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The effects of five different defaunation methods on biogeochemical properties of intertidal sediment

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Abstract

Various methods have been used to remove organisms from sediments to investigate structure and function of faunal assemblages in intertidal habitats. Nevertheless, little is known about how these treatments affect properties of the sediments themselves, although changing these properties may cause changes in the assemblages independently of other hypotheses being tested. This study assesses the efficacy of defaunation and effect on selected biogeochemical properties of five different methods of defaunating soft muddy sediments in an estuary. The methods were: removal and freezing of sediment, removal and oven-heating, freezing in situ with liquid N_2, spraying with formalin and spraying with hydrogen peroxide. The first four of these methods have been used in previous studies, whilst the fifth was considered to be a potentially useful defaunator because it does not leave toxic residues. The first two methods required sediment to be brought back to the lab, disrupting the natural structure of the sediment; the last three were done in situ, with much less disturbance.

Variables measured to assess effects of the treatments on the sediment were: amount of water, grain-size, total carbohydrate, suspension index (relative erosion rate), erosion threshold, chlorophylls-*a* and -*b*, colloidal carbohydrate, F_o (minimal fluorescence) and F_v/F_m (photosynthetic yield). There were no significant effects of any treatment on the first 4 variables. For the others, effects of defaunation varied from treatment to treatment and with time after treatment. Generally, the greatest disturbance was to the microphytobenthos (MPB, measured by chlorophyll and fluorescence) and related variables. For most treatments, recovery was rapid, but the effects of formalin and H_2O_2 persisted for a few days. Effects on physical properties of the sediment were largely minor and insignificant, removal and freezing or heating, however, caused major changes to the sediments because of the disturbances involved. Choosing the appropriate method of defaunation is very important if interpretations are not to be confounded between the effects of defaunation *per se* and any effects of changes in properties of sediments caused by the method used to defaunate experimental areas.

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1 Introduction

Intertidal areas, such as salt-marshes, mangrove forests and mudflats support diverse assemblages of organisms, many of which are intimately associated with the sediments (reviewed by Lopez and Levington, 1987). Biota alter the properties of and processes in sediments (Black et al., 2002), which have important consequences for associated fauna. Many organisms inhabiting sediment secrete extra-cellular polymeric substances (EPS), which are particularly effective stabilising agents, altering cohesion and erodibility of the sediment (Dade et al., 1990; Decho, 1990; Tolhurst et al., 2002; de Brouwer et al., 2005). Bioturbation by animals, e.g. burrowing and feeding, can significantly alter properties of sediments, such as erodibility, porosity, permeability and grain-size (Meadows and Tait, 1989; Meadows et al., 1990). Erosion on mudflats is mediated by the interplay between biological and physical processes (de Brouwer et al., 2000; Defew et al., 2002; Tolhurst et al., 2006b) and can be significantly altered by fauna (Austen et al., 1999; Widdows et al., 2000; Andersen et al., 2002) and flora (Friend et al., 2003; Tolhurst et al., 2006a). Often, an organism can have multiple synergistic or antagonistic influences on properties and processes that may vary temporally and/or spatially (e.g. Fernandes et al., 2006).

Equally important are the responses of organisms to different properties of the sediment. For example, bioturbation may be reduced in areas with increased amounts of food (Taghon and Green, 1990), patterns of distribution may vary with sediment grain-size (Snelgrove and Butman, 1994; Thrush et al., 2003) or algae on the substratum (Chapman and Tolhurst, 2004) and settlement may be enhanced by chemical cues from the substratum (Fitt and Coon, 1992).

This intimate association between biological, physical and biogeochemical properties of sediments results in a highly complex system, making understanding the structure and functioning of intertidal sediments difficult (Gray, 1974; Lopez and Levinton, 1987; Woodin et al., 1995; Black et al., 2002; Chapman et al., 2010). Manipulative experiments of the fauna, flora or their habitats offer a robust framework for improving

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understanding of these habitats. For example, one may need to remove some or all of the organisms from sediments to assess the effects of disturbances on rates or methods of recovery (Levin, 1984; Negrello Filho et al., 2006), on the strength of biological interactions (Thrush et al., 1992), or on functions such as nutrient fluxes (Biles et al., 2002) and erosion processes (de Deckere et al., 2001). Such experiments often require defaunation or removal of the animals from the sediment.

Previous studies in intertidal soft sediments have used a variety of different approaches to defaunate sediments (Table 1). There is no standardised methodology to defaunation, even within a given approach and different approaches are often combined (Table 1). The efficacy of these methods in killing fauna can be implicitly tested within the experiment (by comparison to control treatments; Thrush et al., 1996), or are assumed (Levin, 1984). Their effects on the properties of sediments themselves have rarely been investigated (although see Thrush et al., 1996; de Deckere et al., 2001; Slocum and Plante, 2006) despite it being known that properties of sediment influence behaviour of epi- and infauna.

It is unclear if the different approaches used to defaunate sediments (Table 1) result in differences in the properties of the sediments. Any method of defaunation that alters properties of sediments may also alter post-treatment migration, settlement and recovery of an assemblage, because of responses to the changed sediment, rather than because of direct effects of defaunation. Choosing the appropriate method of defaunation is very important if interpretations are not to be confounded between the effects of defaunation *per se* and any effects of changes in properties of sediments caused by the method used to defaunate experimental areas.

Ultimately, the most appropriate method of defaunation will depend on the particular hypotheses being tested. For many experiments, the best method will be the one that removes all the fauna (or a specific subset of the fauna), whilst minimising impacts on sediments. There will, however, also be experiments where the main requirement is to minimise the effects on one or more specific properties of the sediment. For example, removing animals to test hypotheses about the effects of bioturbation on grain-size

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requires a method of defaunation that minimises changes to grain-size at the start of the experiment.

The time-scale over which different defaunating techniques operate is also important. To maintain defaunated patches for some period (a "press" disturbance), a persistent defaunator may be appropriate. Alternatively, a non-persistent defaunator would be more appropriate to study recovery immediately after defaunation (a "pulse" disturbance).

