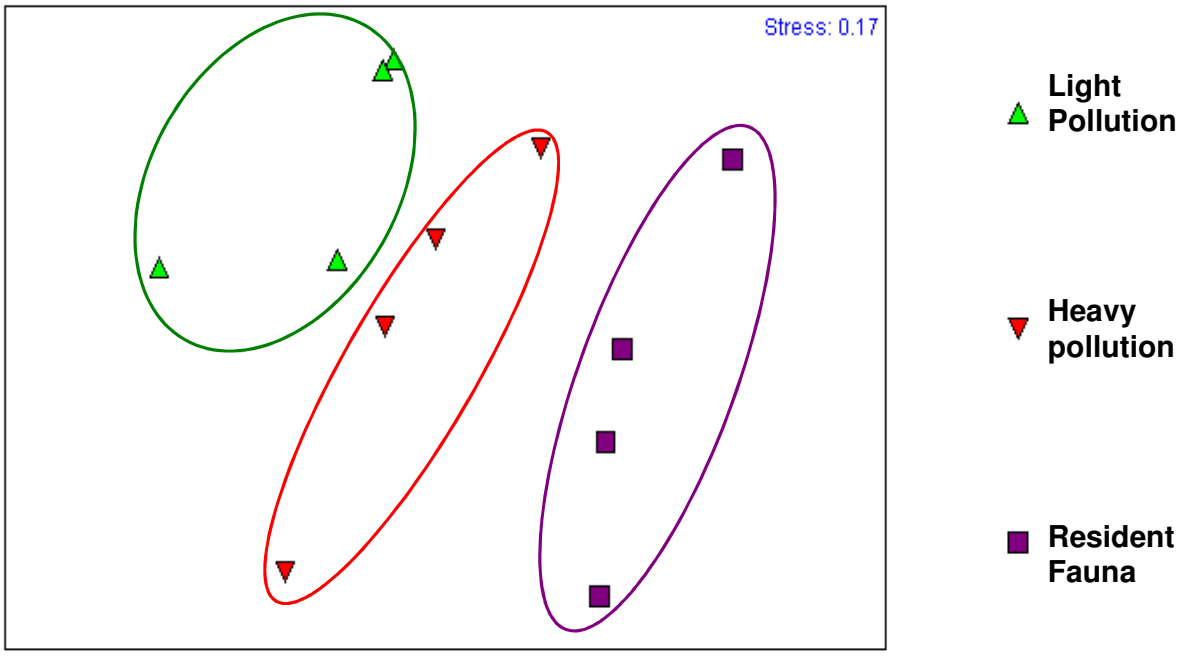


FIGURE 14. nMDS ordination of multivariate assemblages collected from the reference site responding to the three treatments – A) 'Light Pollution', B) 'Heavy Pollution' and C) 'Resident Fauna' (n=4). Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

TABLE 11. Final composition of fauna collected from the reference site and transferred into mesocosms for one month and subjected to three different treatments - 'Light pollution', 'Heavy pollution' and 'Resident fauna' (n=4). Figures marked with an asterisk differ significantly ($P<0.05$)

| | Abundance | | Richness | | Diversity | |
|---|---|---|---|---|---|---|
| Treatments | Mean | S.E. | Mean | S.E. | Mean | S.E. |
| Lightly Pollution | 92 | 7 | 8.25 | 0.48 | 1.1 | 0.1 |
| Heavily Pollution | 68* | 9 | 5.75 | 1.44 | 0.8 | 0.1 |
| Resident fauna | 38* | 2 | 5.75 | 0.48 | 1.2 | 0.1 |

SIMPER analysis suggests that sediment toxicity led to a reduction in certain taxa (Table 12). A reduction in bivalves was the most conspicuous effect, contributing 32% of the difference between the sediment treatments. Semelid abundance fell by 78%, the only statistically significant change within this treatment group. Corbulids and Nuculanids lost 51% and 45% respectively, however corbulids were completely absent in the natural grab communities and seem to have a very patchy distribution among the samples. Maldanid and sigalionid polychaetes dropped in abundance by 15% and 67% respectively, although sigalionid density was initially less than one per sample and so the difference may be attributable to stochastic variation. Crustaceans were all reduced in the polluted sediment; callianasids fell by 83% and other decapods by 100%.

Fauna transferred into unsieved sediment was significantly less abundant (38%) after 1 month than fauna transferred into sieved polluted sediment (Table 11). The main effect was a significant loss of maldanids (56%) (Table 12). A non-significant effect was also detected in the remaining bivalve families, which suffered a further 50% reduction in abundance in the presence of the resident fauna on top of the 60% reduction recorded in the sieved polluted sediment.

TABLE 12. Mean abundance of individual taxa identified by SIMPER as contributing most to the dissimilarity between treatment groups of reference assemblages. The table shows the percentage difference in abundance and contribution to dissimilarity.

