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MBACI sampling of an episodic disturbance: stormwater effects on algal epifauna

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Abstract

Whilst it has been well established that stormwater and associated contaminants negatively impact the quality of recipient waters, the ecological effects of individual stormwater pulses in marine environments are relatively unknown. In this study, the impacts of stormwater outfalls upon water quality and epifaunal invertebrates inhabiting the alga Sargassum linearifolium are assessed through an MBACI sampling program. Water quality and the abundance of mobile algal epifauna were recorded at three control sites and three impact sites every 10-12 weeks during dry weather and opportunistically within 24 h of large rain events (> 50 ml rainfall in 24 h) and again 4 d after the rain event. Sampling took place during two periods over two separate years and this included four large rainfall events. Following rainfall, salinity dropped rapidly at impact sites close to stormwater outfalls, whilst turbidity increased. Declines in salinity at control sites were slight and turbidity did not differ to 'before' periods. The abundance of epifaunal gastropods and polychaetes were reduced at impact sites 24 h after rain events, but not control sites. The abundance of copepods, amphipods and ostracods, however, were reduced at both control and impact sites for up to 4 d following rainfall. Reductions of these taxonomic groups could not be attributed to stormwater outfalls. Whilst short-term impacts of stormwater runoff were identified for some faunal groups, impacts were not identified for the majority. Instead, effects were harbour wide (i.e. at control and impact sites), probably in response to the physical disturbance of heavy seas associated with large rain events.

Keywords: Stormwater; Environmental monitoring; MBACI; Algal epifauna, Pulse disturbance

Accepted

1. Introduction

Stormwater is a largely uncontrolled and unregulated source of contamination that has been implicated in the deterioration of water quality in freshwater (Lieb and Carline, 2000) and marine systems (Bay et al., 2003) and in the ongoing degradation of benthic marine habitats where contaminants may accumulate (Birch and Taylor, 1999, Carr et al., 2000). This complex effluent may contain many toxic contaminants including heavy metals, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and pesticides (Makepeace et al., 1995). In regions such as Port Jackson, Australia, stormwater runoff is known to be one of the major sources of heavy metals (Bickford et al., 1999). There is also the potential for deleterious impacts upon marine organisms through reduced salinity around stormwater outfalls.

Laboratory tests have established the toxicity of prolonged exposures to stormwater effluent in a variety of marine and aquatic fish and invertebrates (Carr, et al., 2000, Ellis et al., 1995, Hatch and Burton Jr., 1999, Kszos et al., 2004, Skinner et al., 1999). Fish mortality has also been noted following periods of intense storm runoff (Magaud, et al., 1997). However, stormwater runoff events occur sporadically and may be relatively short-lived with pulses lasting from minutes to hours (Brent and Herricks, 1999, Burton Jr. et al., 2000) and the responses of communities of marine organisms to pulses of stormwater in the field remain largely unknown. Pulses of chemical contaminants and freshwater entering marine systems have the potential to cause immediate impacts localized around individual stormwater outlets (Brent and Herricks, 1999, Burton Jr., et al., 2000). Predicting the ecological impacts of pollutants upon field

populations in receiving environments will be greatly enhanced by knowledge gained from ecological impact assessments (Schroeter et al., 1993). There have been few such studies addressing the effects of stormwater in marine systems.

The current study assessed the short-term (1-4 d) ecological impacts of stormwater events upon the mobile invertebrates that inhabit macroalgae. To date, there have been no studies that have monitored the ecological impacts of stormwater upon mobile algal epifaunal assemblages. The detection of ecological impacts in communities that are both spatially and temporally variable remains a major challenge in applied ecology (Schroeter, et al., 1993). The unpredictable nature of episodic events, such as stormwater, poses additional difficulties (Beck, 1996, McCahon and Pascoe, 1990), effectively enforcing opportunistic approaches. Nevertheless, the continued development of 'before-after, control-impact' (BACI) sampling designs has improved our ability to detect environmental impacts (Green, 1979, Schroeter, et al., 1993).

