

# Importance of bioturbators for biodiversity maintenance: indirect effects of fishing disturbance

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**ABSTRACT:** The relative impact on macrofaunal diversity of 7 naturally co-occurring bioturbators has been examined in a benthic mesocosm experiment. The bioturbators chosen were selected because of their potential vulnerability to the disturbances associated with demersal trawling. The experiment was conducted at the Solbergstrand mesocosm (Norwegian Institute for Water Research) using subtidal sediment collected from Bjørnehodebukta, a small sheltered bay in the Oslofjord. For 3 of the bioturbating species (*Brissopsis lyrifera*, *Aphrodita aculeata* and *Amphiura chiajei*) a positive, linear relationship was demonstrated between bioturbator abundance and measures of species richness (number of species and Margalef species richness). It was suggested that the presence of these bioturbating species increased oxygen penetration into the sediment, leading to an enhancement in the ability of benthic systems to process organic material. This had a beneficial effect on the associated fauna in terms of maintaining levels of diversity. For *Nephtys caeca*, highest species diversity was seen in the low abundance treatments. Predation by *N. caeca* on small polychaetes is proposed as a possible explanation of the lower diversity in high-density treatments. It is concluded that *B. lyrifera*, *A. aculeata*, *A. chiajei* and *N. caeca* are functionally similar with respect to setting the diversity of the associated macrobenthic community and may therefore fulfill the same role within the benthic ecosystem. The presence of *Calocaris macandreae*, *Nuculana minuta* and *Astarte sulcata* had no detectable effect on diversity. Trawling has been shown to reduce the abundance of bioturbating species, and the current study demonstrates important implications of this loss on the maintenance of diversity. In addition, the interactions between bioturbation, diversity and organic enrichment highlight the need for management of anthropogenic impacts within the coastal environment in a holistic way rather than by isolation.

**KEY WORDS:** Diversity · Macrofauna · Bioturbation · Trawling · Disturbance · Mesocosm · Eutrophication · Oslofjord

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## INTRODUCTION

The disturbances associated with benthic trawling have widespread impacts on soft sediments and the communities that inhabit them (Rijnsdorp et al. 1998). A large number of studies have been conducted to assess these impacts (e.g. Jennings & Kaiser 1998, Frid et al. 1999, Jennings et al. 2002) and most have reported a shift from a highly diverse community with large-bodied

animals and a large number of tube-builders in undisturbed areas to communities dominated by small-bodied species, particularly polychaetes, in places that are heavily trawled. A significant depletion of megafauna (large [ $>10$  mm] epifauna or infauna) following trawling has also been demonstrated by a number of authors (De Groot & Lindeboom 1994, Hill et al. 1996, Kaiser et al. 1996, 1998). Megafaunal species are particularly vulnerable because they are typically long-

lived and take a relatively long time to reach a reproductive age (e.g. Buchanan 1964, 1967, Fenaux 1970, Munday 1992). Such reproductive strategies mean that any impact on the population of these species may still be evident years after the fishing disturbance occurred. In such circumstances even moderate fishing might reduce or destroy megafaunal populations.

As megafaunal species feed and move, they inevitably disturb the fabric of the sediment; a disturbance known as bioturbation. These bioturbating species, such as burrowing shrimps and echinoids, are instrumental in setting and maintaining infaunal diversity in soft-sediment environments (Widdicombe et al. 2000). Experimental evidence to support this has been provided from both field- and laboratory-based studies, which have demonstrated strong links between individual bioturbating species and their associated communities (e.g. Posey 1986, Warwick et al. 1986, 1990, Posey et al. 1991, Widdicombe et al. 2000). Whilst the primary effects of trawling, such as the direct mortality of species due to damage from the fishing gear, has been well studied (e.g. Jennings & Kaiser 1998, Frid et al. 1999, Jennings et al. 2002), the secondary effects of trawling, in particular the removal of bioturbating organisms, is less well understood.

On mixed substrata, the passage of a trawl can destroy structural species such as anemones, bryozoans and sponges and, in doing so, degrade the spatial complexity of the habitat (Tupper & Boutilier 1995). This in turn has been shown to reduce the survivorship of juvenile commercial fish species (Tupper & Boutilier 1995). The presence of epifaunal, structural species in mixed substrata is visually apparent, making it easy to appreciate the increased complexity that such species add to the habitat. In softer sediments, where the majority of organisms live below the surface, the habitat heterogeneity and 3-dimensional structuring provided by the bioturbators (Widdicombe et al. 2000) is not so immediately obvious. It is conceivable that, for the maintenance of spatial complexity, losing large bioturbators from muddy areas may be equivalent to losing epifaunal species such as bryozoans and sponges from areas of coarser, mixed sediment.

Using a manipulative mesocosm experiment, the sediment and associated macrofauna from an untrawled site were subjected to the different types of bioturbation generated by 7 naturally co-occurring infaunal bioturbators. The bioturbators chosen are potentially vulnerable to trawling and the experiment examined their relative impact on diversity. By carefully controlling the type and density of bioturbators present, this study explored the potential implications on the associated macrofauna of bioturbator loss due to trawling. This is the first attempt to directly compare such a large number of bioturbators at a range of realistic field densities.