*Treatments – light pollution versus heavy pollution,* Average dissimilarity = 29%

| Family | Lightly polluted sediment Av. Abund | Heavily polluted sediment Av. Abund | Difference % | Contrib % |
|---|---|---|---|---|
| Corbulidae | 14.25 | 7 | 51 | 13.17 |
| Semelidae | 2.25 | 0.5 | 78* | 9.68 |
| Nuculanidae | 2.75 | 1.5 | 45 | 8.78 |
| Maldanidae | 62.25 | 52.75 | 15 | 8.76 |
| Callianasidae# | 1.5 | 0.25 | 83 | 8.41 |
| Sigalionidae | 0.75 | 0.25 | 67 | 6.09 |
| Decapoda (excluding #) | 0.75 | 0 | 100 | 5.97 |
| Ophiuroidea | 0.5 | 0 | 100 | 4.96 |

*Treatments – heavily polluted sediment, sieved versus unsieved,* Average dissimilarity =

| Family | Sieved sediment Av. Abund | Unsieved sediment Av. Abund | Difference % | Contrib % |
|---|---|---|---|---|
| Maldanidae | 52.75 | 23 | 56* | 26.68 |
| Corbulidae | 7 | 4 | 43 | 11.4 |
| Nuculanidae | 1.5 | 1.25 | 17 | 8.15 |
| Semelidae | 0.5 | 0.25 | 50 | 5.36 |

Transformation: Semelids = Sqrt (Y), Maladanids = Log (Y+1). * significant change ($P<0.05$)

Manipulating fauna collected from the polluted site to test $H_0$ 6

The global effect of treatment on fauna from the polluted site was not significant (Table 13; P (perm) >0.05). The ordination plot shows a general scatter of samples, with no clear distinction between assemblages from the different treatments (Fig 15). Thus, fauna from the heavily polluted site were able to establish equally well in sediments from

the reference site either with or without their resident assemblages. It should be noted however that the natural community was dominated by only two species (*Cirriformia filigera* -78%, *Corophium ascheriscicum* -16%), and the latter did not survive the experimental procedure (as with other gammarid amphipods in the reference assemblages). It should be noted therefore that the treatment effects were largely being trialled on a single species, the consistently dominant cirratulid, *C. filigera*, and it is this species that was equally abundant within all treatment groups.

TABLE 13. Permutational multivariate ANOVA to test the significance of differences in multivariate assemblages collected from the polluted site responding to the three treatments – A) 'Light pollution', B) 'Heavy pollution' and C)'Resident fauna' (n=4).

| Source | df | MS | Permutations | F | P(perm) |
|---|---|---|---|---|---|
| Treatments (A, B, C) | 3 | 704 | 9999 | 1.3136 | 0.2519 |
| Residual | 12 | 536 | | | |

Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

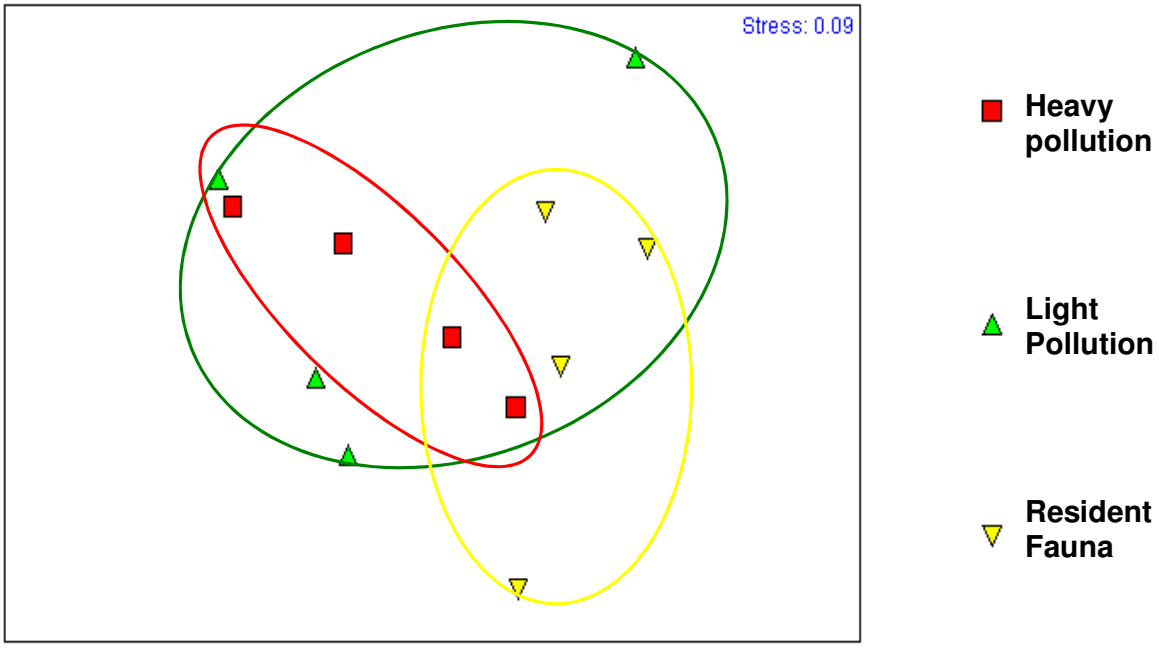


FIGURE 15. nMDS ordination of multivariate assemblages collected from the polluted site responding to the three treatments – 'Light pollution', 'Heavy pollution' and 'Resident fauna' (n=4). Analysis based on Bray-Curtis matrix derived from square-root transformed data at family level.