Algal beds dominate hard substrates in temperate marine habitats and support diverse assemblages of mobile invertebrates including crustaceans, molluscs and polychaetes (Martin-Smith, 1994) which form a major component of coastal biodiversity (Taylor, 1998). The epifauna of algal beds are highly mobile and posses the ability to respond rapidly to perturbations (Martin-Smith, 1994). As such, they are a perfect candidate for assessing the ecological effects of short-lived pulse disturbances. In the current study we examined the effects of storm water pulses through a multiple before-after-control-impact (MBACI) sampling program (Keough and Mapstone, 1997) within the outer reaches of Port Jackson (Sydney Harbour), Australia. The MBACI sampling design was modified to incorporate multiple 'after' times in order to

approximate durations of effects in potentially impacted communities. The Sydney stormwater drainage system was built during the 20th century and consists of over 20 000 km of concrete drains and more than 200 outlets emptying to marine habitats (Brown, 2005). We asked two specific questions. First; how do water quality variables (salinity and turbidity) differ through time ('before' vs. 'after' periods) at sites receiving and not receiving stormwater inputs? Second; how does the abundance of common algal epifauna associated with the common brown alga *Sargassum linearifolium* differ through time ('before' vs. 'after') at sites receiving and not receiving stormwater inputs?

Biological impacts in the present study may be evidenced by a range of responses including short (< 4 d) and long-term (> 4 d) reductions in abundance at impact sites which do not occur at control sites, or slower recovery from storm related physical disturbances (e.g. heavy seas) at impact sites than control sites. We define biological impacts as changes in the abundance of organisms at impact sites from 'before' to 'after' periods that differ from changes at control sites across the same period.

2. Methods

2.1. Sampling design

In order to assess the impacts of stormwater upon the abundance of epifaunal communities a multi-site, multi-time monitoring program known as MBACI was run (Downes et al., 2002). The inclusion of multiple sites and census times is an attempt to improve upon inappropriate spatial and temporal replication from which earlier BACI designs suffered (Downes, et al., 2002, Underwood, 1994).

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All sampling sites were shallow rocky reefs (2-5 m in depth) containing algal communities dominated by the brown alga Sargassum linearifolium. Algal beds were approximately 20 x 20 m at all sites. Three impact sites were chosen in the outer reaches of Port Jackson including Edwards Beach, Balmoral Beach and Shark Bay (Fig 1). The incorporation of multiple impact sites reduces the possibility of confusing natural fluctuations in sampled communities with anthropogenic impacts (Schroeter, et al., 1993). Impact sites were selected which had similar catchment activities (all impact sites receive runoff from predominately urban areas) and were directly adjacent to stormwater drains which emptied onto algal dominated rocky reefs. Three control sites were also selected, including Chinamans Beach, Rocky Point and Steel Point (Fig 1). Control sites were located in separate embayments or on different headlands from stormwater drains (at least 300 m away from storm drains) and were chosen on the basis of having similar algal communities (dominated by *S. linearifolium*), catchment developments (predominantly urban), aspect and degree of exposure as impact sites. The abundance and taxonomic composition of epifaunal communities at all sites were found to be similar through pilot sampling conducted in early 2004.

Sampling took place over two periods across two consecutive years; May-December in 2004 and May-September in 2005 (no rain events occurred in the later part of 2005) which coincided with the highest abundance of *S. linearifolium* (Poore and Steinberg, 1999). During these periods, all sites were sampled every 8-12 weeks on randomly chosen dry days ('before' dates). Randomized sampling dates at irregular frequencies are desirable as they avoid potentially coinciding and thus confounding natural cycles in sampled communities (Stewart-Oaten and Murdoch, 1986). The only

constraint upon the random selection of dry sampling dates was that they were preceded by at least 24 h of zero precipitation and no substantial rainfall (maximum ~ 5 mm) occurred in the week prior to collecting 'before' samples. A total of seven 'before' dates were sampled.

All sites were also sampled within 24 h of a rain event ('after' dates). At least 50 mm of rain within a 24 h period at any of the sites constituted a rain event. Rain figures from the Australian Bureau of Meteorology website

(www.bom.gov.au/hydro/flood/nsw/sydney_metro.shtml) for Mosman and Rose Bay weather stations estimated rainfall received over a 24 h period at the northern and southern sites respectively. We observed strong flows emanating from all drains when sampling 24 hr after rainfall. Drains were not observed to flow under dry sampling conditions. Sites were once again sampled 4 d after such a rain event in order to establish approximate time scales of recovery. A period of four days following sampled rain events was chosen as individuals of the alga *S. linearifolium* are re-colonised within one or two days of complete defaunation associated with small-scale experimental disturbances (Poore, 2005, Roberts and Poore, 2006). A total of four rain events were sampled throughout the two sampling periods.

