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Field and laboratory simulations of storm water pulses: Behavioural avoidance by marine epifauna

David A. Roberts*, Emma L. Johnston, Stefanie Müller, Alistair G.B. Poore

School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia

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Brief storm water pulses trigger avoidance response in mobile epifauna due to the inundation of freshwater.

Abstract

Epifaunal communities associated with macroalgae were exposed to storm water pulses using a custom made irrigation system. Treatments included Millipore® freshwater, freshwater spiked with trace metals and seawater controls to allow for the relative importance of freshwater inundation, trace metals and increased flow to be determined. Experimental pulses created conditions similar to those that occur following real storm water events. Brief storm water pulses reduced the abundance of amphipods and gastropods. Freshwater was the causative agent as there were no additional effects of trace metals on the assemblages. Laboratory assays indicated that neither direct nor latent mortality was likely following experimental pulses and that epifauna readily avoid storm water. Indirect effects upon epifauna through salinity-induced changes to algal habitats were not found in field recolonisation experiments. Results demonstrate the importance of examining the effects of pulsed contaminants under realistic exposure conditions and the need to consider ecologically relevant end-points.

Keywords: Field simulation; Storm water; Algal epifauna; Avoidance

1. Introduction

One of the most useful yet underutilized approaches to assess the impacts of contamination events upon assemblages of organisms are small-scale field simulations (McCahon et al., 1991; Underwood, 1995). Field simulations are appealing as they allow entire assemblages to be exposed to chemical contaminants under realistic exposure conditions (McCahon and Pascoe, 1990). They also allow for the manipulation of factors which may contribute to the toxicity of individual contamination events, such as the composition of contaminants, or the duration and intensity of pulse exposures. Yet despite their advantages, such studies are relatively rare in the marine literature (Johnston et al., 2003).

The identification of toxic effects of chemical contaminants in marine and aquatic environments has largely been the domain of ecotoxicology, a field which relies heavily upon standardized laboratory based testing protocols (Johnston and Keough, 2002). Laboratory based toxicity testing generally involves a 24–96 h exposure of a single species to a single chemical contaminant maintained at a constant concentration (Cairns, 1983; Chapman, 1995a). While such studies are an invaluable means of assessing the potential or relative toxicity of specific contaminants, the ecological relevance of such an approach has been questioned (Breitholtz et al., 2006). Standardized toxicity testing may fail to simulate the complexity of real world toxicant exposure scenarios where the dilution, dispersion and degradation of contaminants and the behaviour of organisms may change the effective duration of exposure (Brent and Herricks, 1999; Ellis et al., 1995; Moriarty, 1999). Moreover, interactions between organisms in complex communities may influence their responses to contaminants

* Corresponding author. Tel.: +61 2938 53447; fax: +61 2938 51558.
E-mail address: d.roberts@student.unsw.edu.au (D.A. Roberts).

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(DeAngelis, 1996; Johnston and Keough, 2003). In cases where contamination occurs in discrete pulses, such as storm water, exposure scenarios may be more realistically simulated in a field experiment than the laboratory.

Storm water is a pulse disturbance that is capable of conveying large quantities of chemical contaminants to recipient environments in a short amount of time (Magaud et al., 1997; Makepeace et al., 1995). These contaminants may accumulate in sediments (Birch, 2000) and macroalgae (Roberts et al., 2006) and have been linked to the chronic degradation of biological communities in soft sediment habitats (Carr et al., 2000; Morrisey et al., 2003; Schiff and Bay, 2003). Little is known, however, about the short-term effects of storm water pulses in recipient environments following periods of heavy rainfall. Storm water events are typically discrete and relatively short (hours to days) and produce exposures to contaminants potentially lasting from only minutes to hours (Brent and Herricks, 1998; Burton et al., 2000).

In shallow temperate marine environments, hard substrates are dominated by dense macroalgal beds which support abundant and diverse assemblages of mobile epifauna (Taylor, 1998a). Epifaunal assemblages are typically dominated by crustaceans such as amphipods, copepods and isopods, small molluscs and polychaetes (Martin-Smith, 1994) which form an important component of temperate food webs (Taylor, 1998a). Storm water outfalls frequently drain directly onto macroalgal beds and runoff may alter abiotic conditions for the period of the event (Roberts et al., in press). Our previous research in Sydney Harbour, Australia has found that both the physio-chemical properties of marine waters and the abundances of several key taxa in epifaunal communities were affected by storm water events. Sites adjacent to storm water drains were characterized by both low salinities and high turbidities following rainfall, as well as reduced abundances of polychaetes, gastropods and copepods for up to 4 days (Roberts et al., in press). However, the mechanisms by which these responses arise are not known and, in particular, it is unclear whether fauna are responding to the chemical contaminants or freshwater inundation. Furthermore, responses could be explained by direct mortality and/or behavioural avoidance (escape) of storm water plumes. There is also the potential for indirect effects of storm water pulses which may result from stresses implied upon algae through reduced salinity which alters their suitability as habitat for epifauna (Kakinuma et al., 2006).