# 5. Discussion

A number of models have been proposed relating pollution to macrobenthic succession within the marine environment (Pearson & Rosenberg, 1978; Ferraro *et al.*, 1991; Lee *et al.*, 2006). Lee (2006) summarises the response of fauna to increasing pollution in terms of a progressive loss of biological richness, regression of sensitive and proliferation of tolerant species, culminating in dominance by opportunistic species. The heavily polluted site of this study is consistent with this model. The soft-sediment assemblages at the heavily polluted site differed significantly from those sampled at the less polluted reference site. Both sites supported a similar density of fauna. However, in accordance with other studies (e.g. Rygg, 1985b; Agard *et al.*, 1993; Stark, 1998b) there was a decline in faunal richness and diversity and a concomitant increase in dominance of a few species at the polluted site. The disparity in taxonomic richness was apparent at all taxonomic levels, including phyla, but was particularly pronounced at family and species level.

## 5.1 Regression of sensitive and proliferation of tolerant species

Several studies of the effects of heavy metal pollution on macrobenthic assemblages have suggested that impacts are characterised by a move from assemblages dominated by crustaceans and molluscs to dominance by subsurface-dwelling, surface deposit feeding annelids (Rygg, 1985a; Warwick & Clarke, 1993; Gaston *et al.*, 1998). In keeping with these findings, crustaceans were far less abundant and diverse at the polluted site and molluscs were largely absent, except for small numbers of the introduced, pollution tolerant bivalve *Corbula gibba* (Talman et al., 1999). Suspension and surface deposit feeders almost exclusively dominated at the polluted site and no infaunal deposit feeders

or carnivores were detected. Deposit-feeding animals may be at particular risk of harm in contaminated sediments due to the high volumes ingested in bulk feeding. Deposit feeders play an important role in sediment bioturbation and the recycling of nutrients (Gaston *et al.*, 1998). These changes signal a significant shift in the macrobenthic community structure of this site which could have implications for, sediment oxygenation and energy cycling and transfer between trophic levels (Gaston *et al.*, 1998).

Although both sites were dominated by polychaetes, both numerically and in terms of species richness, the diversity was greater at the reference site, with eight families represented compared with four at the polluted site. At the polluted site 96% of polychaetes belonged to a single species of cirratulid, *Cirriformia filigera*. This species was consistently present at high densities throughout the sampling period (mean, 1679 $m^{-2}$), contributing up to 97% of the total faunal abundance in some samples. *C. filigera* has been reported from South America, South Africa and both sides of the north Atlantic coast (Pardo & Amaral, 2004), which suggests that it was introduced to Australia. Very little has been reported about the biology and ecology of this species, other than the fact that it is tolerant of muddy sediments with low oxygen concentrations (Pardo & Amaral, 2004). Inglis & Kross (2000) reported higher densities of cirratulids within more urbanised estuaries in northern Queensland and two closely related congeners have been positively associated with pollution, *C. tentaculata* (George, 1964) and *C. setosa* (Trannum *et al.*, 2004b).

It is difficult to say how a soft-bodied species that lives directly within the sediment is able to tolerate such high levels of contamination without absorbing large concentrations through its skin or ingesting toxins with food. *C. filigera* is a burrowing species, remaining just below the sediment surface, feeding selectively on surface deposits by extending specialised grooved feeding palps (Pardo & Amaral, 2004). The fact that it feeds at the

surface rather than on subsurface deposits may afford it some protection from ingestion of contaminants (Trannum *et al.*, 2004b). Exposure to extremely high concentrations of metals may also have resulted in the selection of local populations with metal tolerance (Hummel & Paternello, 1994; Trannum *et al.*, 2004b). The evolution of heavy metal resistance has been shown to occur in as little as four generations in some benthic annelids (Klerks & Levinton, 1989). Subsurface dwellers, such as *C. filigera,* which regularly encounter toxic metals would be one of the trophic groups most likely to develop tolerance (Rygg, 1985a). Their persistence at the polluted site throughout this study and in a previous year (Macleod & Helidoniotis, 2006), suggests that they possess some form of physiological adaptations to reduce the effects of absorbed toxicants. There are several mechanisms by which the susceptibility of individuals to toxicants may be reduced, including sequestration, enhanced detoxification and improved repair systems (see Maltby, 1999).

The consistently elevated abundances of *C. filigera* recorded were probably a response to an accumulation of unexploited resources at the polluted site. Both the reference and polluted sites had a similar degree of organic enrichment (total organic carbon 3.8% w/w DMB v 4.5%) (Macleod & Helidoniotis, 2006); however, far fewer taxa were present at the polluted site to exploit these resources. Therefore, the polluted site is likely to provide a good source of nutrients to those organisms tolerant of high levels of metals, such as *C. filigera*.

### 5.2 Dominance by opportunistic species

Life-history traits may also influence the ability of organisms to exploit underutilised resources within polluted environments. Communities in polluted areas are often dominated by species with r-selected traits such as small body size high fecundity and

short generation times (Trannum *et al.*, 2004b; Lee *et al.*, 2006). These traits enable species to rapidly colonise disturbed environments, and opportunistically exploit underutilised resources, frequently reaching very high densities as a result (e.g. Pearson & Rosenberg, 1978; Gaston *et al.*, 1998; Lenihan *et al.*, 2003; Stark *et al.*, 2003a; Mucha *et al.*, 2004). The two species responsible for much of the temporal variability at the polluted site, *Corophium acherusicum*, a gammarid amphipod, and, *Euchone limnicola*, a sabellid polychaete, share the characteristics of 'weedy' r-type species. Both species were introduced to Australia, probably via ballast or hull-fouling communities, have a worldwide distribution (Poore & Storey, 1999; Talman *et al.*, 1999) and have been associated with polluted environments (NOAA, 2000; Cohen *et al.*, 2001; Carvalho *et al.*, 2005).