2.2. Water quality and biological sampling

Sampling involved collecting biological samples and water quality data. At each site, 10 replicate readings of salinity and turbidity were taken *in situ* using a Yeo-Kal[®] Model 611 Intelligent Water Quality Analyzer. Swimmers deployed the probe at haphazardly selected locations within the algal bed from which algal samples were

collected whilst an assistant on shore noted the readings. At each site and on each date, 7 individuals of *Sargassum linearifolium* were also collected while snorkeling (selected at random from algal patches within 20 m of the stormwater outfall). Samples were transferred quickly to 1 L watertight containers with resident epifaunal invertebrates, transported to the laboratory and fixed in a 5% formalin solution. Prior to counting, algal samples were rinsed in fresh water and sieved through 300 μ m mesh to retain invertebrates. Epifauna were then sorted to groups including gammarid amphipods, anemones, copepods, gastropods, isopods, ostracods and polychaetes using a dissecting microscope and finally stored in 70% ethanol. Algal wet weights were recorded and counts converted to densities (# organisms/gram algae). Algal wet weights were analysed statistically to check for any possible bias in sample size. The wet mass of algal samples collected did not differ between the levels of any factors or interactions of interest (P > 0.120 in all cases).

2.3. Data analyses

Analyses of variance (ANOVA) contrasted temporal patterns of abundance and water quality at control and impact sites. Only taxa that displayed overall mean abundances > 0.10 individuals/gram of algae at control sites during dry weather were formally analysed. These taxa included gammarid amphipods, anemones, copepods, gastropods, isopods, ostracods and polychaetes. The total abundance of all individuals collected, which included rarer taxa (<0.10 individuals/gram), was also analysed. The main interaction of interest in an MBACI design for identifying ecological impacts is the interaction between control/impact sites and before/after times (Downes, et al., 2002).

This interaction was examined using planned comparisons testing for differences in abundance between before and after sampling times at control and impact sites separately. Four comparisons were run for each variable; before rain vs. 24 h after rain and before rain vs. 4 d after rain at control sites, and again for data from impact sites. Planned comparisons were tested over the appropriate error term taken from the full model (Quinn and Keough, 2002).

Responses considered to be indicative of biological effects were determined *a priori*. Short-term biological impacts of stormwater were defined as significant reductions in abundance at impact sites 24 h, but not 4 d after rain events, in the absence of significant time effects at control sites. Long-term biological impacts of stormwater were defined as significant reductions in abundance at impact sites both 24 h and 4 d after rain events, in the absence of significant time effects at control site at control sites. Short-term biological impacts of rain events *per se* were defined as significant reductions in abundance at significant reductions in abundance at both control and impact sites 24 h, but not 4 d after rain events whilst long-term impacts would persist for 4 days at control and impact sites. Contrasting temporal patterns of change in water quality variables at control and impact sites confirmed the appropriate choice of control locations.

Analyses of variance and planned comparisons were performed using the statistical package SYSTAT[®] Version 10 (SPSS Inc.). The assumptions of normality and heterogeneity of variance were tested for each variable by examining residual histograms and scatterplots of estimates vs. residuals respectively (Quinn and Keough, 2002). When necessary, data were log transformed to satisfy the assumptions of ANOVA. Data for

salinity were negatively skewed and required reflection prior to transformation. (Quinn and Keough, 2002).

3. Results

3.1. Effects of stormwater upon water quality

Water quality parameters showed different temporal patterns at control and impact sites. Salinity was significantly lower at both control and impact sites 24 h after rainfall. Reductions in salinity were substantially greater at impact sites than control sites (reduced from 34 ppt to approximately 27 ppt and 32 ppt respectively) (Table 1, Fig. 2a). Salinities at control and impact sites were still reduced after 4 d (Table 1, Fig 2a). However, salinity at impact sites showed strong signs of recovery to dry weather conditions (Table 1, Fig 2a). Turbidity was significantly higher at impact sites 24 h after rainfall, than during 'before' periods (Table 1, Fig. 2b). In contrast, turbidity at control sites 24 h after rainfall did not differ from 'before' periods and was significantly lower 4 d after rainfall (Table 1, Fig. 2b).