## MATERIALS AND METHODS

**Bioturbating species.** The 7 species of bioturbators selected for this study were *Brissopsis lyrifera*, *Nuculana minuta*, *Calocaris macandreae*, *Amphiura chiajei*, *Nephtys caeca*, *Aphrodita aculeata* and *Astarte sulcata*. These species were chosen as they represent a range of bioturbation mechanisms, are potentially vulnerable to fishing, and are abundant in the Oslofjord.

The burrowing heart urchin *Brissopsis lyrifera* (Forbes, 1841) is a non-selective, infaunal deposit-feeder (Nichols 1959) which can burrow to a depth of 10 cm and is known to ingest both surface and deep sediment (Lawrence 1987). By 'bulldozing' through the sediment, it has a major impact on sediment structure and stability. In addition, by creating a respiratory funnel in the upper 3 cm of sediment (De Ridder & Lawrence 1982), *B. lyrifera* increases the depth of oxygenation. The presence of this species has been shown to enhance both local heterogeneity and diversity in a macrobenthic community (Widdicombe & Austen 1998).

The bivalve *Nuculana minuta* (Müller, 1776) is also a subsurface 'bulldozing' deposit feeder and it may, therefore, be expected that bioturbation caused by this species and that caused by *Brissopsis lyrifera* will have similar effects on diversity. However, *B. lyrifera* (max. length 6 cm) is a much larger organism than *N. minuta* (max. length 3 cm), it moves faster and, consequently, its movements will displace more sediment and any response in diversity to its bioturbation may differ in magnitude from that seen for *N. minuta*.

The thalassinidean shrimp *Calocaris macandreae* (Bell, 1846) constructs deep, complex burrows with multiple surface openings (Nash et al. 1984). These shrimps rarely emerge onto the sediment surface, obtaining the majority of their food from within the burrow; subsurface sediment from as deep as 215 mm may be expelled during normal feeding activities and burrow maintenance (Nash et al. 1984).

The brittlestar *Amphiura chiajei* (Forbes, 1843) lies buried in the sediment with its disc at 4 to 6 cm depth, with 1 or 2 arms stretched up above the sediment to collect food at the surface. Food particles are then transported along the arms to its mouth, and ingested (Buchanan 1964). Other arms may be extended deeper into the sediment below the disc. *A. chiajei* is particularly slow-growing, with a typical increase in disk diameter of 0.25 mm yr<sup>-1</sup> compared to 1.6 mm yr<sup>-1</sup> for the closely related species *A. filiformis* (Buchanan 1964).

The burrowing polychaete *Nephtys caeca* (Fabricus, 1780) is a large, predatory worm, 15 to 25 cm long, that creates non-permanent burrows, marked only by mucilage lining, as it moves through the sediment in search of food. This species is highly mobile and will

create sediment mixing to a depth in excess of 15 cm. This species lives for up to 15 yr (Caron et al. 1995).

*Aphrodita aculeata* (L., 1758) is a large (up to 20 cm long), oval-bodied polychaete worm. It is an active, mobile predator (Mettam 1980) but, unlike *Nephtys caeca*, this species operates close to or actually on the sediment surface.

*Astarte sulcata* (da Costa, 1778) is a medium-sized (shell length up to 3 cm), suspension-feeding bivalve that remains near the sediment surface with the upper edge of its shell protruding slightly into the water column. It feeds by filtering out suspended or resuspended material through short siphons at the sediment surface. This bivalve is considered less motile and functionally very different from the other species, but nevertheless is functionally representative of a large number of benthic animals. Its inclusion in the current study will provide a valuable comparison between the effect of mobile deposit-feeders and a less mobile suspension-feeder.

All the bioturbating species used during the current study are common within the study area (Oslofjord); however they also occur in many other areas throughout the North Sea, Irish Sea and English Channel, where fishing disturbance is absent or low. Where these precise species are not present it is not unusual to find closely related, functionally similar organisms in their place. The sediment and associated community selected for the experiment were from a well-bioturbated area that is subjected to relatively high levels of organic enrichment (Mirza & Gray 1981). The benthic fauna of this area has been described as typical of that found in other sites in Western Europe and probably also of large areas of the European continental shelf (Mirza & Gray 1981, Valderhaug & Gray 1984). Consequently, the results and conclusions from the current study may have the potential to be applied to a large number of temperate, coastal areas in which fishing impact may occur.

**Experimental set-up.** The experiment was carried out in one of the benthic mesocosms of the NIVA (Norwegian Institute for Water Research) Marine Research Station, Solbergstrand, Oslofjord, Norway. The mesocosm used is a 5 × 7 m indoor, epoxy-resin-coated concrete basin in which a constant depth of 100 cm is maintained with a continuous flow of water pumped from 60 m depth from the Oslofjord. The system has been described in detail by Berge et al. (1986).