There is very little specific biological information about these species, particularly *E. limnicola*. *Corophium sp*. have frequently been found to be opportunistic (Harris and Musko, 1999, in Macleod & Helidoniotis, 2006) and *C. acherusicum* has shown rapid recruitment, reaching very high densities in areas subjected to pulse pollution events, (Flemer *et al.*, 1995; Dumbauld *et al.*, 2001). Heavy metal resistance has not been reported but tube dwelling, suspension feeding fauna such as these are insulated to some extent from direct contact with contaminated sediments which may prolong their survival (Aller, 1980, in Gaston *et al.*, 1998). Both species recorded sequential 'boom-bust' dynamics, experiencing large blooms in abundance for approximately a month, followed by rapid declines. Declines may have resulted from temporary resource exhaustion due to their rapid rise in abundance (in conjunction with consistently high densities *C. filigera*); the effects of an escalating toxin load, or, perhaps these patterns of temporal variability simply reflect seasonal recruitment events.

## 5.3 Mechanisms responsible for observed relationships between sediment chemistry and fauna

Field evidence suggested that the distinctive patterns of biota recorded at the polluted site were primarily a function of the severity of sediment contamination. Correlative patterns cannot prove causal relationships, however, as assemblage patterns may be confounded by the inherent spatial and temporal variability of soft-sediment systems, or other influencing factors (Morrisey *et al.*, 1996; Stark, 1998b; Lindegarth & Underwood, 1999). Therefore, cause-effect hypotheses about the nature of the relationship between assemblages and contamination at the polluted site were tested experimentally in laboratory mesocosms.

The most valuable information was provided by those taxa that were not overly sensitive to handling and housing within mesocosms. Crustacean groups such as tanaids, cumaceans and amphipods did not survive within mesocosm controls and Stark (1998a) recorded a similar susceptibility of these crustacean groups to mesocosm conditions. In contrast, a number of mollusc, polychaete and decapod crustacean families were more resistant to these procedural effects and showed marked variation between experimental treatments.

### *5.3.1 The direct effects of sediment toxicity on infaunal assemblages*

Fauna from the reference site were significantly less diverse and abundant after one month in mesocosms containing sieved sediment (with no resident fauna) from the polluted site than in mesocosms with sediment from the reference site. As in the field study, sediments from the polluted site supported less families derived from fewer phyla. Semelid bivalves were significantly less abundant (78%) when subjected to heavily polluted sediment. Several other taxa, in particular nuculanid bivalves, maldanid and

sigalionid polychaetes; brittle stars; callianasids and other decapod crustaceans, contributed to the dissimilarity between treatments, all being fewer in the polluted sediments. These results are consistent with other experimental studies which have found similar adverse effects of heavy metals on crustaceans (Morrisey *et al.*, 1996; Stark, 1998a; Lenihan *et al.*, 2003), echinoderms (Rygg, 1985b; Olsgard, 1999; Lenihan *et al.*, 2003) and bivalves (McClusky *et al.*, 1986; Stark, 1998a; Damiens *et al.*, 2006).

Recent work showed that fauna collected at the reference site was consistent with communities found throughout the middle and lower reaches of the Derwent Estuary (Macleod & Helidoniotis, 2006). Our work suggests that many of these taxa are unable to establish within highly contaminated sediments and would therefore be excluded from the polluted site by the direct effects of toxicity. An organisms sensitivity to heavy metal toxicity may be related to physiological sensitivity (Wang *et al.*, 2002), trophic function (Selck *et al.*, 1998; Wang *et al.*, 2002) or life history characteristics (Lenihan *et al.*, 2003). Heavy metals may alter recruitment dynamics (Watzin & Roscigno, 1997) and induce avoidance behaviours in colonising adults and settling juveniles (Morrisey *et al.*, 1996).

Given the extreme levels of heavy metal contamination within sediments at the polluted site it is perhaps surprising that any animals survive there at all. Although the sediments clearly have a significant effect on biota, they still support high densities of fauna, albeit with greatly reduced diversity. Concentrations of heavy metals at this site are among the highest in the world (Coughanowr *et al.*, 2001) and mixtures of metals can be even more toxic than would be predicted from individual toxicities (Hagopian-Schlekat *et al.*, 2001). Toxicity tests carried out on a benthic algae and gammarid amphipod showed that the metal concentrations found within these sediments were acutely toxic, however, the sediments were aggressively shaken before the test animals were added, which is likely to have released sediment bound toxins (personal

communication, Derwent Estuary Program). Chemicals must be bioavailable to result in toxicity to benthic organisms (Liber *et al.*, 1996). The toxicity of metals contained within sediments is related to the concentration of free ions available for bioassimilation (Trannum *et al.*, 2004b) and possibly a function of pore water availability (Liber *et al.*, 1996). Sediments at the polluted site are muddy (>85% clay), with a moderate load of organic carbon (4.5% w/w DMB) (Macleod & Helidoniotis, 2006). As metals are tightly bound within fine sediments, particularly clays (Watzin & Roscigno, 1997) and organic carbon is known to complex zinc and other metals (Liber *et al.*, 1996), these factors may go some way to protecting fauna from the most severe effects of the toxicants. It seems likely that biota survives at the polluted site because of a combination of metal tolerance and some reduction in the bioavailable toxin component of the sediments.