Given the interconnected nature of processes controlling properties of sediments, making a decision about the appropriate method is difficult. This study tested the effects of 5 different methods of defaunation (with appropriate procedural controls for disturbance) on selected sedimentary properties over 15 days to identify any short-term changes to sediments caused by effects of the defaunation method itself. These included two common methods (removal and freezing or oven-heating) which are assumed to cause complete defaunation, but which also cause major disturbance to the physical integrity of the sediment. Three methods of treating sediment in situ were also tested, because they may cause less disruption to the sediment surface. These were freezing with liquid N_2 and poisoning with formalin or hydrogen peroxide. In terms of "press" and "pulse" treatments, formalin may leave toxic residues in the sediment, but liquid N_2 rapidly evaporates and H_2O_2 breaks down into water and oxygen.

2 Materials and methods

2.1 Defaunation treatments

The experiment was done on an intertidal mudflat at Tambourine Bay, in the upper reaches of Sydney Harbour over 15 days, from 23 October 2003 (day 0), to 27 October 2003 (day 4), to 7 of November 2003 (day 15). Details of the site and its location can be found in Chapman and Tolhurst (2007).

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2.2 Experimental design

Twenty seven 30 cm × 30 cm plots, at least 5 m apart, were marked. Five days prior to setting up the experiment, sediment was collected from six randomly-selected plots, by carefully removing it to a depth of 10 cm and taking it back to the laboratory. Three of these samples were each thoroughly mixed (keeping the sediment from each plot separate) before being placed in an oven at 120 °C. The other three were similarly mixed and frozen at −80°C. The samples were left for 5 days. On the day the experiment started, these samples were taken into the field and placed into the original 6 plots (after excavating any recently deposited sediment to a suitable depth); so that the introduced sediment was flush with the surroundings. There was no attempt to replace any sediment into the same plot from which it was removed.

The remaining 18 plots were allocated to six other treatments, including the procedural controls ($n = 3$ per treatment). For treatments where the plots were poisoned in situ (i.e. using formalin or H_2O_2) or frozen in situ (using liquid N_2), the plots were surrounded by a frame of metal pushed into the sediment to a depth of ∼1 cm to prevent spread of the treatment to surrounding mud. Three replicate plots were then sprayed with 300 ml of 37 % formalin or 300 ml of 35 % v/v H_2O_2 or 4 l of liquid N_2 was poured into the frame.

There were three procedural controls: (PC1) sediment collected from a plot and then replaced into each plot after approximately 1 h with minimal disturbance to the structure of the sediment; (PC2) sediment removed the day prior to the experiment, taken to the laboratory and mixed, but not heated nor frozen and then replaced in the plots the day the experiment started; (PC3) plots surrounded with the metal frame and the surface of the mud sprayed with 300 ml of estuarine water to wet, but not poison, the surface of the sediment. These provided controls for the major disturbances associated with the various treatments. Measurements were taken on the day the experiment started (day 0), a minimum of 20 min after treatment.

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2.3 Properties of the sediments

A suite of variables that are routinely measured and which are generally considered important in the ecology and sedimentology of mudflats were examined. These were: amounts of water, grains <63 µm (i.e. the mud fraction) and grains >63 µm (i.e. the sand and coarser fraction), organic matter, chlorophylls-*a* and -*b*, total and colloidal carbohydrates. These were all expressed per surface area to avoid problems associated with using measures expressed as contents (Perkins et al., 2003; Tolhurst et al., 2005) and because the exact volume of cores was not available to calculate concentrations.

Two replicate cores ~2 mm depth were collected from each plot at each time of sampling using the contact-core technique (Honeywill et al., 2002). Surface cores were used because this is where the majority of the microphytobenthos are found and using deeper cores masks patterns (Kelly et al., 2001), also many invertebrates are known to respond to cues associated with the surface of the substratum when colonizing different habitat (Fitt and Coon, 1992; Woodin et al., 1995; Hardege et al., 1998). The same plots were sampled at each time, so care was taken not to sample the same spot twice. Immediately prior to taking these cores, the area was dark-adapted for 15 min and the F_o (minimal fluorescence) and Yield (F_v/F_m) were measured using a Heinz Walz Pulse Amplitude Modulation fluorometer (PAM; Honeywill et al., 2002; Consalvey et al., 2005).

Colloidal carbohydrates (the water-soluble fraction) and total carbohydrates were measured using the sulphuric acid-phenol Dubois assay (Dubois et al., 1956) as per methods outlined in Underwood et al. (1995) and expressed as glucose equivalents using a standard curve. Approximately 0.2 g of freeze-dried sample was used for the colloidal assay and 0.0025–0.005 g for the total carbohydrate assay.

In addition to measuring fluorescence using PAM, chlorophylls-*a* and -*b* were measured spectrophotometrically from a sub-sample of sediment using a dimethyl formamide (DMF) extraction, following the equations of Porra et al. (1989).

$$\text{Chlorophyll-a} = 12(A_{664} - A_{750}) - 3.11(A_{647} - A_{750})$$

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$$\text{Chlorophyll-b} = 20.78(A_{647} - A_{750}) - 4.88(A_{664} - A_{750})$$

where A are the absorptions at the specified wavelengths.

Organic matter was measured by weighing, then ashing the remaining sediment in a furnace at 450°C to obtain the weight of burnt material; this allowed back calculating of the amount of organic matter in the original mass of the sample. The ashed sediment was then washed through a 63 µm sieve, the sieve containing the sand was then freeze-dried and reweighed to determine the amount of sand (>63 µm) and mud (<63 µm) by mass; to maintain consistency in the data, these were expressed per surface area.

A Cohesive Strength Meter (CSM) was used to measure the erosion threshold and relative erosion rate (S_i) of the sediment (Tolhurst et al., 1999; Vardy et al., 2007). These measures correlate well with measures of shear strength using a fall-cone apparatus (Watts et al., 2003). Two replicate CSM measures were taken in each plot at each time.

Approximately 20 min after the treatments were set up; the plots which were poisoned were sampled to test the efficacy of methods in killing fauna compared to three untreated control plots. The macrofauna were sampled using 11.5 cm diameter cores to a depth of 10 cm. In the laboratory, samples were sieved carefully over a 500 µm sieve without being preserved and then examined under the microscope on the same day. All macrofauna were counted and scored as living or dead (where possible). Because freezing and drying at high temperatures have been evaluated previously (see Table 1), it was assumed these methods would kill all organisms.

2.4 Statistical analysis

For analysis, the treatments were split into two groups: (1) those that physically disturbed the sediment (removal to the laboratory for freezing or heating), their procedural controls (PC1 and PC2) and the undisturbed treatment; (2) those treatments where liquid was sprayed onto the sediment (formalin, liquid N_2, H_2O_2), PC3 and the undisturbed treatment. The same undisturbed plots were used to compare with each of these groups.