3.2. Effects of stormwater upon epifauna

Four types of response to stormwater were identified in epifaunal taxa; short-term reductions in abundance at impact sites, delayed recovery at impact sites following rainfall, broad scale reductions in abundance across both control and impact sites and, finally, no identifiable ecological impacts. Of all taxa analysed, only the response of gastropods and polychaetes were suggestive of an impact of stormwater. The abundance of both groups was significantly lower at impact sites 24 h after rainfall, than at impact

sites before rainfall (Table 2, Fig. 3d,g). In contrast, the abundance of gastropods and polychaetes after rain events at control sites did not differ significantly from abundances during dry periods (Table 2, Fig. 3d,g). After 4 d, the abundance of polychaetes had recovered to dry weather conditions whilst gastropod abundances were still substantially reduced, although not significantly so (Table 2, Fig. 3d,g). While no other taxa demonstrated significant responses to stormwater, a subtle effect was detected for copepods. The abundance of copepods was significantly lower at both control and impact sites 24 h after rainfall. However, recovery at impact sites was slower than at control sites. While copepod abundance was no longer significantly reduced at control sites after 4 d, abundances were still significantly lower than during 'before' times after 4 d at impact sites (Table 2, Fig. 3b).

The abundance of gammarid amphipods (the most numerically dominant taxa), ostracods and the overall abundance of epifaunal communities were significantly lower at both control and impact sites within 24 h of heavy rainfall than during dry conditions (Table 2, Fig. 3c,f,h). Reductions of gammarids, and the entire epifaunal community were of a greater duration than those identified for polychaetes and gastropods, with lowered abundances persisting for at least 4 d following rain events with no signs of recovery (Table 2, Fig. 3c,h). Ostracods recovered more rapidly (Table 2, Fig. 3f). The abundance of anemones and isopods were highly variable at all sampling times and were unaffected by rain events (Table 2, Fig. 3a,e).

4. Discussion

The detection of ecological impacts of stormwater in marine systems has proven difficult despite anecdotal evidence that toxicants such as heavy metals and hydrocarbons may have widespread impacts (Morrisey, et al., 2003). The majority of studies have considered the ecological impacts of accumulated contaminants (particularly in sediments), with little consideration of effects of individual pulses of stormwater upon recipient communities. Transient influences of stormwater pulses upon various aspects of the physical environments have been identified but impacts upon biological communities may not necessarily result. For example, Schiff and Bay (2003) sampled infaunal habitats offshore from drainage catchments following large rain events and found stormwater to alter sediment composition and contaminant concentrations in benthic habitats. However, infaunal assemblages sampled from sites within impacted regions were both abundant and healthy (Schiff and Bay, 2003).

In the present study, whilst stormwater was found to affect water quality variables, biological effects of runoff were relatively subtle and for the majority of taxa, could not be detected. Rather, the data presented in the current study show obvious and substantial impacts of actual rain events which occur across all sampling sites. Salinity at impact sites decreased substantially within 24 h of rainfall, whilst turbidity increased. Salinity dropped slightly at control sites after rain, and turbidity did not differ from the 'before' period. In contrast to patterns in water quality, stormwater did not have consistent effects upon the abundance of epifauna, with a variety of responses identified. Gastropods and polychaetes were the only taxa for which localized pulses of stormwater were found to have negative ecological impacts. Both taxa recovered very rapidly from pulses with the detectable impacts identified 24 h after strong rain events, no longer

evident after 4 d. Such a rapid recovery suggests that reductions in the abundance of epifauna were due to emigration of mobile organisms away from stormwater plumes, rather than direct mortality. Further research examining the role of behavioural avoidance of stormwater pulses in the field by mobile epifauna is required. The speed of recovery also suggests that impacts were confined to a relatively small region surrounding stormwater drains (the current study sampled a region of approximately 20 x 20 m around stormwater drains). Thus it appears that ecological impacts of stormwater pulses in this part of the harbour have limited spatial and temporal extent.