Using a Day grab, muddy sand was collected from 30 m depth in Bjørnehodebukta, a sheltered bay in the inner part of Oslofjord. This sediment was

used to fill food-quality plastic buckets (36 cm diameter, surface area = 0.1 m<sup>2</sup>), to a depth of 30 cm. It has been shown that soft-sediment communities can be patchy at scales greater than the area sampled by a single grab (Kendall & Widdicombe 1999). Therefore, if each bucket had been filled with sediment from a single grab, a high level of dissimilarity between buckets would have occurred. By using the sediment from a number of different grabs to fill each bucket, this heterogeneity was reduced and ensured that each bucket had a similarly high number of species at the start of the experiment. As the sediment was added to the buckets, any conspicuous bioturbating species were removed. The buckets were placed in the mesocosm on 7 March 2002 and the sediment was allowed to settle for at least 2 d. Into each bucket, 1 of 7 species of bioturbators was added at either high or low densities (Table 1). These densities were adjusted to actual published values for the natural field densities of each species. The high densities were representative of the maximum density recorded whilst the low densities were representative of the most commonly reported densities. These 14 'treatments' were each replicated 4 times with an additional 7 control buckets containing no bioturbators. Bioturbators were collected on the eastern side of Håøya Island, Oslofjord, during the period 8 to 16 March 2002. Most bioturbators were introduced to the treatment buckets within 24 h of collection.

After the bioturbators had been added, the experiment ran for 21 wk. The sediments and communities of Oslofjord receive a relatively large input of organic material during the summer months (Rosenberg et al. 1987). In order to reproduce these conditions within the mesocosm, each bucket received three 3.21 g doses of freeze-dried, powdered *Entromorpha* sp. algae (3.9% N and 31% C). These were added on 15 June, and on 5 and 26 July 2002.

**Sampling.** On 7 March 2002, 4 macrofauna 'field' samples were taken at the sediment collection site using a 0.1 m<sup>2</sup> Day grab, and sieved over 0.5 mm mesh. The residue was preserved in 10% formaldehyde solution. On 17 March 2002, 3 of the 7 control buckets es-

Table 1. Treatment densities (individuals bucket<sup>-1</sup>) for each of the 7 bioturbating species. For number of individuals m<sup>-2</sup> multiply treatment density by 10

Bioturbator	Organism type	Low density	High density
<i>Nuculana minuta</i>	Bivalve	8	32
<i>Astarte sulcata</i>	Bivalve	3	12
<i>Brissopsis lyrifera</i>	Heart urchin	1	4
<i>Amphiura chiajei</i>	Brittle star	7	28
<i>Aphrodita aculeata</i>	'Sea mouse' polychaete	1	4
<i>Nephtys caeca</i>	Polychaete	1	4
<i>Calocaris macandreae</i>	Burrowing shrimp	1	4

tablished within the mesocosm were sampled to ascertain the macrofaunal diversity present in the buckets at the start of the experiment. The sediment from each bucket was preserved in 10% formaldehyde solution. By comparing these 'initial' samples with the 'field' samples taken from Bjørhodebukta, an assessment of how successfully the natural field communities were transplanted into the mesocosm system could be made.

Final sampling in the mesocosm took place from 9 to 14 August 2002. The sediment from each bucket was sieved over 0.5 mm mesh and the residue was preserved in 10% formaldehyde solution. During this sampling period, the initial samples collected and preserved in March were sieved and the residue was returned to 10% formaldehyde solution.

In the laboratory, all organisms contained within the residue were extracted under a binocular microscope and identified to the lowest possible taxonomic level. The numbers and identities of bioturbators in each bucket at the end of the experiment were noted and compared to the original number added to ascertain bioturbator survival and identify buckets contaminated by other species of bioturbator.

**Data analysis.** Number of species ( $S$ ), number of individuals ( $NI$ ), Margalef species richness ( $d$ ) and Pielou's evenness ( $J'$ ) were calculated for each sample using PRIMER (Plymouth routines in multivariate ecological research) v5.0. More than one measure of diversity was used in order to appreciate how bioturbation may act on a community. Measures of species richness ( $S$  and  $d$ ) enable an evaluation of diversity change based on the number of species present whilst a measure of evenness ( $J'$ ) indicates how the individual organisms are distributed amongst the species present. Statistical analyses of these indices were performed using MINITAB 13 for Windows computer package. Due to the survival rate of the bioturbating test species being less than 100%, relationships between bioturbator abundance and the above indices were examined using linear regression analysis. Where visual interpretation indicated a potential non-linear relationship, and sufficient replication allowed, pair-wise ANOVA was used to further explore the relationship.

Multivariate data analyses followed the methods described by Clarke (1993) using PRIMER v5.0. Analysis was carried out on both untransformed and presence/absence data, using the Bray-Curtis similarity measure, to determine the effects of treatments on different components of the community. Analysis of untransformed data is sensitive to changes in the abundance of the dominant species, whilst analysis of presence/absence data detects changes in community structure due to the identities of all the species present without being unduly influenced by dominant, high-

abundance species. ANOSIM (analysis of similarities) (Clarke & Warwick 2001) was carried out to test for treatment effects. Similarity percentages (SIMPER) analysis was used to identify the species responsible for any dissimilarity between different treatments (Clarke & Warwick 2001).