In the present study we address four specific questions using a combination of field simulations of storm water events and laboratory based experiments: (1) Are responses of epifauna to storm water pulses triggered by the inundation of freshwater, or the metal contaminants within storm water? (2) Are reductions in the abundance of mobile epifauna following storm water pulses a result of direct mortality or behavioural avoidance of plumes? (3) Do organisms exposed to brief storm water plumes suffer latent mortality? and (4) Are there indirect effects of storm water pulses upon epifauna due to exposure of algal habitats to reduced salinities?

2. Materials and methods

2.1. Study sites and organisms

Field experiments were conducted at Frenchmans (33°59′14″S, 151°13′48″E) and Congwong Bays (33°59′24″S, 151°14′08″E), which are located within Botany Bay, Sydney, Australia. The sites are protected rocky reefs with a maximum depth of 4 m. Brown algal beds cover hard substrates at both sites, with *Sargassum linearifolium* (Turner) C. Agardh being the most abundant species during the winter months. This alga is host to a diverse epifaunal assemblage of molluscs, crustaceans and polychaetes (Poore et al., 2000; Roberts and Poore, 2006).

2.2. Field-based experimental exposures

Mobile invertebrates inhabiting *S. linearifolium* were exposed to mimic storm water pulses in the field in order to assess responses to freshwater inundation and heavy metal contaminants. The field experiment consisted of four separate treatments (eight replicates per treatment): a seawater control (‘seawater’), freshwater free of metallic contaminants (‘fresh’), freshwater spiked with copper and zinc (‘spiked’) and an un-manipulated control (‘un-dosed’). Field dosing involved pumping site-collected seawater (seawater control), or freshwater (spiked or un-spiked) from containers on the shore through a sub-tidal irrigation system (constructed of 12 mm plastic hosing) which was directed onto algal patches. By comparing responses of epifauna to spiked and un-spiked freshwater treatments, the relative importance of freshwater inundation and metal contaminants as causative agents could be determined. Un-manipulated algae were collected from patches not disturbed by the experimental setup to test for effects of the setup of the irrigation system. Seawater controls tested the effects of increased water flow resulting from pumping which may dislodge epifauna from algal habitats.

Mimic effluent was used in place of actual storm water to facilitate consistency in contaminant concentrations across replicates. The toxicity of storm water can vary greatly across brief time scales making the collection of effluent with consistent contaminant levels difficult (Katznelson et al., 1995). Furthermore, the toxicity of storm water may be affected by various factors associated with laboratory storage (USEPA, 2002). We specifically considered the ecological effects of trace metals which are amongst the most toxic and commonly identified contaminants within storm water (Makepeace et al., 1995).

Mimic storm water was created by spiking water from a Millipore® reagent grade purification system (catalogue # ZFMQ230U4) with known concentrations of copper and zinc. These metals are amongst the most commonly identified metals in storm water and generally occur at the highest concentrations (Duncan, 1999; Makepeace et al., 1995). Stock solutions (1000 mg L^{-1}) were created by dissolving $CuSO_4$ (UNILAB®) and $ZnSO_4 \cdot 7H_2O$ (SIGMA®) in Millipore water. Un-spiked Millipore water was then transported to the field site in clean plastic 15 L containers. At the time of dosing, the appropriate amounts of copper and zinc stock solutions were mixed through the Millipore water to create mimic effluent. Nominal concentrations of copper and zinc in the mimic effluent prior to delivery were 150 μg L^{-1} and 580 μg L^{-1}, respectively. These values represent the maximum mean copper and zinc concentrations from storm water samples from an extensive review of storm water constituents (Makepeace et al., 1995). Much of the metal load found in storm water runoff may be associated with particulates such that metal concentrations in our mimic effluent represent relatively high levels of dissolved contaminants.