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3 Results

3.1 Efficacy of defaunation

Taxa were analysed only if clearly identifiable as alive or dead. There were no living animals in the samples that had been treated with formalin and all animals in the undisturbed treatment were alive. The mean proportion of live animals in plots treated with liquid N_2 or H_2O_2 were 0.48 (SE 0.10; $n = 6$) and 0.63 (SE 0.01), respectively. Therefore, the only treatment in the field to kill all fauna was formalin, but the other methods killed approximately half to two-thirds of the fauna. Overall, the most susceptible fauna were small crustaceans (such as amphipods) and polychaetes, where 0–42 % of these survived the different treatments. In contrast, very few nemerteans, bivalves or gastropods died.

To test whether liquid N_2 or H_2O_2 killed different taxa *pro rata* or selectively, the numbers of animals remaining alive were compared with those in the undisturbed treatments using standardized counts (i.e. relative proportions of taxa in the different samples). Assemblages differed between the two treatments and between the treatments and the undisturbed control (PERMANOVA = NPMANOVA in Anderson, 2001), $P < 0.05$. Liquid N_2 killed most taxa, but neither capitellid nor nephtyiid polychaetes. H_2O_2 had little effect on oligochaetes, mainly affecting insect larvae and crustaceans.

3.2 Effects on sediments that were physically disturbed

Using the freezer or oven necessitates removing sediment to the laboratory with associated disturbances to the structure of the sediment. All variables were therefore analysed for these two treatments, with their appropriate controls; Undisturbed sediment, PC1 (removing and replacing sediment) and PC2 (removing, mixing and replacing sediment).

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The water content, mud and sand fractions, organic matter, total carbohydrates, chlorophyll-*b* and erosion rate of the sediments were not affected by any of the treatments (analyses of variance for each variable; differences among treatments were not significant, $P > 0.10$ for each variable at each time). With the exception of erosion rate and total carbohydrate, mean values were consistent across treatments (illustrated for sand and chlorophyll-*b* in Fig. 1a, b, respectively), so these results were not attributable to any problems of lack of power (i.e. there were no trends that might have been detected as significant with more replication). Although not significant, erosion rate was increased in the oven and PC1 and PC2 treatments on day 0 and average total carbohydrate was considerably larger in the control than all other treatments on day 0.

The erosion threshold was increased significantly by the freezing on day 0 (analyses of variance, $P < 0.01$; SNK tests, $P < 0.05$; Fig. 1c), but there was no significant difference between the undisturbed sediment and that heated in the oven, nor with the procedural controls. The mean values for all treatments on day 15 were consistently large (compare days 0 and 4 with day 15 in Fig. 1c); these values are consistent, however, with results obtained from other estuaries (Tolhurst et al., 2006a) and at other times in these sites (Tolhurst et al., 2010).

Chlorophyll-*a*, F_o and F_v/F_m were all significantly reduced by defaunating sediment in the freezer or oven (F_o and chlorophyll-*a* illustrated in Fig. 1). These variables were, however, also reduced in both procedural controls and there was no significant difference between the two experimental treatments and these controls (analyses of variance, $P < 0.01$; SNK tests, $P < 0.05$). Any consequent interpretation of these variables or effects on fauna cannot therefore be attributed to defaunation, but rather to the disturbance of the sediment when it is removed and later replaced.

Freezing the sediment significantly reduced amounts of colloidal carbohydrate, but to a similar degree as in the two procedural controls. Treating the sediment in the oven, in contrast, significantly increased amounts of colloidal carbohydrates (analyses of variance, $P < 0.01$; SNK tests, $P < 0.05$; means in Fig. 1f).

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By day 4 and later, on day 15, there were no longer any significant differences in treatments from the undisturbed sediment or the procedural controls for F_o, F_v/F_m nor colloidal carbohydrates (Fig. 1d, f); analyses of variance, $P > 0.10$), although F_o in the oven treatment was decreased compared to the other treatments on day 4. In the freezer and oven treatments, the mean amounts of chlorophyll-*a* continued to be decreased compared with undisturbed treatment and controls on day 4, but measures had recovered and were no longer significantly different from controls by day 15 (analysis of variance; $P < 0.01$; means in Fig. 1e).

3.3 Effects on sediments treated in situ

With the exception of F_v/F_m on day 0, which was significantly reduced, comparisons of the procedural control (PC3) with undisturbed sediment showed no significant differences (analyses of variance, SNK tests, all $P > 0.10$; means in Fig. 2). Effects of treatments described below are therefore attributable to defaunation, not to changes caused by the treatments. As for the disturbed sediments, there were no significant effects of any treatments on water content, the mud and sand fractions, organic matter, total carbohydrates, chlorophyll-*b* or erosion rate (analyses of variance, $P > 0.10$; illustrated for chlorophyll-*b* in Fig. 2a), although average amounts of total carbohydrate were smaller in the liquid N_2, H_2O_2 and procedural controls.

Application of H_2O_2 significantly reduced the mean amounts of chlorophyll-*a*, F_o and F_v/F_m compared with controls (Fig. 2b, c, d; analyses of variance, $P < 0.01$; SNK tests, $P < 0.05$). These reductions persisted until day 4 (Fig. 2b, c) except for F_v/F_m which was no longer different from the undisturbed control (Fig. 2d). In contrast, mean amount of chlorophyll-*b* was significantly reduced on day 4 in the H_2O_2 treatment (analysis of variance, SNK tests, $P < 0.01$; Fig 2a), but not on days 0 nor 15. Colloidal carbohydrate and erosion threshold were not affected by application of H_2O_2 at any time (analysis of variance, $P > 0.10$, Fig. 2e, f).

Using formalin to defaunate sediments caused a significant increase in erosion threshold and colloidal carbohydrate on day 0 (Fig. 2e, f) and decreases in F_o and

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F_v/F_m (Fig. 2c, d; analyses of variance and SNK tests for each variable, $P < 0.01$). On day 4, the reduced values of F_o and F_v/F_m persisted and there were, now, reduced values of chlorophyll-*a* and -*b* and colloidal carbohydrates, despite colloidal carbohydrate having been increased at the start of the experiment (means in Fig. 2; all analyses, $P < 0.01$). Total carbohydrate was, on average, considerably less, but this difference was not significant. None of these effects persisted to day 15, by which time no variables showed any significant differences from the controls (Fig. 2).