Despite detectable impacts upon gastropods and polychaetes, temporal fluctuations in the abundance of the epifaunal community as a whole could not be attributed to stormwater as the responses of epifauna to rain events were comparable at both control and impact sites. Although water quality parameters were clearly impacted by stormwater, the majority of taxa demonstrated either no response to stormwater pulses or significant reductions at both control and impact sites. Together the water quality and biological data suggest that whilst some ecological impacts of stormwater pulses are evident, generally, the factors that determine epifaunal abundance in algal habitats were operating across broader spatial scales than the factors that determine local water quality following heavy rainfall. It is possible that deleterious environmental conditions associated with large rain events masked any direct effects that stormwater may have upon epifaunal communities. All rain events sampled generally coincided with heavy seas, whilst conditions during dry weather sampling were typically calm.

Mobile marine assemblages have received much less attention than their sessile counterparts with respect to the role of disturbance in structuring communities (Sousa,

2001). There is however, some evidence to suggest that the mobile epifauna of algal beds are negatively affected by turbulent seas. Night time sampling of dispersing amphipods in algal beds found greater numbers in the water column during heavy seas than calm conditions, suggesting dislodgement of amphipods from seaweeds by strong currents and large waves (Fincham, 1974). In addition, the abundance of epifauna is frequently lower on algae at exposed sites than the same algal habitat in protected areas (Sánchez-Moyano et al., 2000, Tararam and Wakabara, 1981, Yassini et al., 1995). It is likely therefore, that the reductions identified in epifaunal abundance after rainfall can in part be attributed to heavy seas which co-occur with large storms.

The ability of an MBACI design to detect anthropogenic impacts relies in part upon the appropriate selection of control sites. Control sites must be selected that are located far enough away from the putative contaminant sources so as to be unaffected by it (Downes, et al., 2002). In the present study, control sites showed evidence of ecological impact however, water quality data suggested that the location of control sites was appropriate for this study. Previous research has found salinity to be the most accurate means of identifying plumes of stormwater (Washburn et al., 2003). The combined salinity and turbidity measurements recorded in the current study suggest that stormwater plumes did not extend to control sites. This study therefore highlights the dual considerations when selecting and locating appropriate control sites. Reference locations in monitoring studies must be located outside the zone of potential environmental impact, but also close enough to impacted sites so as to be similarly affected by broader scale processes that would influence the temporal patterns of abundance in monitored communities. For example, in the present study control sites were located such that they

were outside the influence of stormwater plumes, but in a region that would be similarly affected by physical disturbances associated with storms. Had control sites been located further afield (e.g. in an adjacent estuary) the impact of stormwater drains is likely to have been overestimated.

In the present study, we have attempted to approximate time scales of recovery of epifaunal communities from large rain events. Recovery from disturbance in algal epifaunal communities involves re-colonisation by dispersing epifauna in the water column or organisms crawling from alga to alga (Taylor, 1998) and can be very rapid, with recovery from complete defaunation within days (Poore, 2005). Epifaunal communities as a whole were reduced throughout the outer reaches of a large harbour in the present study. In a system where recovery is reliant upon re-colonisation of small dispersers, it is likely that the speed at which communities recover is dependant in part upon the spatial scale of disturbance. Recovery from a disturbance event on a harbourwide scale is likely to be much slower than recovery from localized disturbances. This appears to hold true for the dominant fauna in our study (amphipods and copepods); abundances of which were still depressed four days after a rainfall event.

The impacts of stormwater pulses on algal epifauna in this study appear to be subtle and quite localized. Contaminants within stormwater may pose a greater threat to marine organisms through their tendency to accumulate over time (Bay, et al., 2003, Carr, et al., 2000). Experimentally increased copper levels in the tissues of the alga *S*. *linearifolium* results in reduced colonisation, feeding and survivorship of *Peramphithoe parmerong*, an abundant herbivorous amphipod (Roberts et al., 2006). Consequently, the ecological impacts of contaminants in surface runoff may become more evident when

sampled across longer time scales than individual rain pulses. The impact of individual pulses of contaminants may also decrease with time, as background levels of contamination increase, resulting in constant stresses upon exposed communities, and subsequent development of resistance (Bryan and Hummerstone, 1971, Shavyrina et al., 2001). Further research is required in order to link the findings of laboratory based stormwater toxicity tests with the results obtained from field monitoring programs.