In order to dose epifaunal communities, 12 V water pumps (LVM Amazon® SP10 Series) were placed in buckets of effluent and connected to batteries (DiaMec® DM12–18). Each replicate received 45 L of mimic storm water or seawater, which took approximately 5 min to pump. At the end of the dosing period, a single *S. linearifolium* was collected from within 30 cm of the outlet to the irrigation system and placed in a sample jar. Replicate dosing trials were separated by 5 m. Algal samples were preserved in a 5% formalin solution. Preserved algal samples were rinsed with freshwater to remove invertebrates and these were then sorted to taxonomic groups including amphipods, copepods, gastropods, polychaetes and ostracods. Algal samples were blotted dry with paper toweling and weighed to the nearest 0.1 g. The abundance of each taxonomic group was analysed as number per gram algae. The wet mass of algal samples did not differ between experimental treatments ($F_{3,28} = 0.288$, $P = 0.834$).

Salinity, turbidity, dissolved oxygen and temperature readings were measured at the outlet of the irrigation system for each replicate before pumping commenced until after the pulse had passed, and within un-manipulated patches of algae. These measurements were taken with a Yeo-Kal® Model 611 Intelligent Water Quality Analyzer (set to log automatically at 2 min intervals). Seawater samples were taken from two randomly chosen replicates from each treatment at the outlet of the irrigation system following the 5 min exposure. Samples were acidified with 75 μL HNO_3 (Ajax FineChem®) and analysed for total copper and zinc by way of ICP-AES at the NATA accredited National Measurement Institute in Sydney, Australia.

2.3. Laboratory based experimental exposures

Reduced abundance of an organism following exposure to storm water could be explained by direct mortality or behavioural avoidance (i.e. escape). To distinguish between these possible mechanisms, epifaunal assemblages were exposed to the same treatments in the laboratory as in the field (again with eight replicates per treatment). Individual algae of 10 cm height were collected from the field in 1 L containers with resident communities intact and transported directly to a temperature controlled laboratory (20 °C). Once in the laboratory, algae were attached to sandstone blocks (to replicate the substrate in the field) and placed in 8 L aquariums within separate flow through systems containing 3 L of seawater from the collection site.

Following a 30 min acclimation period, each alga was exposed to one of three dosing solutions: seawater collected from the field site, seawater diluted to 28 ppt with Millipore water, or seawater diluted to 28 ppt with metal spiked Millipore water (field dosing reduced salinity to approximately 28 ppt). Dilutions were stored in plastic header tanks and pumped into the aquaria using Eheim® 1000 Series pumps for a period of 5 min at a rate of 8 L/min.

Immediately following the pulse exposure, the alga was removed from the aquarium and preserved with resident epifauna in a 5% formalin solution (individuals remaining on the alga were assumed to be alive). Following exposures, aquarium water was filtered through a 300 μm sieve to collect organisms that had vacated seaweeds. Dispersing individuals were immediately viewed under a dissecting microscope to ascertain whether individuals were dead or alive (death was determined as no response when gently prodded). Live animals were rinsed twice in clean seawater and placed in separate 250 ml jars with 200 ml of clean seawater without food to gauge latent mortality over a 24 h period.

The number of organisms alive in the water column (dispersers) and on algae (non-dispersers) was recorded following 5 min exposures. The number of dispersers from each taxonomic group was standardized by the total abundance of that taxa (sum of dispersers and organisms alive on algae) to provide a proportion of individuals from each taxonomic group that chose to disperse (i.e. leave their algal habitat) in each treatment. The number of dispersing epifauna that died in the 24 h latent period was also recorded and was standardized by the number of individuals that dispersed to provide a proportion of dispersing individuals from each taxonomic group that experienced latent mortality in each treatment. Wet mass of algal samples did not differ between experimental treatments ($F_{2,21} = 0.381$, $P = 0.688$). Water samples were collected from two randomly selected replicates for each treatment and processed and analysed as described earlier for samples from field exposures.

2.4. Colonisation of salinity stressed algae

Storm water runoff may have indirect effects upon epifauna through the alteration of algal habitats. To test this, the colonisation of salinity stressed algae by epifauna was examined in a field experiment repeated on three randomly selected dates in July 2006. Prior to each trial, 18 *S. linearifolium* were collected and returned to a temperature controlled laboratory (20 °C). Algae were defaunated using the liquid insecticide carbaryl (1-napthyl-*N*-methylcarbamate). Carbaryl is toxic to amphipods at concentrations as low as 1 $\mu g\,L^{-1}$ (Shacklock and Croft, 1981) and is not known to affect algae (Carpenter, 1986). Four *S. linearifolium* at a time were placed in 4 L of carbaryl solution (1 g carbaryl/L filtered seawater) and left for 5 min while being shaken repeatedly. Following defaunation, algae were thoroughly rinsed in clean, filtered seawater to remove superficial carbaryl solutions. This treatment successfully removes over 95% of amphipods and copepods, 80% of gastropods and over 70% of polychaetes (personal observations).