### *5.3.2 The effects of metals on community structure and interspecific interactions*

Metals have the potential to cause not only the direct mortality of resident or recruiting organisms, but through such direct mortality, to indirectly alter the structure of communities and thus modify the suite of interspecific biological interactions (Keough & Quinn, 1998; Adams, 2005). Milward et al. (2004) showed that exposure to contaminants can lead to reduced abundances of some species due to the induction of ecological changes in competitive hierarchies within the communities. In our work, manipulative tests showed that interspecific interactions led to a greater decline in abundance of fauna from the reference site than could be accounted for by sediment toxicity alone.

Polychaetes were the group most affected by ecological interactions within polluted sediment. Maldanids, the dominant polychaete family and most abundant group within the reference fauna, were only moderately affected by the direct effects of toxicity,

declining by 15% within the sieved polluted sediment. However, they lost a further 56% of their abundance in the unsieved treatment. The unsieved treatment contained large abundances of *C. filiger*a and it is possible that their presence within the sediments prevented the establishment, or in some way compromised the survival of the maldanids. There results raise the possibility that some fauna could be excluded from the polluted site because of interspecific interactions with the modified community rather than by direct toxicity. Resource competition may have been responsible for the decline in maldanids, but further manipulative tests would be needed to draw any meaningful conclusions from this data.

While maldanids were able to establish at higher densities in toxic sediments without other macrofauna present, the abundances of *C. filigera* were unaffected by the presence or absence of resident communities in either heavily or lightly polluted sediments. This raises interesting questions about why that particular species is so abundant at the polluted site but not in other parts of the estuary. The short time period of the experiment may have been insufficient for interaction effects to develop since, although the abundance of *C. filigera* was unaffected by different treatment combinations, the condition of individuals was markedly poorer in treatments with sediments from the reference site with the resident fauna intact than in other treatments.

A recent survey recorded this species at comparable densities at only one other site in the Derwent, a shallow bay subject to hypoxia (Macleod & Helidoniotis, 2006). The highly localised distribution of this species within pollution hotspots essentially lacking other macro infauna, suggests that its distribution and dispersal capabilities may be governed by interspecific interactions. Carman et al. (1997) suggested that differential tolerances to contaminant exposure can result in the competitive release of more resistant species. The dominance of *C. filigera* at the contaminated site is likely to result from its reduced

susceptibility to metal toxicants compared with other macrobenthic organisms, enabling populations to utilise unexploited resources. However, given that resources available to an individual are finite, increasing maintenance costs due to defence and repair processes in metal resistance would leave fewer resources available for growth and reproduction (Maltby, 1999). Under less polluted conditions the attributes that contribute to *C. filigera*'s dominance in polluted sediments would likely confer a physiological cost rather than a survival advantage (Piola & Johnston, 2006), possibly affecting their ability to compete for limited resources. This implies that there is a trade-off associated with metal resistance; the benefits of exploiting resources in environments that few other organisms can tolerate weighed against metabolic costs, which possibly diminish an organisms competitive abilities under more benign circumstances.

This work suggests that the distinctive patterns of infaunal assemblages associated with highly contaminated sediments result from the specific responses of organisms to the combined effects of: bioavailability of metals; individual and population level resistance to toxicants; life-history characteristics and interspecific interactions.

## 5.4 Review of methodology and suggestions for further research

### *5.4.1 Whole sediment treatments*

Most experimental studies have focused on the impacts of an individual pollutant, such as copper (Morrisey *et al.*, 1996; Stark, 1998a; Lenihan *et al.*, 2003; Trannum *et al.*, 2004b), zinc (Ward & Hutchings, 1996; Watzin & Roscigno, 1997) and cadmium (Trannum *et al.*, 2004b). In this work, we investigated the effects of whole sediments on assemblage characteristics. Sediments recorded a large range of pollutants at extremely high levels of contamination and it was not possible to categorise individual effects of pollutants. It is unlikely that distinguishing between contaminant effects would have

added to our understanding of the system, moreover, efforts to monitor and assess pollution impacts are beginning to shift from identifying single-contaminant to multi-contaminant effects (Lenihan *et al.*, 2003).

### *5.4.2 The mesocosm approach*

The response of animals to sediment contamination within a 7-litre mesocosm was potentially very different from that shown by animals in much larger contaminated areas. And, although, the only possible response to treatments was a loss of abundance as there was no possibility of recolonisation of new species or recruitment of juveniles (Stark, 1998a; Trannum *et al.*, 2004b), the responses of fauna provided convincing insights into the complex effects of contaminants on natural communities.

The mesocosms employed in the laboratory experiments provided a very useful tool for manipulating fauna and testing treatment effects. They were simple, cheap to construct, easy to replicate and allowed conditions to be controlled across samples.

### *5.4.3 Stochastic variation between samples*

Due to equipment restrictions the sample size was small which has implications regarding the adequacy of assessments of within-location variation (Morrisey *et al.*, 1994). However, the SIMPER analysis showed that assemblages in replicated grabs within a given site had a high degree of similarity, suggesting that samples were representative of the natural community.

The design of the experiments meant that fauna were not enumerated before treatments began so that perceived changes in abundance may actually reflect initial stochastic variation in abundances of various taxa across treatments and samples (Stark, 1998a). However, the response of fauna to treatment effects was consistent across samples suggesting that the abundance changes recorded reflected treatment effects.

### *5.4.4 Further research*

There is little specific biological information about *Cirriformia filigera* in the literature. More work is needed on the physiology, life history and ecology of this species in order to understand the mechanisms by which this species tolerates such high levels of sediment contamination and whether it poses a bioaccumulation risk.