## RESULTS

### Impact of experimental set-up on macrofaunal diversity and community structure

A summary of the diversity values for field, initial and control samples is shown in Table 2. For measures of species diversity, i.e. number of species ( $S$ ) and species richness ( $d$ ), no significant differences were observed between field and initial samples ( $S$ :  $F = 3.18$ ,  $p = 0.135$ ;  $d$ :  $F = 5.79$ ,  $p = 0.061$ ) or between field and control samples ( $S$ :  $F = 4.69$ ,  $p = 0.074$ ;  $d$ :  $F = 3.27$ ,  $p = 0.121$ ). However, the species diversity of the control samples was significantly lower than that of the initial samples ( $S$ :  $F = 50.91$ ,  $p = 0.001$ ;  $d$ :  $F = 33.25$ ,  $p = 0.002$ ). This indicates that a small, but insignificant, increase in species diversity occurred when the communities were established in the mesocosm and that species diversity in control treatments subsequently declined over the course of the experiment to a level equivalent or lower than that of naturally occurring field levels.

For number of individuals there were no significant differences for field versus initial ( $F = 0.77$ ,  $p = 0.420$ ) or initial versus control ( $F = 0.70$ ,  $p = 0.441$ ). No significant differences were observed for measures of evenness. Multivariate analysis (ANOSIM) demonstrated no significant differences between the community structure of the field and the initial samples, either in the relative abundance of the numerically dominant species (untransformed data;  $R = 0.481$ ,  $p = 0.057$ ) or in the identity of the rare species (presence/absence data;  $R = 0.481$ ,  $p = 0.086$ ). Comparisons of the initial and control samples (ANOSIM) indicated that the mesocosm community did not change significantly over the course of the experiment in response to any factors other than those being deliberately manipulated

Table 2. Measures of diversity for field, initial and control samples (means  $\pm$  95% CI)

Parameter	Field	Initial	Control
No. of species ( $S$ )	27 $\pm$ 4.0	31.3 $\pm$ 0.6	22 $\pm$ 2.1
No. of individuals	215 $\pm$ 38.9	189 $\pm$ 61.8	161 $\pm$ 19.2
Species richness ( $d$ )	4.82 $\pm$ 0.64	5.85 $\pm$ 0.45	4.13 $\pm$ 0.38
Evenness ( $J'$ )	2.43 $\pm$ 0.20	2.60 $\pm$ 0.09	2.05 $\pm$ 0.19

(untransformed data:  $R = 0.037$ ,  $p = 0.429$ ; presence/absence data:  $R = 0.278$ ,  $p = 0.143$ ).

### Bioturbator survival and treatment integrity

Of the 60 treatment buckets, 6 were discarded from the analyses as they contained a *Calocaris macandreae* individual in addition to the bioturbators constituting the expected bioturbator treatment. These treatments were 1 high-density *Brissopsis lyrifera*, 1 high-density *Aphrodita aculeata*, 1 high-density *Amphiura chiajei*, 2 low-density *Astarte sulcata* and 1 high-density *A. sulcata*. Observations from this study and previous experiments (Widdicombe & Austen 2003) have indicated that it is not unusual for *C. macandreae* to leave treatment areas during the first few weeks of an experiment. After this time any shrimps still present tend to remain for the full duration of the experiment. Widdicombe & Austen (2003) showed no significant difference in macrofauna community structure or diversity between the areas that had been abandoned by shrimps and control areas that had never contained shrimps. It is reasonable, therefore, to use the number of shrimps present at the end of the experiment as the true treatment condition. Due to the abandonment of buckets by 1 or more shrimps, the densities of shrimp in some of the *C. macandreae* treatments were reduced. This resulted in 5 control treatments, 3 treatments containing a single *C. macandreae*, 3 treatments with 2 *C. macandreae* and a single treatment containing 3 *C. macandreae*. The other bioturbator that displayed a notable loss from the treatment buckets was *Nephtys caeca*. This resulted in 6 treatments containing a single *N. caeca* and 2 treatments containing 3 *N. caeca*. One *B. lyrifera* low-treatment bucket contained an additional *N. caeca*; this bucket was excluded from the analysis. No bioturbator losses were observed in the other treatments.