Liquid nitrogen was the least effective treatment for killing the fauna (see above). Also had minimal effects upon the sediment properties. It only significantly influenced one variable in the sediments, reducing F_v/F_m on day 0 (Fig. 2d; SNK test, $P < 0.05$), but this reduction did not persist to day 4 (analyses of variance at days 4 and 15 showed no significant differences among treatments).

The figures only present an illustrative subset of the data, the full data set for all variables and treatments is given in Table 2.

4 Discussion

Because muddy sediments are complex and interactive systems, a change to one component of sediment is likely to have major impacts on other components. Nevertheless, most studies that defaunate sediment to test hypotheses about disturbances, recovery, colonization, etc., assume that responses of fauna colonizing these sediments are not confounded by responses to any abnormal conditions created in the sediment by the defaunation process. Thus, Thistle (1981) and Beukema et al. (1995, 1999) noted that the abnormally large densities of infauna that developed in defaunated sediment immediately after deployment and which subsequently declined to background levels could have been due to lack of competitors, or increased resources, but did not address whether the methods of defaunation changed these resources directly, rather than by the removal of animals.

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4.1 Treatments that physically disturbed the sediment

Despite the many methods that have been developed to defaunate sediment (Table 1), air-drying, freezing, or oven-heating are commonly used, yet this study shows that they significantly alter the properties of the sediment. They also generally kill flora in addition to fauna. Here, freezing and oven-drying sediment were compared to undisturbed plots, in addition to sediment that had only been removed from the plots overnight and mixed, or removed and replaced in situ within an hour with minimal mixing.

None of these defaunation methods had major impacts on any of the physical sedimentary components, i.e. amounts of water, or the mud or sand components. The sediment placed in the plots was saturated with water from the surrounding sediment soon after deployment, explaining the similarity in water content between the undisturbed sediment and the oven-dried sediment. All treatments reduced amounts of mud and increased sand on day 0 compared to the undisturbed control, although the changes were small and non-significant. This probably represents disturbance effects altering sediment structure, or mixing of deeper coarser sediment into the surface 2 mm of sediment. Where stratification of grain size with depth is more pronounced, effects of mixing would be expected to be greater.

There were slight decreases in organic matter in all treatments compared to the undisturbed control on day 0. These decreases probably reflected the small decreases in mud (organic matter is often associated with fine sediment) and are quite likely also due to mixing. There were large decreases in total carbohydrates in all treatments compared to the undisturbed control, although there was a large amount of variability and these were not significant. This also probably represents mixing of the carbohydrate found at the surface (associated with filamentous green algae) throughout the 10cm depth of the treated sediment; this is supported by the reductions seen in the measures of algal biomass.

The major effects of these treatments were on the microphytobenthos on the surface of the sediment. This was shown as a decrease in chlorophyll-*a* (but not chlorophyll-*b*)

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and the two PAM measures, F_o and F_v/F_m. Although one might expect freezing or heating to destroy microphytobenthos, this decrease was not significantly greater than that in the sediment that was simply disturbed and mixed. Because these microphytobenthos are most abundant in the top few mm of the sediment surface (Tolhurst, 2012), it is not surprising that mixing the sediment reduced the amount of chlorophyll measured on the surface. Where colonization of fauna is influenced by amounts of microphytobenthos (Stocks and Grassle, 2001); treatments that mix the surface layers of the sediments into deeper sediment will potentially be confounded. The disturbed sediment will not only have no (or fewer) fauna, but also less surface microphytobenthos. Although reduced, the amounts of chlorophyll-*a* measured chemically were not reduced to zero, even in the treatment that had been oven-dried for 4 days. This probably represents measurement of chlorophyll degradation products remaining after the experimental treatments, because the decline in fluorescence from the PAM (which measures the photosynthetic activity) was proportionally much greater.

Unlike diatom-dominated European estuaries, these habitats are dominated by filamentous green algae (Murphy et al., 2004, 2008). Colonization of treated plots by microphytobenthos was, however, relatively rapid, with recovery in many treatments after 4 days and recovery among all treatments by 15 days. Previous work has shown that diatoms colonise defaunated sediments very rapidly, especially in the absence of grazers (Tolhurst et al., 2008) and this study shows this to be true for other types of microphytobenthos and in the field situation. This suggests that after 15 days colonization by fauna would not be affected by changes to microphytobenthos; but, because earlier rates of faunal colonization may have been altered by the biomass of algae, recovery of the assemblage in the defaunated sediment might have already been compromised. It is also unknown if the species composition of the microphytobenthos was altered, which may also affect colonisation by fauna.

Freezing and oven-heating had different effects on other sedimentary properties. Freezing significantly increased the erosion threshold on day 0, although erosion rate was not altered. The cause of this is unknown, but may reflect the effects of freezing on

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the sediment structure. The oven treatment decreased erosion threshold and erosion rate, whilst freezing decreased colloidal carbohydrate; but similar changes occurred in the procedural controls, indicating these changes were caused by the physical disruption of the sediment fabric. Oven-heating also increased amounts of colloidal carbohydrate on day 0, probably due to breakdown of dead biota. These effects had, however, disappeared by day 4, indicating that any effects on colonization associated with changes to the erosion threshold of the sediment, or the amounts of colloidal carbohydrate, would be short-lived and have minimal influence on interpretation of experiments done over longer periods. Artefacts such as these may, however, affect measures of short-term colonization or introduce initial effects that persist through time i.e. by facilitating the recruitment of one species over another.

The short-term increase in amounts of colloidal carbohydrate in sediments heated in the oven or treated with formalin may have been due to the breakdown of insoluble carbohydrates, possibly by bacteria and/or fungi. The rapid decline to ambient amounts within 4 days, suggests rapid utilization of this resource and redistribution into the food web as the sediments are recolonised. Similar rapid changes in colloidal carbohydrates have been found before in frozen sediments (Tolhurst et al., 2008). This was not seen in the frozen treatment in this study, probably because the times of measurement after treatment were different. Further work is required to understand the cause(s) of these changes.

4.2 Treatments where liquid was sprayed onto the sediment

To assess methods of defaunation that cause less disturbance to the structure of the sediment, methods of defaunating the sediment in situ (formalin, liquid nitrogen and H_2O_2) were also evaluated. Again, there were only minor effects on the sedimentary properties other than the microphytobenthos.