This study generated interesting questions about the nature of interspecific interactions within heavily contaminated sediments that could be further investigated by manipulating specific combinations of fauna and sediment in laboratory mesocosms.

Mesocosms could also be used to investigate the effects of changes in system ecology on ecosystem function by comparing nutrient processing and assimilation rates within these sediments and communities.

# 6. Conclusions

This study demonstrates the value of combining multiple methods, in this case field surveys and laboratory experiments, to explore relationships between anthropogenic pollution and biological change. The field component established a qualitative link between high levels of heavy metal contamination within sediments and the composition of infaunal assemblages, consistent with other studies. We were subsequently able to develop hypotheses regarding the mechanisms responsible for the observed effects that could be tested within the laboratory.

Although, exposing animals to sediment contamination within a 7-litre mesocosm does not mimic natural conditions, the responses of fauna provided convincing insights into the complex effects of contaminants on natural communities. The distinctive patterns of infaunal assemblages recorded at the polluted site and in treatments within the lab are likely to represent the response of individual components to the combined effects of multiple abiotic and biotic factors, including:

- The influence of sediment characteristics on the bioavailability of toxicants.
- Differential sensitivity to heavy metal toxicants resulting in a regression of sensitive species and persistence of populations of tolerant species.
- The competitive release of pollution tolerant organisms and their response to reduced resource exploitation resulting in very high densities.
- Opportunistic responses of r-type species.

Results from this work will add to the body of local evidence concerning biological functioning with the Derwent Estuary and provide globally relevant information regarding the ecological impacts of pollution on marine soft-sediment assemblages.

## 7. Literature cited


Adams, S. (2005) Assessing cause and effect of multiple stressors on marine systems. *Marine Pollution Bulletin*, **51**, 649-57.

Agard, J., Gobin, J., & Warwick, R. (1993) Analysis of marine macrobenthic community structure in relation to pollution, natural oil seepage and seasonal disturbance in a tropical environment. *Marine Ecology Progress Series*, **92**, 233-43.

Aller, R. (1980). Relationships of tube-dwelling benthos with sediment and overlying water chemistry. In *Marine Benthic Dynamics* (eds K. Tenore & B. Coull), pp. 285-309. University of South Carolina Press, Columbia, SC.

ANZECC (2000) Australian and New Zealand guidelines for Fresh and Marine Water Quality, Volume 1 - the Guidelines, Australia and New Zealand Environment and Conservation Council.

Carman, K., Fleeger, J., & Pomarico, S. (1997) Response of a benthic food web to hydrocarbon contamination. *Limnology and Oceanography*, **42**, 561-571.

Carvalho, S., Moura, A., Gaspar, M., Pereira, P., Da Fonseca, L., Falcao, M., Drago, T., Leitao, F., & Regala, J. (2005) Spatial and inter-annual variability of the macrobenthic communities within a coastal lagoon (Obidos lagoon) and its relationship with environmental parameters. *Acta Oecologica*, **27**, 143-159.

Clarke, K. & Warwick, R. (2001) *Change in marine communities: an approach to statistical analysis and interpretation.* PRIMER-E, Plymouth, UK.

Cohen, B., McArthur, M., & Parry, G. (2001). Exotic Marine Pests in the Port of Melbourne, Victoria. Marine and Freshwater Resources Institute, Melbourne.

Coughanowr, C., Green, G., & Alomes, G. (2001). Derwent Estuary Program Environmental Management Plan. DPIWE, Hobart.

Damiens, G., Mouneyrac, C., Quiniou, F., His, E., Gnassia-Barelli, M., & Romeo, M. (2006) Metal bioaccumulation and metallothionein concentrations in larvae of *Crassostrea gigas*. *Environmental Pollution*, **140**, 492-499.

Drake, J., Huxel, G., & Hewitt, C. (1996) Microcosms as models for generating and testing community theory. *Ecology*, **77**, 670-677.

Draper, N. & Smith, H. (1981) *Applied regression analysis*, 2nd edn. John Wiley and Sons, New York, U.S.A.

Dumbauld, B., Brooks, K., & Posey, M. (2001) Response of an Estuarine Benthic Community to Application of the Pesticide Carbaryl and Cultivation of Pacific Oysters (*Crassostrea gigas*) in Willapa Bay, Washington. *Marine Pollution Bulletin*, **42**, 826-844.

Ferraro, S., Cole, R., & Schults, D. (1991) Temporal changes in the benthos along a pollution gradient: discriminating the effects of natural phenomena from sewage and industrial wastewater effects. *Estuarine Coastal Shelf Science*, **33**, 383-407.

Flemer, D., Stanley, R., Ruth, B., Bundrick, C., Moody, P., & Moore, J. (1995) Recolonization of estuarine organisms: Effects of microcosm size and pesticides. *Hydrobiologia*, **304**, 85-101.

Fletcher, R., Reynoldson, T., & Taylor, W. (2001) Maintenance of intact sediment box cores as laboratory mesocosms. *Environmental Pollution*, **115**, 183-189.

Gaston, G., Rakocinski, C., Brown, S., & Cleveland, C. (1998) Trophic function in estuaries: response of macrobenthos to natural and contaminant gradients. *Marine and Freshwater Research*, **49**, 833-46.