Algae were then exposed to one of three salinity treatments: a seawater control (34 ppt), mid-range salinity (30 ppt) and low-range salinity (25 ppt) for a period of 12 h (six replicates per treatment). Salinity dilutions were created by diluting freshly collected seawater with appropriate amounts of Millipore water. Nine 5 L aquaria containing 4.5 L of diluted or undiluted seawater were used for salinity exposures, with three aquaria per level of the salinity treatment (two algae per aquaria). Aquaria were arranged randomly under a Power-Glo® aquarium light, covered with cling wrap to avoid evaporative loss which may alter salinity levels and aerated for the entire period of the stress treatment.

Following stress treatments, algae were marked with flagging tape and returned to the field for 4 days to allow for recolonisation by mobile fauna. Individual thalli were randomly attached to one of three plastic mesh plots (45 × 35 cm) by cable ties. Mesh plots were attached to the reef by SCUBA divers with masonry nails. After the 4-day colonisation period algal samples were collected in 1 L containers.

Samples were preserved and processed as described for the first field experiment. Epifauna were sorted to the following taxonomic groups: amphipods, copepods, gastropods and polychaetes. Wet mass of algal samples did not differ between levels of the salinity treatment ($F_{2,4} = 0.524$, $P = 0.628$).

2.5. Data analyses

Data were analysed using a series of planned comparisons, which were determined *a priori*. Only taxa that displayed mean abundances of >0.5 individuals/g algae in un-manipulated controls were formally analysed. These taxa included amphipods, copepods, gastropods, ostracods and polychaetes. In the field experiment, the first planned comparison contrasted un-dosed control with seawater controls. If no significant response was identified, seawater controls were then contrasted with un-spiked and spiked freshwater treatments, respectively. In cases where un-dosed and seawater controls differed (indicating an artifact of the experimental methodology) further analyses were not conducted. In the laboratory experiment, two planned comparisons were performed: control vs. un-spiked freshwater and control vs. spiked freshwater. For the salinity stress experiment ostracod recolonisation was minimal and so only amphipods, gastropods, copepods and polychaetes were analysed. Data were analysed by a two factor ANOVA with 'date' as a random factor and 'salinity' as a fixed factor.

Analyses of variance and planned comparisons were performed using SYSTAT® Version 10 (SPSS Inc.). Planned comparisons tested for specific effects over the error term from the full ANOVA (Quinn and Keough, 2002). 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. Correct error terms for the mixed model (salinity stress experiment) were taken from Zar (1999).

3. Results

3.1. Physio-chemical parameters of experimental exposures

The experimental dosing system successfully simulated brief runoff events in the field and in the laboratory. In the field experiment, salinity dropped rapidly from ambient levels (approximately 34 ppt) to 28 ppt in plots receiving spiked and un-spiked freshwater treatments (Fig. 1a). These reductions persisted for the duration of the trial (5 min). Two separate trials were run for metal spiked freshwater treatments as initial