As for the physically disturbed treatments, changes to the physical properties were minor and non-significant. Small decreases in mud in the treatments that sprayed liquid on the sediment may have been due to physio-chemical dispersion, as the fresh liquid

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winnowed fine grained sediment away, similar to some of the processes associated with rainfall (Tolhurst et al., 2006c, 2008). As for the physically mixed treatments, changes in the organic matter mirrored the changes in the amount of mud.

Liquid N_2 had very minor effects on the sediment, only reducing F_v/F_m, showing it could temporarily inhibit photosynthesis, but had no persistent effects, even on the microphytobenthos. Chlorophyll-*a* and both measures of florescence using the PAM were considerably reduced by H_2O_2, highlighting its extreme toxicity and localised surface action. Effects on chlorophyll-*a* and F_o persisted till the 4th day, although F_v/F_m had recovered, but these differences had disappeared by the 15th day. Formalin may have initially "fixed" the microphytobenthos, causing no change in biochemical chlorophylls and only a slight decrease in F_o, but a large decrease in the F_v/F_m (a measure of photosynthetic activity), indicating the microphytobenthos was dead. Subsequently, all measures of microphytobenthos as well as both carbohydrates were reduced on day 4, showing the effects of formalin to be more persistent than the H_2O_2 treatment. The reduction in carbohydrates is probably due to the lack of photosynthetic activity (as shown by the F_v/F_m) and slow colonisation by fauna. Formalin also caused an increase in the erosion threshold and amounts of colloidal carbohydrate on day 0. This increase in colloidal carbohydrate may be a result of secretion of EPS as a physiological response to poisoning. An increase in EPS would also explain the increased erosion threshold, as EPS stabilises sediment (Tolhurst et al., 2002; de Brouwer et al., 2005). Both these variables decreased by day 4 reflecting the persistent nature of formalin in preventing growth of microphytobenthos. All of these effects had disappeared by the 15th day, again indicating that colonization by fauna into sediments treated in these ways may be confounded over the short-term, but not after a week or so.

5 Conclusions

Laboratory freezing and oven treatments significantly altered biogeochemical properties, physically disturbed the sediment and had persistent effects on biogeochemical

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properties; they are, however, excellent defaunators. Formalin had significant persistent effects on the sediment, but because it was more effective at killing the fauna than was H_2O_2, it would be preferable if complete defaunation was required. Although liquid N_2 had the least effects on the sediment, it was also not very effective at killing the fauna. Therefore, its positive aspect of minimal effect on the sediment is unlikely to outweigh the negative aspect of incomplete defaunation (presuming complete defaunation is the goal). Repeated or larger applications of liquid N_2 may, however, improve defaunation, but this requires further investigation.

Because many models relate faunal abundance to that of microflora, e.g. grazing decreasing micro-algae (Daborn et al., 1993; Hillebrand et al., 2002), bioturbation and excretions of fauna releasing nutrients that may alter algal abundance (Dyson et al., 2007) etc.; the fact that so many of these treatments reduced the amounts of algae is a concern. These data show there are serious consequences for interpretation of experiments that use these methods to test hypotheses about defaunation. This is illustrated in simplified form in Fig. 3. The left-hand column illustrates some measure of a property of the sediment (e.g. amount of chlorophyll-*a*); the right-hand column is some variable associated with the fauna (e.g. the number of species present). At the time of the arrow on the x-axis, sediment is defaunated. In Fig. 3a, this is successful (the faunal measure becomes 0) and causes no changes to the sediment. The recovery of the fauna is complete when the dashed line recovers to the solid line representing control conditions. This can be interpreted in relation to experimental removal of previous fauna (i.e. all of the grey region is about responses to defaunation).

In contrast, in Fig. 3b, defaunation was only partially successful and, again, caused no artefactual changes to the sediments. Obviously, no hypothesis about defaunation can be tested. Changes in fauna are in response to partial removal of unknown amounts of unplanned components of the fauna.

The experimental results in this paper are illustrated in Fig. 3c. Defaunation is complete, but also alters properties of the sediment. Sediment recovers to natural conditions at the arrow above the line, where the dashed line meets control conditions. Prior

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to that (the dotted region in the right-hand graph), responses by the fauna are due to the confounded influences of defaunation and changed properties of the sediment. After the sediment recovers (the arrow above the line), it may be possible to interpret changes to the fauna to be a response to defaunation because there are no further confounding influences of altered sediment. Whether or not this is a valid inference depends entirely on whether the influences of altered sediment, *per se*, on changes to fauna, persist after the sediment has recovered. Persistent effects can occur, for example, if altering sediments alters cues for settlement of certain species, which then influence subsequent colonization or survival and therefore patterns of recovery of other fauna. Without detailed experimental evidence about influences on early stages of colonization of defaunated sediment where no alterations of properties of sediments had occurred, it would be very difficult, if not impossible, to make logically justifiable inferences.

Care is required in the interpretation of experiments that use currently available techniques for defaunation. Results must always be considered in terms of effects on the sediments in addition to faunal processes influenced by defaunation itself.

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Table 1. Summary of some of the various different approaches used to defaunate sediments, there is no standard methodology even when the same technique is used (JEMBE is Journal of Experimental Marine Biology and Ecology; MEPS is Marine Ecology Progress Series).