### Impact of bioturbating species on macrobenthic diversity and community structure

Significant relationships between bioturbator abundance and species diversity ( $S$  and  $d$ ) were observed for 3 species: *Brissopsis lyrifera*, *Aphrodita aculeata* and *Amphiura chiajei* (Fig. 1). For each of these species, the greater the abundance of bioturbators, the higher the species diversity of the associated fauna. For *Nephtys caeca* the relationship between bioturbator abundance and species diversity was not linear (Fig. 1). In the low-density treatments, species diversity was higher than in the controls. This difference was significant for species richness ( $F = 5.15$ ,  $p = 0.05$ ), but not for number of species ( $F = 4.13$ ,  $p = 0.07$ ). At the

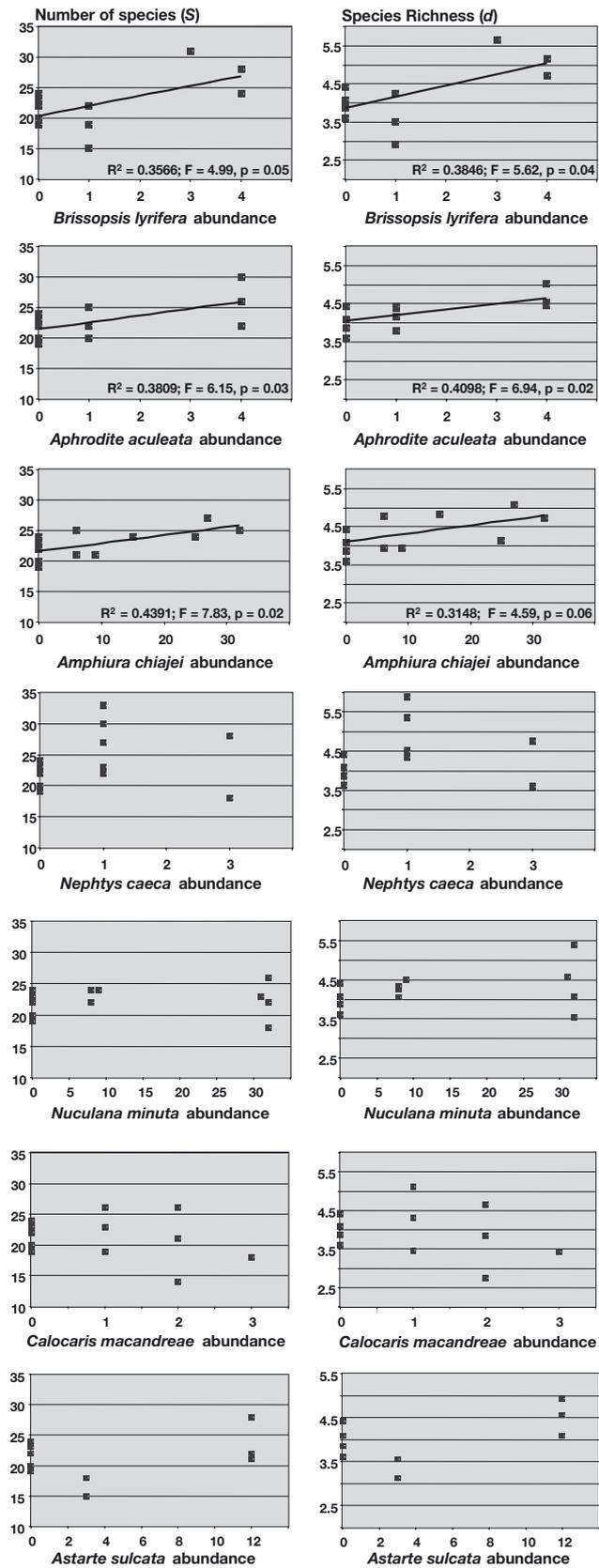


Fig. 1. Impact of bioturbator abundance on species diversity

highest *N. caeca* density, species diversity appeared to fall back to a level similar to that of the controls. No significance testing could be performed for the high-density treatments as there were only 2 replicates. No relationships were observed between species diversity and the abundance of *Calocaris macandreae*, *Nuculana minuta* or *Astarte sulcata* (Fig. 1).

No relationships were observed between the abundance of any of the bioturbating species and either Pielou's evenness or number of individuals (Fig. 2).

Multivariate analysis of both untransformed and presence/absence data failed to identify any patterns of community change in response to different bioturbator treatments.

## DISCUSSION

This study has demonstrated that the presence of 4 bioturbating species, *Brissopsis lyrifera*, *Aphrodite aculeata*, *Amphiura chiajei* and *Nephtys caeca*, at densities equivalent to those observed in the field, had a significant, positive impact on the diversity of associated macrobenthic communities. Of these species, 3 (*B. lyrifera*, *A. aculeata* and *N. caeca*) are large, highly mobile organisms that actively move through the upper 10 cm of sediment in similar ways (Caron et al. 1996, Hollertz et al. 1998), and with respect to bioturbation these 3 species may be considered functionally similar. The type of movement these species employ has traditionally been termed 'bulldozing' and the species that operate in this way as 'bulldozers'. Whilst this definition is not a totally accurate representation of their locomotory behaviour it still offers a good shorthand description of the way in which they impact on their environment.