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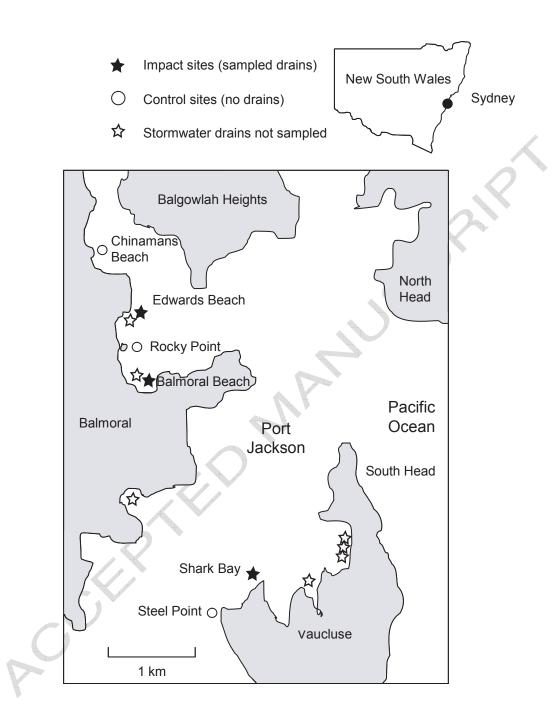
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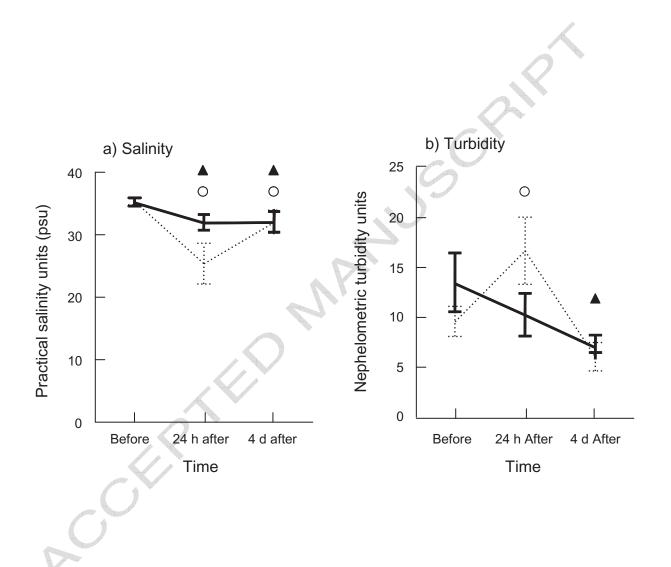
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Figure 1. Location of storm water drains (open stars), control (open circles) and impact (closed stars) sampling sites within the outer reaches of Port Jackson. Sites with stormwater drains that were not sampled did not drain onto algal beds, or drained onto beds that did not contain *Sargassum linearifolium*. Only drains with a diameter of approximately 0.75 m are shown, smaller drains were not identified near control sites.

Figure 2. Temporal changes in a) salinity and b) turbidity at control (solid line) and impact sites (dashed line). Turbidity is expressed as nephelometric turbidity units \pm standard error (n = 3 per observation, means of site averages). Planned comparisons contrasted salinity and turbidity at control and impact sites separately. Means marked with triangles indicate a significant difference exists between the control site mean at that time and the control site data from before periods. Means marked with circles indicate a significant difference exists between the impact site data from before periods.

Figure 3. Temporal changes in the abundance of a) anemones, b) copepods, c) gammarids, d) gastropods, e) isopods, f) ostracods, g) polychaetes and h) the total community at control (solid line) and impact sites (dashed line). Data are mean densities \pm standard error (n = 3 per observation, means of site averages). Planned comparisons contrasted the abundance of taxonomic groups at control and impact sites separately. Means marked with triangles indicate a significant difference exists between the control site mean at that time and the control site data from before periods. Means marked with circles indicate a significant difference exists between the impact site data from before periods.