Reference	Method
Levin (1985), Ecology, 65: 1185–1200	Air dried
Whitlatch and Zajac (1985), MEPS, 21: 299–311	Air dried 1 week
Wu and Shinn (1997), Mar. Biol., 128: 475–487	Air dried 1 month
Lee (1999), Est. Coast. Shelf. Sci., 49:703–712	Air dried for several weeks
Lu and Wu (2000), Mar. Biol., 136: 291–302	Air drying 1 month
Sevidge and Taghon (1988), JEMBE, 115: 137–155	Sieved 300 µm, frozen
Chandler and Fleeger (1983), JEMBE, 69: 175–188	Repeatedly frozen (−20˚C) 3 or more times
Thrush and Roper (1988), JEMBE, 116: 219–233	Frozen 10 days
Berge (1990), MEPS, 66: 103–115	Frozen
Olafsson and Moore (1990), MEPS, 65: 241–244	Large animals picked out and frozen
Pechenick and Cerulli (1991), JEMBE, 151: 17–27	Sieved, frozen
Thrush et al. (1992), JEMBE, 159: 253–265	Sieved 2 mm and frozen
Olafsson and Moore (1992), MEPS, 84: 161–171	Frozen
Snelgrove et al. (1992), MEPS, 182: 149–159	Frozen and freshwater
Turner et al. (1997), JEMBE, 216: 51–75	Frozen
Hall and Frid (1998), Aquat. Ecol., 33:333–340	Frozen −18 ˚C for 48 h
Hsieh and Hsu (1999), MEPS, 177: 93–102	Frozen −70 ˚C for 7 days (twice), mixed
Ford et al. (1999), MEPS, 191: 163–174	Frozen at 18 ˚C for 12 h
Kline and Stekoll (2001), Mar. Env. Res., 51: 301–325	Frozen 1 wk −20 ˚C, 1 week room temp, wash with freshwater and salt water, refrigeration at 4 ˚C for 6 weeks, Frozen −20 ˚C 1 day
Bolam and Fernandes (2002), JEMBE, 269: 197–222	Frozen-refrozen 6 times −20 ˚C
Flemer et al. (2002), Hydrobiol., 485: 83–96	Frozen and air dried
Hansen and Kristensen (1998), Est. Coast Shelf. Sci., 45: 613–628	Overlying water purged with N_2, inducing anoxia that kills the animals or forces them up. Restored to original condition with O_2
Gilbert et al. (1996), Chemosphere, 33: 1449–1458	N_2 method
Heilskov and Holmer (2001), J. Mar. Sci., 58: 427–434	N_2 method
Thrush et al. (1996), Ecology, 77: 2472–2487	Covered with black plastic and concrete slabs for 3 weeks
Beukema et al. (1999), J. Sea. Res., 42: 235–254	Cover with synthetic material for 3 months
Gamenick et al. (1996), MEPS, 144: 73–85	Covered with PVC foil 1 month
Stocks and Grossle (2001), MEPS, 221: 93–104	Sediment enclosed in plastic bags for 2 to 3 weeks
Gallagher et al. (1983), Ecology, 64: 1200–1216	Commercially purchased sand
Bostrom and Bonsdorff (2000), MEPS, 205:123–128	Commercially purchased sand
Fegley (1988), JEMBE, 123: 97–113	Washed with distilled water and equal # of dryings at 50 ˚C in an oven
Kern (1990), MEPS, 60: 211–223	Baked at 200 ˚C for 3 h
Zhou (2001), JEMBE, 256: 99–121	Combustion at 500 ˚C for 6 h
Sandnes et al. (2000), MEPS, 197: 151–167	30 % NaCl solution
Hansen and Blackman (1991), MEPS, 75: 283–291	Anoxia. Corers sealed for 24 h, macrofauna migrated up and removed
Christensen at al (2000), MEPS, 192: 203–217	Sieving (only removal of macrofauna)
Service and Bell (1987) JEMBE 114: 49–62	Moving a rake over the area for 20 min
Crowe at al. (1987), MEPS, 41: 61–69	Immersion in freshwater 5-d, homogenize
Negrello Filho et al. (2006), JEMBE, 328: 240–250	30 ml of 40 % formalin added to cores in situ

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Table 2. Means and (SE) of the 12 variables for each treatment, for 0, 4 and 15 days after treatment.

Day 0	Control	Frozen	Oven	PC1	PC2	Formalin	Liquid N_2	H_2O_2	PC3
Chlorophyll-*a*	2.34	0.83	0.60	1.14	0.97	2.47	1.83	0.91	2.07
($\mu g\,cm^{-2}$)	(0.27)	(0.09)	(0.03)	(0.18)	(0.09)	(0.28)	(0.38)	(0.10)	(0.30)
Chlorophyll-*b*	0.72	0.53	0.94	0.65	0.55	0.67	0.55	0.57	0.61
($\mu g\,cm^{-2}$)	(0.06)	(0.06)	(0.09)	(0.09)	(0.04)	(0.07)	(0.09)	(0.05)	(0.08)
Colloidal carbohydrate	22.70	5.07	32.68	8.93	7.34	52.00	17.76	30.66	22.33
($\mu g\,cm^{-2}$)	(3.59)	(0.84)	(3.18)	(1.88)	(0.95)	(10.04)	(3.68)	(5.47)	(5.51)
Total Carbohydrate	1380	728	658	810	852	1159	621	660	801
($\mu g\,cm^{-2}$)	(403)	(163)	(71)	(67)	(229)	(287)	(99)	(178)	(171)
Water	104.6	87.0	81.7	89.0	93.8	91.4	100.4	73.8	97.7
($mg\,cm^{-2}$)	(8.5)	(2.9)	(4.6)	(2.5)	(4.6)	(5.6)	(9.3)	(7.3)	(6.1)
Organic matter	7.6	6.8	7.2	6.1	6.8	7.2	5.9	5.1	6.5
($mg\,cm^{-2}$)	(1.3)	(0.5)	(0.3)	(0.9)	(0.8)	(0.8)	(0.8)	(0.9)	(1.0)
Sand	209.7	236.1	229.3	227.5	221.0	211.2	207.9	212.2	232.2
($mg\,cm^{-2}$)	(11.2)	(9.3)	(6.1)	(8.2)	(7.8)	(11.0)	(20.3)	(4.3)	(3.7)
Mud	38.6	26.6	28.0	26.1	28.9	29.7	25.9	21.9	31.1
($mg\,cm^{-2}$)	(6.2)	(3.4)	(1.4)	(2.5)	(2.9)	(3.8)	(3.9)	(3.5)	(4.5)
F_o	324.50	18.17	17.44	15.42	11.60	9.37	7.13	14.66	201.50
	(57.50)	(6.15)	(3.12)	(3.94)	(4.18)	(4.53)	(2.23)	(2.93)	(55.11)
Yield F_v/F_m	0.54	0.33	0.27	0.23	0.26	0.87	0.33	0.26	0.30
	(0.04)	(0.17)	(0.08)	(0.04)	(0.04)	(0.25)	(0.07)	(0.08)	(0.07)
Relative erosion rate	6.04	5.68	15.33	140.17	73.00	207.83	273.67	126.00	6.72
(S_i)	(1.20)	(1.06)	(5.34)	(54.24)	(12.93)	(36.44)	(33.41)	(40.69)	(1.22)
Erosion threshold	0.46	0.92	0.25	0.38	0.24	0.06	0.12	0.03	0.46
(Nm^{-2})	(0.06)	(0.27)	(0.17)	(0.03)	(0.09)	(0.02)	(0.07)	(0.02)	(0.09)

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Table 2. Continued.