George, J. (1964) On some environmental factors affecting the distribution of *Cirriformia tentaculata* (Polychaete) at Hamble. *Journal of the Marine Biological Association of the United Kingdom*, **44**, 383-388.

Green, G. & Coughanowr, C. (2003). State of the Derwent Estuary: a review of pollution sources, loads and environmental quality data from 1997 to 2003. Department of Primary Industries, Water and Environment, Hobart, Tasmania.

Hagopian-Schlekat, T., Chandler, G., & Shaw, T. (2001) Acute toxicity of five sediment-associated metals individually and in a mixture, to the estuarine meiobenthic harpacticoid copepod *Amphiascus tenuiremis*. *Marine Environmental Research*, **51**, 247-264.

Harris, R. & Musko, I. (1999) Oxygen consumption, hypoxia, and tube-dwelling in the invasive amphipod *Corophium curvispinum*. *Journal of Crustacean Biology*, **19**, 224-234.

Hirst, A. (2004) Broad-scale environmental gradients among estuarine benthic macrofaunal assemblages of south-eastern Australia: implications for monitoring estuaries. *Marine and Freshwater Research*, **55**, 79-92.

Hummel, H. & Paternello, T. (1994). Genetic effects of pollutants on marine and estuarine invertebrates. In *Genetics and evolution* (ed A. Beaumont). Chapman and Hall, London.

Inglis, G. & Kross, J. (2000) Evidence for systemic change in the benthic fauna of tropical estuaries as a result of urbanization. *Marine Pollution Bulletin*, **41**, 367-376.

Islam, M. & Tanaka, M. (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. *Marine Pollution Bulletin*, **Vol. 48**, 624-649.

Johnston, E., Keough, M., & Qian, P. (2002) Maintenance of species dominance through pulse disturbances to a sessile marine invertebrate assemblage in Port Shelter, Hong Kong. *Marine Ecology Progress Series*, **226**, 103-114.

Keough, M. & Quinn, G. (1998) Effects of periodic disturbances from trampling on rocky intertidal algal beds. *Ecological Applications*, **8**, 141-161.

Klerks, P. & Levinton, J. (1989) Rapid evolution of metals resistance in a benthic oligochaete inhabiting a metal-polluted site. *Biological Bulletin*, **176**, 135-141.

Lancellotti, D. & Stotz, W. (2004) Effects of shoreline discharge of iron mine tailings on a marine soft-bottom community in northern Chile. *Marine Pollution Bulletin*, **48**, 303-312.

Lee, H., Bailey-Brock, J., & McGurr, M. (2006) Temporal changes in the polychaete infaunal community surrounding a Hawaiian mariculture operation. *Marine Ecology Progress Series*, **307**, 175-185.

Lenihan, H., Peterson, C., Kim, S., Conlan, K., Fairey, R., McDonald, C., Grabowski, J., & Oliver, J. (2003) Variation in marine benthic community composition allows discrimination of multiple stressors. *Marine Ecology Progress Series*, **261**, 63-73.

Liber, K., Call, D., Markee, T., Schmude, K., Balcer, M., Whiteman, F., & Ankley, G. (1996) Effects of acid-volatile sulfide on zinc bioavailabilty and toxicity to benthic macroinvertebates: a spiked-sediment field experiment. *Environmental Toxicology and Chemistry*, **15**, 2113-2125.

Lindegarth, M. & Hoskin, M. (2001) Patterns of distribution of macro-fauna in different types of estuarine, soft-sediment habitats adjacent to urban and non-urban areas. *Estuarine Coastal Shelf Science*, **52**, 237-247.

Lindegarth, M. & Underwood, A. (1999) Using an experimental manipulation of contaminants in intertidal sediments. *Ecotoxicology*, **8**, 495-501.

Lindegarth, M. & Underwood, A. (2002) A manipulative experiment to evaluate predicted changes in intertidal, macro-faunal assemblages after contamination by heavy metals. *Journal of Experimental Marine Bology*, **274**, 41-64.

Macleod, C. & Helidoniotis, F. (2006). Biological Status of the Derwent and Huon Estuaries. Tasmanian Aquaculture and Fisheries Institute, Hobart.

Maltby, L. (1999) Studying stress: the importance of organism-level responses. *Ecological Applications*, **92**, 431-440.

McClusky, D., Bryant, V., & Campbell, R. (1986) The effect of temperature and salinity on the toxicity of heavy metals to marine and estuarine invertebrates. *Oceanography and Marine Biology Annual REview*, **24**, 481-520.

Milward, R., Carman, K., Fleeger, J., Gambrell, R., & Portier, R. (2004) Mixtures of metals and hydrocarbons elicit complex responses by a benthic invertebrate community. *Journal of Experimental Marine Biology and Ecology*, **310**, 115-130.

Morrisey, D., Stark, J., Howitt, L., & Underwood, A. (1994) Spatial variation in concentrations of heavy metals in marine sediments. *Australian Journal of Marine and Freshwater Research*, **45**, 177-184.

Morrisey, D., Underwood, A., & Howitt, L. (1996) Effects of copper on the faunas of marine soft-sediments: an experimental field study. *Marine Biology*, **125**, 199-213.

Mucha, A., Vasconcelos, M., & Bordalo, A. (2004) Vertical distribution of the macrobenthic community and its relationship to trace metals and natural sediment characteristics in the lower Douro estuary, Portugal. *Estuarine Coastal Shelf Science*, **59**, 663-673.