Roberts et al.- Fig. 3

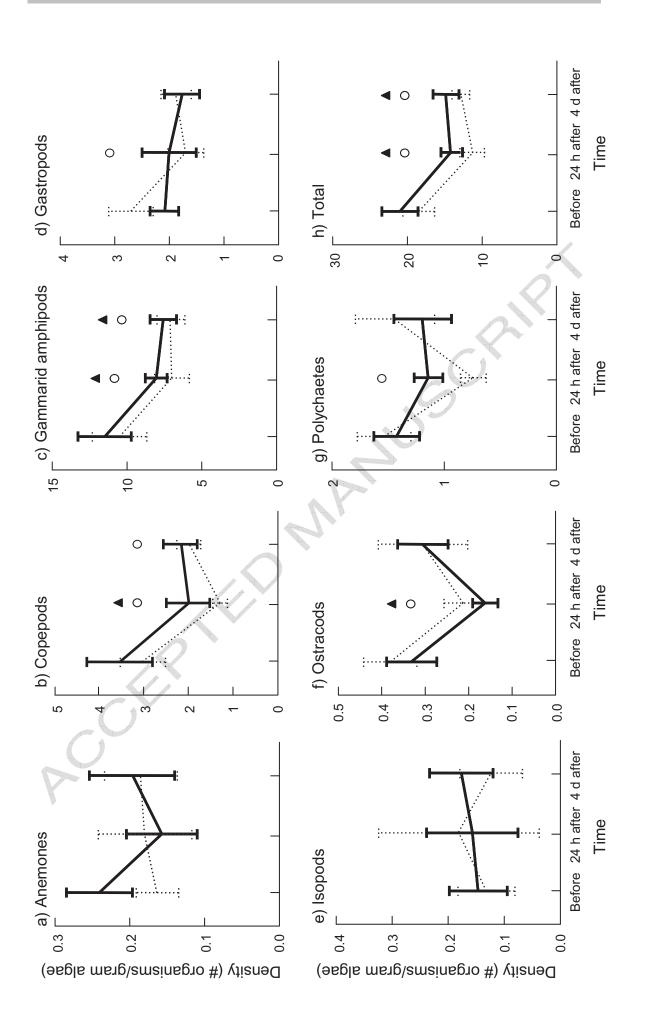


Table 1. Planned comparisons for water quality parameters contrasting temporal changes at control and impact sites. Appropriate error terms were taken from the omnibus ANOVA.

		Salinity ^b		Turbidity ^a	
	df	MS	р	MS	р
Control sites					
Before vs. 24 h After	1	4.003	0.006	3.382	0.389
Before vs. 4 d After	1	6.986	0.001	26.619	0.034
Impact sites					
Before vs. 24 h After	1	35.557	0.000	24.386	0.040
Before vs. 4 d After	1	7.720	0.001	19.364	0.061
Error	8	0.282		4.073	

. Bo Data are ppt (salinity) and nephelometric turbidity units (turbidity). Bold figures are statistically significant

Table 2. Planned comparisons contrasting temporal changes in the abundance of

 epifaunal taxa at control and impact sites. Appropriate error terms were taken from the

 omnibus ANOVA.

		Anemones ^a		<u>Copepods^a</u>		Gammarids ^a		Gastropods ^a	
	df	MS	р	MS	р	MS	р	MS	Р
Control sites									
Before vs. 24 h After	1	2.345	0.169	14.949	0.043	4.293	0.030	2.715	0.298
Before vs. 4 d After	1	0.642	0.452	7.863	0.120	13.034	0.002	0.864	0.548
Impact sites									
Before vs. 24 h After	1	0.209	0.664	70.324	0.001	21.713	0.000	19.003	0.019
Before vs. 4 d After	1	0.131	0.730	17.208	0.033	7.732	0.008	6.020	0.136
Error	8	1.027		2.599		0.623		2.195	
		<u>Isopods^a</u>		Ostracods ^a		Polychaetes ^a		<u>Total^a</u>	
	df	MS	р	MS	р	MS	р	MS	р
Control sites									
Before vs. 24 h After	1	0.053	0.674	11.050	0.017	0.032	0.894	7.647	0.024
Before vs. 4 d After	1	0.272	0.350	0.437	0.567	0.541	0.586	7.438	0.026
Impact sites				N					
Before vs. 24 h After	1	0.000	0.995	9.072	0.026	23.844	0.006	33.414	0.000
Before vs. 4 d After	1	0.238	0.380	0.358	0.603	1.241	0.416	8.532	0.019
Error	8	0.276		1.224		1.684		0.995	

Data are mean abundances (# of individuals/gram of algae). Bold figures are statistically significant (P <

0.05).

^aLog transformed

o^c