In areas such as Oslofjord, which receive high levels of organic input (Rosenberg et al. 1987), the high bacterial oxygen demand generated by microbial decomposers may have a negative impact on the macrofauna by lowering oxygen levels. However, by increasing the depth of oxygen penetration in organically enriched sediments, bioturbation can supply the additional oxygen required during the processing of the additional organic material (Aller 1994), thus reducing the impact of oxygen depletion on macrofauna species sensitive to low oxygen levels whilst also stimulating the activity of aerobic microbial decomposers and accelerating carbon processing. In addition, organic material buried within the deeper anoxic sediments degrades 3.6 times faster when re-exposed to oxic conditions than when left in anoxic conditions (Hulthe et al. 1998). Therefore, the physical mixing generated by bioturbators will facilitate this re-exposure and result in increased carbon degradation. A recent study by Widdicombe &

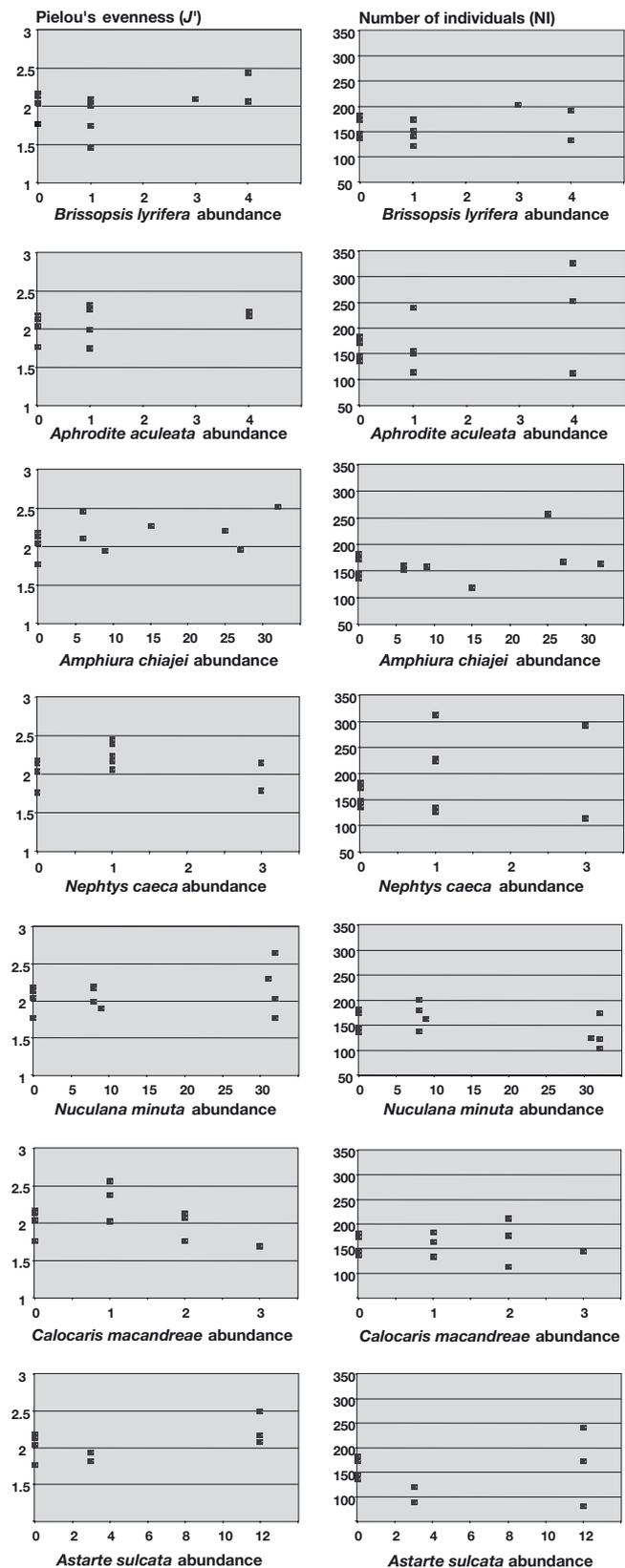


Fig. 2. Impact of bioturbator abundance on evenness diversity and faunal abundance

Austen (2001) provided experimental evidence that a physical disturbance similar to bioturbation could maintain diversity in the presence of high organic input. The ameliorating effect of bioturbation by the polychaete *Capitella capitata* has even been shown to 'recondition' the organically polluted sediments that result from intensive aquaculture (e.g. Chareonpanich et al. 1994). It is not unreasonable to assume from the results of the current study and an appreciation of the species locomotory activities, that *Brissopsis lyrifera*, *Aphrodita aculeata* and *Nephtys caeca* may be enhancing diversity through this combination of oxygenation and sediment mixing.

The brittle star *Amphiura chiajei* buries its disk to a depth of 6 cm (Hollertz et al. 1998), resulting in increased oxygen penetration. Compared with the 3 'bulldozing' species (*Brissopsis lyrifera*, *Aphrodita aculeata* and *Nephtys caeca*) *A. chiajei* is less mobile and would therefore be unable to influence as large an area of sediment when present at similar densities. However, *A. chiajei* occurs at much greater densities than the other 3 species, and this was accounted for in the treatment densities used. The current study shows that the impact on diversity of *B. lyrifera* and *A. aculeata* at densities of 40 individuals  $m^{-2}$  was broadly equivalent to that of *A. chiajei* at densities of around 300 individuals  $m^{-2}$ . So although *A. chiajei* is not a 'bulldozer', when present at realistic field densities it may still be responsible for increased sediment oxygenation and may be considered an important species in maintaining macrobenthic diversity.