Day 4	Control	Frozen	Oven	PC1	PC2	Formalin	Liquid N_2	H_2O_2	PC3
Chlorophyll-*a*	1.83	1.26	0.98	2.22	1.69	0.55	1.87	0.89	1.83
($\mu g\,cm^{-2}$)	(0.20)	(0.11)	(0.21)	(0.17)	(0.11)	(0.05)	(0.15)	(0.08)	(0.16)
Chlorophyll-*b*	0.67	0.62	0.69	1.02	0.79	0.30	0.61	0.36	0.58
($\mu g\,cm^{-2}$)	(0.05)	(0.09)	(0.10)	(0.10)	(0.07)	(0.06)	(0.03)	(0.06)	(0.05)
Colloidal carbohydrate	19.67	9.81	8.84	15.28	11.34	2.04	11.60	9.15	18.59
($\mu g\,cm^{-2}$)	(2.83)	(1.10)	(1.41)	(3.35)	(1.99)	(0.66)	(0.97)	(1.33)	(3.71)
Total Carbohydrate	953	605	787	1098	819	203	802	510	578
($\mu g\,cm^{-2}$)	(212)	(76)	(111)	(186)	(166)	(50)	(300)	(190)	(156)
Water	105.7	89.8	83.0	98.3	95.3	84.0	93.1	86.2	85.3
($mg\,cm^{-2}$)	(7.8)	(4.7)	(4.3)	(2.7)	(4.9)	(2.8)	(5.2)	(7.4)	(4.4)
Organic matter	6.6	5.8	6.7	8.5	6.3	3.8	5.6	4.7	4.8
($mg\,cm^{-2}$)	(0.7)	(0.5)	(0.9)	(0.5)	(0.7)	(0.3)	(0.6)	(1.2)	(0.4)
Sand	221.9	219.0	208.6	197.9	203.3	219.8	209.3	203.6	211.0
($mg\,cm^{-2}$)	(16.6)	(17.2)	(8.7)	(6.9)	(8.4)	(6.5)	(7.9)	(12.8)	(9.9)
Mud	37.6	27.6	32.6	36.5	32.0	15.1	31.1	20.3	25.1
($mg\,cm^{-2}$)	(4.4)	(2.0)	(5.6)	(1.9)	(3.9)	(2.4)	(4.1)	(5.7)	(3.4)
F_o	156.33	120.83	59.00	224.17	137.00	4.67	231.33	55.50	305.66
	(36.86)	(15.23)	(29.01)	(34.43)	(19.62)	(3.22)	(21.74)	(13.30)	(64.21)
Yield F_v/F_m	0.66	0.68	0.59	0.61	0.65	0.06	0.57	0.67	0.59
	(0.04)	(0.02)	(0.13)	(0.02)	(0.02)	(0.06)	(0.02)	(0.06)	(0.01)
Relative erosion rate	6.63	11.20	10.60	5.35	7.07	19.51	7.92	14.66	5.38
(S_i)	(1.87)	(3.32)	(2.75)	(1.32)	(2.03)	(4.44)	(2.79)	(4.11)	(0.59)
Erosion threshold	0.52	0.46	0.47	0.53	0.51	0.35	0.64	0.42	0.47
(Nm^{-2})	(0.11)	(0.06)	(0.11)	(0.13)	(0.08)	(0.10)	(0.08)	(0.07)	(0.07)

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Table 2. Continued.

Day 15	Nothing	Frozen	Oven	PC1	PC2	Formalin	Liquid N_2	H_2O_2	PC3
Chlorophyll-*a*	2.21	1.90	1.76	2.00	1.78	2.14	2.19	2.16	2.29
($\mu g\,cm^{-2}$)	(0.18)	(0.25)	(0.10)	(0.16)	(0.18)	(0.32)	(0.19)	(0.31)	(0.15)
Chlorophyll-*b*	0.75	0.68	0.55	0.67	0.61	0.58	0.59	0.60	0.71
($\mu g\,cm^{-2}$)	(0.08)	(0.08)	(0.05)	(0.06)	(0.07)	(0.06)	(0.07)	(0.06)	(0.05)
Colloidal carbohydrate	24.28	18.58	23.16	27.65	15.79	31.42	23.16	22.15	19.93
($\mu g\,cm^{-2}$)	(4.20)	(3.39)	(4.55)	(1.75)	(2.21)	(5.65)	(3.93)	(4.75)	(2.59)
Total Carbohydrate	897	1022	727	1091	750	498	603	1010	994
($\mu g\,cm^{-2}$)	(172)	(127)	(59)	(155)	(85)	(95)	(187)	(188)	(199)
Water	101.7	88.1	82.9	86.1	95.4	87.5	88.4	95.6	94.5
($mg\,cm^{-2}$)	(4.9)	(3.9)	(3.0)	(5.7)	(7.0)	(2.1)	(4.5)	(5.9)	(4.0)
Organic matter	7.7	5.7	5.7	6.8	7.1	4.9	5.5	6.3	6.5
($mg\,cm^{-2}$)	(0.6)	(0.4)	(0.6)	(0.6)	(1.0)	(0.5)	(0.7)	(0.7)	(0.5)
Sand	166.4	177.4	185.0	190.5	154.8	187.1	192.5	192.0	184.8
($mg\,cm^{-2}$)	(17.0)	(11.8)	(9.7)	(13.5)	(12.8)	(13.4)	(12.9)	(7.7)	(5.0)
Mud	43.2	27.8	29.3	26.6	44.2	19.4	28.6	30.7	33.4
($mg\,cm^{-2}$)	(5.0)	(1.5)	(4.3)	(3.0)	(10.3)	(2.7)	(5.4)	(5.2)	(4.2)
F_o	186.33	184.83	295.50	283.50	138.00	498.50	285.00	243.17	278.33
	(40.00)	(24.82)	(58.29)	(44.24)	(28.52)	(48.82)	(39.70)	(54.57)	(31.87)
Yield F_v/F_m	0.67	0.68	0.67	0.66	0.70	0.66	0.65	0.66	0.65
	(0.01)	(0.01)	(0.00)	(0.02)	(0.02)	(0.01)	(0.01)	(0.01)	(0.01)
Relative erosion rate	1.04	0.19	7.32	0.40	0.31	2.70	1.31	0.31	0.20
(S_i)	(0.41)	(0.05)	(6.94)	(0.07)	(0.10)	(1.97)	(0.70)	(0.13)	(0.07)
Erosion threshold	5.30	8.63	6.60	7.01	7.40	5.19	5.71	7.89	7.61
(Nm^{-2})	(1.58)	(0.23)	(1.27)	(0.68)	(1.01)	(1.53)	(1.68)	(0.63)	(0.52)