NOAA (2000). San Francisco Bay California: Benthic Community Assessment. National Ocean Service, Nation Centers For Coastal Ocean Science, Center For Coastal Monitoring And Assessment, Charleston, South Carolina.

Olsgard, F. (1999) Effects of copper contamination on recolonisation of subtidal marine soft-sediments - an experimental field study. *Marine Pollution Bulletin*, **38**, 448-462.

Pardo, E. & Amaral, A. (2004) Feeding behaviour of the cirratulid *Cirriformia filigera* (Dell Chiaje, 1825) (Annelida: Polychaeta). *Brazilian Journal of Biology*, **64**, 283-288.

Pearson, T. & Rosenberg, R. (1978) Macrobenthic succession in relation to organic enrichments and pollution of the marine environment. *Oceanography and Marine Biology Annual REview*, **16**, 229-311.

Piola, R. & Johnston, E. (2006) Differential tolerance to metals among populations of the introduced bryzoan *Bugula neritina*. *Marine Biology*, **148**, 997-1010.

Poore, G. & Storey, M. (1999). Soft Sediment Crustacea of Port Phillip Bay. In *Marine Biological Invasions of Port Phillip Bay, Victoria.* (eds C. Hewitt, M. Campbell, R. Thresher & R. Martin), pp. 150-170. Centre for Research on Introduced Marine Pests, CSIRO Marine Research, Hobart.

Rygg, B. (1985a) Distribution of species along pollution-induced diversity gradients in benthic communities in Nowegian fjords. *Marine Pollution Bulletin*, **16**.

Rygg, B. (1985b) Effect of sediment copper on benthic fauna. *Marine Ecology Progress Series*, **25**, 83-9.

Seitz, R. (1998) Incorporation of soft-sediment systems into a model of marine benthic community regulation. *Marine and Freshwater Research*, **49**, 817-26.

Selck, H., Forbes, V., & Forbes, T. (1998) Toxicity and toxicokinetics of cadmium in *Capitella* sp. I. Relative importance of water and sediment as routes of cadmium uptake. *Marine Ecology Progress Series*, **164**, 167-178.

Stark, J. (1998a) Effects of copper on macrobenthic assemblages in soft sediments: a laboratory experimental study. *Ecotoxicology*, **7**.

Stark, J. (1998b) Heavy metal pollution and macrobenthic assemblages in soft sediments in two Sydney estuaries, Australia. *Marine and Freshwater Research*, **49**, 533-40.

Stark, J., Riddle, M., & Smith, S. (2004) Influence of an Antarctic waste dump on recruitment to nearshore marine soft-sediment assemblages. *Marine Ecology Progress Series*, **276**, 53-70.

Stark, J., Riddle, M., Snape, I., & Scouller, R. (2003a) Human impacts in Antarctic marine soft-sediment assemblages: correlations between multivariate biological patterns and environmental variables. *Estuarine Coastal Shelf Science*, **56**, 717-734.

Stark, J., Snape, I., & Riddle, M. (2003b) The effects of petroleum hydrocarbon and heavy metal contamination of marine sediments on recruitment of Antarctic soft-

sediment assemblages: a field experimental investigation. *Journal of Experimental Marine Bology*, **283**, 21-50.

Talman, S., Bite, J., Campbell, S., Holloway, M., McArthur, M., Ross, D., & Storey, M. (1999). Impacts of Introduced Marine Species in Port Phillip Bay,. CSIRO Marine Research,, Hobart, Tasmania, Australia.

Terlizzi, A., Scuderi, D., Fraschetti, S., & Anderson, M. (2005) Quantifying effects of pollution on biodiversity: a case study of highly diverse molluscan assemblages in the Mediterranean. *Marine Biology*, **148**, 293-305.

Trannum, H., Olsgard, F., Skei, J., Indrehus, J., Øverås, S., & Eriksen, J. (2004a) Effects of copper, cadmium and contaminated harbour sediments on recolinisation of soft-bottom communities. *Journal of Experimental Marine Bology*, **310**, 87-114.

Trannum, H., Olsgard, F., Skei, J., Indrehus, J., Øverås, S., & Eriksen, J. (2004b) Effects of copper, cadmium and contaminated harbour sediments on recolonisation of soft-bottom communities. *Journal of Experimental Marine Bology*, **310**, 87-114.

Underwood, A. (1991) Beyond BACI: Experimental designs for detecting human environmental impacts on temporal variations in natural populations. *Australian Journal of Marine and Freshwater Research*, **42**, 569 - 587.

Vitousek, P., Mooney, H., Luchenco, J., & Melillo, J. (1997) Human domination of earth's ecosystems. *Science*, **277**, 494-503.

Wang, W., Yan, Q., & Wenhong Fan, Y. (2002) Bioavailability of sedimentary metals from a contaminated bay. *Marine Ecology Progress Series*, **240**, 27-38.

Ward, T. & Hutchings, P. (1996) Effects of trace metals on infaunal species composition in polluted intertidal and subtidal marine sediments near a lead smelter, Spencer Gulf, South Australia. *Marine Ecology Progress Series*, **135**, 123-135.

Warwick, R. & Clarke, K. (1993) Increased variability as a symptom of stress in marine communities. *Journal of Experimental Marine Biology and Ecology*, **172**, 215-226.

Watzin, M. & Roscigno, P. (1997) The effects of zinc contamination on the recruitment and early survival of benthic invertebrates in an estuary. *Marine Pollution Bulletin*, **34**, 443-455.