The response of diversity to the presence of *Nephtys caeca* was different to that seen for *Brissopsis lyrifera*, *Aphrodita aculeata* or *Amphiura chiajei*. Increasing the density of *B. lyrifera*, *A. aculeata* and *A. chiajei* caused a linear increase in macrofaunal diversity, whilst the relationship between diversity and *N. caeca* was a 'humpback' curve. This 'humpback' relationship was similar to that described for *B. lyrifera* in a previous mesocosm experiment (Widdicombe & Austen 1998). The fact that no decrease in diversity was seen in the current study for *B. lyrifera* may be due to the densities chosen. In the study by Widdicombe & Austen (1998), the treatment densities were 28 and 71 individuals  $m^{-2}$  compared with the current study of 10 and 40 individuals  $m^{-2}$ . It may be that between 40 and 71 individuals  $m^{-2}$  the negative impacts of sediment disturbance created by *B. lyrifera* become greater than the positive effects of increased oxygenation. Despite the current study demonstrating a positive, linear relationship between diversity and the abundance of both *A. aculeata* and *A. chiajei*, it should not be assumed that this relationship would be maintained indefinitely, and these species too may cause a decrease in diversity at densities greater than those used here.

*Nephtys caeca* and *Aphrodita aculeata* have both been described as active predators (Caron et al. 1996, Mettam 1980). It is important to consider the possible implications of this activity on the current study. Non-specific deposit-feeders such as *Brissopsis lyrifera* may effectively be considered predators, as they indiscriminately consume small macrofaunal species through bulk ingestion of sediments. This non-selective predation has the potential of increasing diversity by reducing competitive exclusion (Widdicombe & Austen 1998). However, predation that is targeted towards specific prey species, as it is the case for *N. caeca* and *A. aculeata*, may have 1 of 2 outcomes, depending on the identity of the prey species. Predation may result in the extinction of the prey species and a subsequent reduction in diversity (this may be of particular relevance in closed mesocosm systems) or, if the target species is a competitive dominant within the community, diversity may increase, as it does through non-selective predation. Polychaetes from the family Nephtydidae are reported to target many different prey species (Clark 1962, Beukema 1987) with *N. caeca* consuming mainly small polychaetes and crustaceans (Caron et al. 1995, 1996). It seems likely that some of the reduction in diversity observed in the high-density *N. caeca* treatments may have been due to predation on particular species of small polychaetes. Additionally, species such as the capitellid *Heteromastus filiformis*, a known prey species of *N. caeca*, were numerically abundant in the current study, and consequently any process that reduces their abundance may have an impact on diversity through competition. The diet of *A. aculeata* is more specialised than that of *N. caeca*, with *A. aculeata* preying mostly on large active polychaetes such as *Nephtys* spp. and *Nereis* spp. (Mettam 1980). These species are rare within the mesocosm system, and therefore the impact on diversity of predation by *A. aculeata* may be less than any impact due to bioturbation. In field sediments where *N. caeca* and *A. aculeata* co-occur implications of their interaction should also be considered. *A. aculeata* predation may reduce the abundance of *N. caeca* and consequently *N. caeca*'s impact on other species. Also, the potential relationship between *N. caeca* and *A. aculeata* and the subsequent consequences for diversity may be heavily influenced by the relative impact of trawling disturbance on these 2 species. *N. caeca* is a deep-burrowing species and will be less affected by disturbances at the sediment surface. In contrast, *A. aculeata* operates at or near the sediment surface and will be vulnerable to damage from most types of benthic fishing gear (Kaiser et al. 1998). Resilience of *N. caeca* to fishing disturbance may also be high, compared to other types of bioturbator, because it is able to regenerate lost body tissue.

The current study failed to demonstrate an effect on diversity resulting from the presence of *Nuculana minuta*, and this may in part be due to the treatment densities chosen for this experiment. A previous experiment by Widdicombe & Austen (1999) demonstrated a 'humpback' relationship between a similar species (*Nuculoma tenuis*) and diversity, with highest diversity occurring at bivalve densities of 612 individuals  $m^{-2}$ . This density is almost double that of the highest treatment density used in the current study. It is therefore possible that, even though *N. minuta* is functionally similar to other mobile deposit-feeders, its small size means that in order to impact on diversity it needs to be present in much higher numbers. As the densities used in the current study were representative of naturally occurring field densities in Oslofjord, the actual densities required before *N. minuta* functions as an ecosystem engineer probably only occur under exceptional circumstances. In other habitats, such as physically controlled glacial bays, nuculid bivalves can be extremely abundant (1500 to 3000 individuals  $m^{-2}$ ), and are the numerically dominant species (Kendall & Aschan 1993, Kendall et al. 2003); they thus may have a greater importance in these areas.