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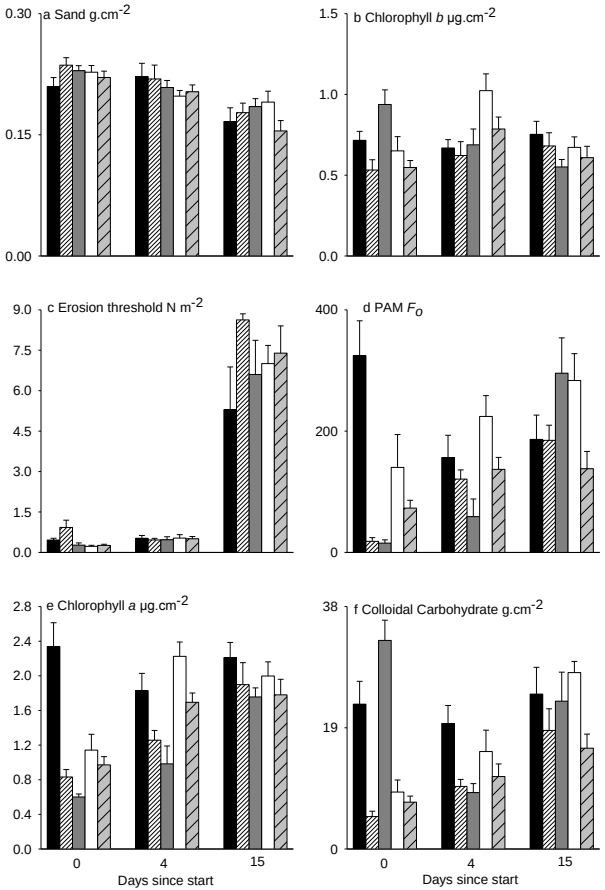

Fig. 1. Means (+ S.E., $n = 3$ plots) of selected variables for sediments that were physically disturbed: black, undisturbed; fine stripe, frozen; grey, oven; white, PC2; coarse striped, PC1 (details in text).

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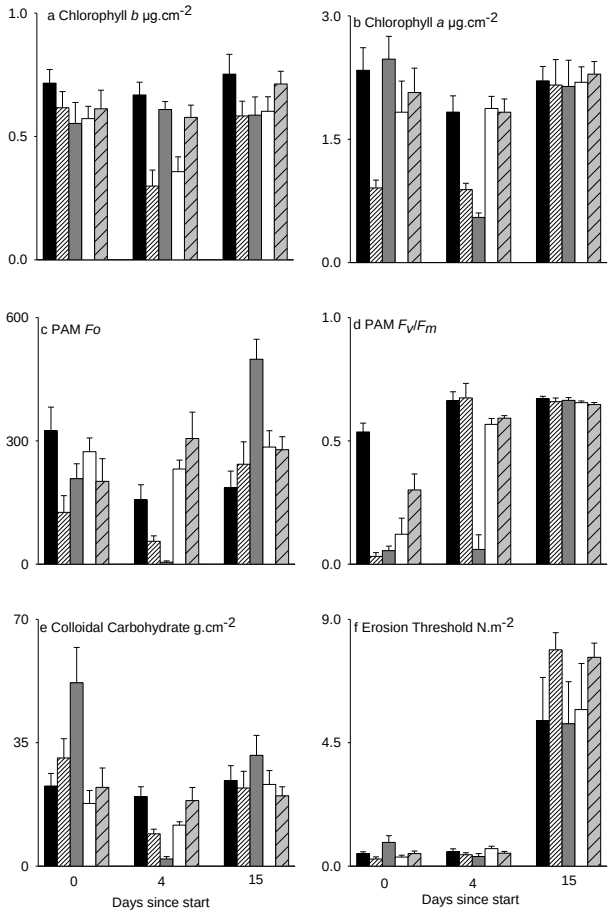

Fig. 2. Means (+ S.E., $n = 3$ plots) of variables for sediments treated in situ: black, undisturbed; fine stripe, H_2O_2; grey, formalin; white, liquid N_2; coarse stripe, PC3 (details in text).

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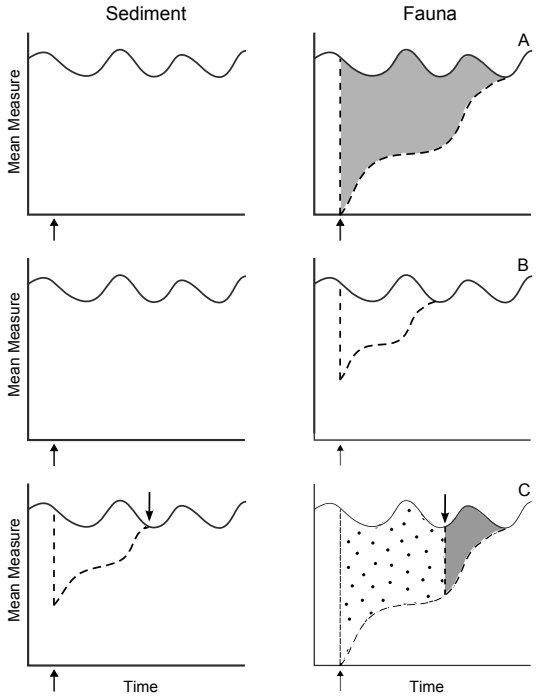

Fig. 3. Hypothetical patterns of responses of sediment and fauna to experimental defaunation at the time of the arrow on the x-axis. Solid lines indicate unaltered, control conditions. Dashed lines indicate responses by sediment or fauna to experimental treatment. **(A)** Defaunation does not affect measures of properties of sediments, defaunation is complete and the grey area indicates that processes are in response to defaunation. **(B)** Defaunation is only partial, even though sediments are unaltered. No valid inferences can be made about effects of defaunation. **(C)** Defaunation is complete, but also alters sediments, which recover by the time shown by the upper arrows. Prior to this (the dotted region), fauna are responding to defaunation and simultaneous alteration of sediment. Subsequently, fauna are responding to influences of defaunation (grey region), but only if influences of altered sediment do not persist.

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