*Astarte sulcata* is a suspension-feeding bivalve and, as such, may be considered functionally different from the other species examined in this study. It was, therefore, not unexpected that an organism with such limited potential for bioturbation displayed no significant effect on diversity. This result supports the findings of Widdicombe & Austen (1999), who observed that the presence of *Abra alba*, another suspension-feeding bivalve, also had no beneficial effects on diversity. The 'non-response' in *A. sulcata* treatments in this study and the observations on *A. alba* in the previous study of Widdicombe & Austen (1999) highlights the importance of bioturbation, as opposed to the consumption of organic material, for maintaining diversity in areas of high organic input such as Oslofjord.

No enhancement of diversity was observed in treatments that contained *Calocaris macandreae* compared with the controls. This result is in partial agreement with a previous study (Widdicombe & Austen 2003), which showed that despite having a significant effect on community structure *C. macandreae* had no significant effect on diversity. These results may appear surprising considering the extensive burrow construction and the resulting oxygenation of deep sediments associated with *C. macandreae*. However, unlike *Brissopsis lyrifera*, *Aphrodita aculeata*, *Nephtys caeca* and *Amphiura chiajei*, which all operate in the upper 10 cm, *C. macandreae* lives within a burrow and generally operates much deeper, so any organic material that is supplied to the sediment surface will not be actively mixed into this layer as it is with the other bioturbators. From

the current study it would appear that bioturbation associated with burrow formation is less important for maintaining diversity than the bioturbation of 'bulldozing' species, a conclusion also reached by Thayer (1983) who stated that 'biological bulldozing is the most effective form of bioturbation'.

By reducing the abundance of the large bioturbating species, trawling will have a long-lasting, indirect impact on the rest of the benthic fauna. Additionally, in areas that frequently become eutrophicated, such as Oslofjord (Rosenberg et al. 1987), the impact of losing key bioturbating species on benthic diversity could be exacerbated. It is important therefore that when considering the potential effects of anthropogenic impacts it is done in a holistic way rather than by considering the different impacts in isolation from each other.

In field observational studies it is often impossible to disentangle the direct and indirect effects of trawling. To demonstrate cause and effect and to distinguish treatment effects from confounding influences, controlled manipulative experiments are necessary. Mesocosms are well suited to such experiments as long as the results they produce are considered subject to the usual caveats. In particular, isolation from many of the influences that maintain community structure and function in the field, particularly the supply of recruits to replace dead and dying individuals, can be seen as compromising the extent to which results from mesocosm experiments can be applied to natural situations. In the current study, however, the method of sediment collection meant that the diversity within each bucket was higher than that found in corresponding areas of natural sediment. This enabled the study to examine whether bioturbation was necessary to maintain levels of diversity, rather than needing to observe an increase in diversity to demonstrate an effect. Now that an effect has been demonstrated, it is important that its relevance to natural situations is validated through field experiments, e.g. the reintroduction of bioturbators into areas where heavy trawling has reduced their numbers.

A surprising outcome of the current study was the failure of multivariate data analysis to identify any changes in macrobenthic community structure in response to different bioturbator treatments despite the fact that univariate measures of diversity clearly showed a response. This is all the more surprising as many authors have shown multivariate techniques to be extremely sensitive to community change, and able to detect underlying differences between groups that are undetectable by univariate techniques (Warwick & Clarke 1991). To understand why this was not the case in the current study we must consider the different ways in which multivariate and univariate techniques identify differences between groups of samples. In the

case of univariate measures, the identity of the species is of limited importance; 2 groups will be considered the same if they both contain the same number of species irrespective of whether they have any species in common or not. For multivariate analysis, however, species identity is critical, with differences between groups being affected not only by the number but also by the type of organisms present. This is particularly important when testing for significant differences between groups, as the sensitivity of the ANOSIM procedure is greatly affected by the degree of dissimilarity within the groups being compared. In the initial set-up for the current mesocosm experiment, the natural variability in species identity and composition of the field community was homogenised by ensuring that each bucket was filled using sediment from several different grabs. However, the identity of these species was not necessarily the same within each bucket, particularly for the less abundant species which would not be regularly distributed between treatments. This meant that whilst the set-up technique was appropriate for an experiment that intended to observe changes in species diversity, identity-dependent measures of community structure could be subjected to too much within-treatment dissimilarity to be able to identify between-treatment differences.

From the results of the current study, it can be concluded that *Brissopsis lyrifera*, *Aphrodita aculeata*, *Amphiura chiajei* and *Nephtys caeca* are important for the maintenance of macrobenthic diversity, and may be fulfilling the same role within the benthic ecosystem. Does it matter, therefore, if the majority of these species are lost, as long as at least 1 representative of this particular functional type remains? Also, what are the implications for diversity of the inevitable interactions that will occur between co-existing bioturbating species? As yet we do not know the answer to these questions, and only through carefully constructed experiments that combine different numbers and types of bioturbators from the same functional group will we be able to examine the role bioturbator diversity plays in the relationship between bioturbation and species diversity.

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