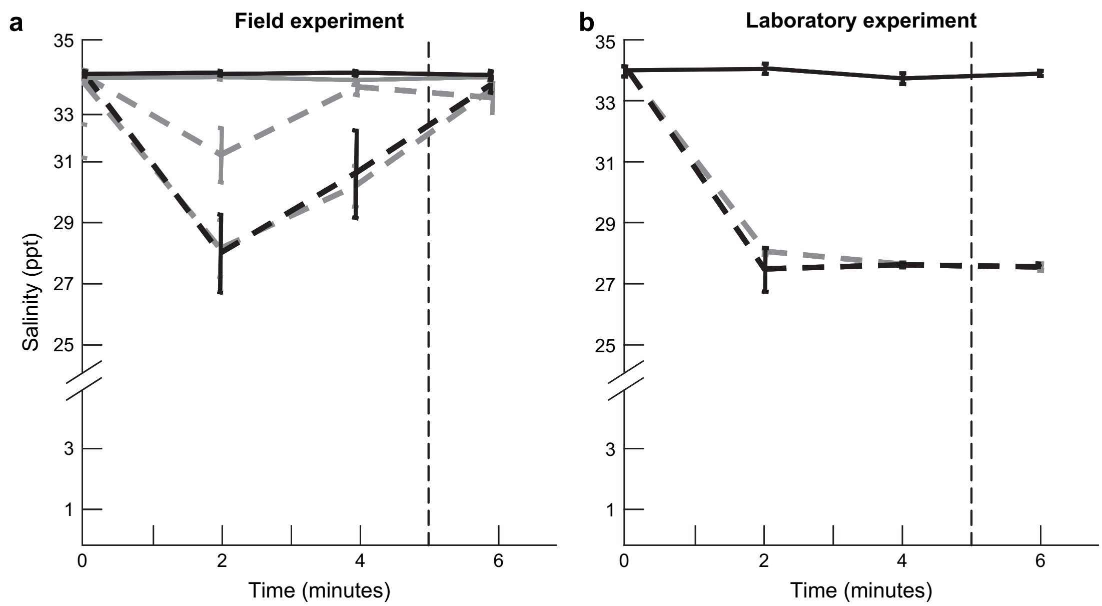

Fig. 1. Experimental storm water pulses created in the experimental exposures in the (a) field and (b) laboratory as traced by salinity measurements. Black and grey unbroken lines represent un-dosed and seawater control treatments, respectively. Black and grey dashed lines show data for freshwater and metal spiked freshwater pulses, respectively. Two separate field trials were run with metal spiked freshwater, which are shown separately in (a). Salinity is expressed in parts per thousand (ppt). Error bars show means ± S.E.

salinity pulses varied between spiked and un-spiked freshwater treatments. The second trial indicated that salinity pulses in spiked and un-spiked freshwater treatments were identical (Fig. 1a). Recovery of salinity was rapid, returning to ambient salinity levels within 2 min of the trial finishing (Fig. 1a). Salinity measured in un-manipulated, and seawater control plots maintained a steady salinity of 34 ppt (Fig. 1a). Water samples collected from the outlet of the irrigation system following the 5 min exposure in the spiked metal treatments contained copper and zinc concentrations of $29 \pm 1.10\ \mu g\ L^{-1}$ and $159 \pm 9.90\ \mu g\ L^{-1}$, respectively. Copper and zinc concentrations were $<5\ \mu g\ L^{-1}$ in all other treatments.

In the laboratory experiment, spiked and un-spiked freshwater treatments were exposed to 5 min pulses of 28 ppt, whilst control treatments maintained a steady salinity of approximately 34 ppt (Fig. 1b). Concentrations of copper and zinc in aquariums at the end of the pulse exposure in the metal spiked treatment were $32 \pm 1.50\ \mu g\ L^{-1}$ and $140 \pm 10\ \mu g\ L^{-1}$, respectively. Copper and zinc concentrations were $<5\ \mu g\ L^{-1}$ in all other treatments. Other physio-chemical parameters including water temperature (22.8–23.7 °C), pH (8.1–8.3), dissolved oxygen (81–87% saturation) and turbidity (4–7 NTU) were not affected by dosing procedures in either the field or laboratory experiments.

3.2. Epifaunal responses to field-based exposures

Pulses of mimic storm water reduced the abundance of amphipods inhabiting *S. linearifolium* to approximately 50% of that found on un-manipulated algae and seawater controls (Fig. 2a, Table 1). Reductions occurred in both metal spiked and un-spiked freshwater treatments (Fig. 2a). Similarly, gastropods were less abundant on seaweeds receiving un-spiked freshwater following the 5 min exposures (Fig. 2b, Table 1). While gastropod abundance was substantially lower in spiked freshwater treatments, this reduction was not statistically significant (Fig. 2b, Table 1). Copepod abundance was significantly greater in the seawater treatment when compared to un-manipulated controls (Fig. 2c, Table 1). Polychaetes and ostracods were not affected by exposure to any of the treatments (Fig. 2d and e, Table 1).

3.3. Epifaunal responses to laboratory based exposures

Amphipods, gastropods and copepods were found in greater numbers in the water column in the laboratory flow through experiment when epifaunal communities were exposed to mimic storm water than when exposed to seawater (Fig. 3a–c, Table 2). Responses were of two types: amphipods and gastropods exhibited the same rates of dispersion when exposed to pulses of metal spiked and un-spiked freshwater, whilst copepods only responded significantly to plumes of freshwater when trace metals were present (Fig. 3a–c). Polychaetes and ostracods showed no significant response to any of the dosing effluents used (Fig. 3d, e, Table 2). No individuals of any taxa died during the pulse exposure. Latent mortality did not differ between experimental treatments for any epifaunal taxa (Table 2). Amphipods and copepods exhibited latent mortality after 24 h of approximately 10% in all treatments (Tables 2 and 3), latent mortality in all other taxonomic groups was negligible (<5%) and did not differ between treatments (Tables 2 and 3).

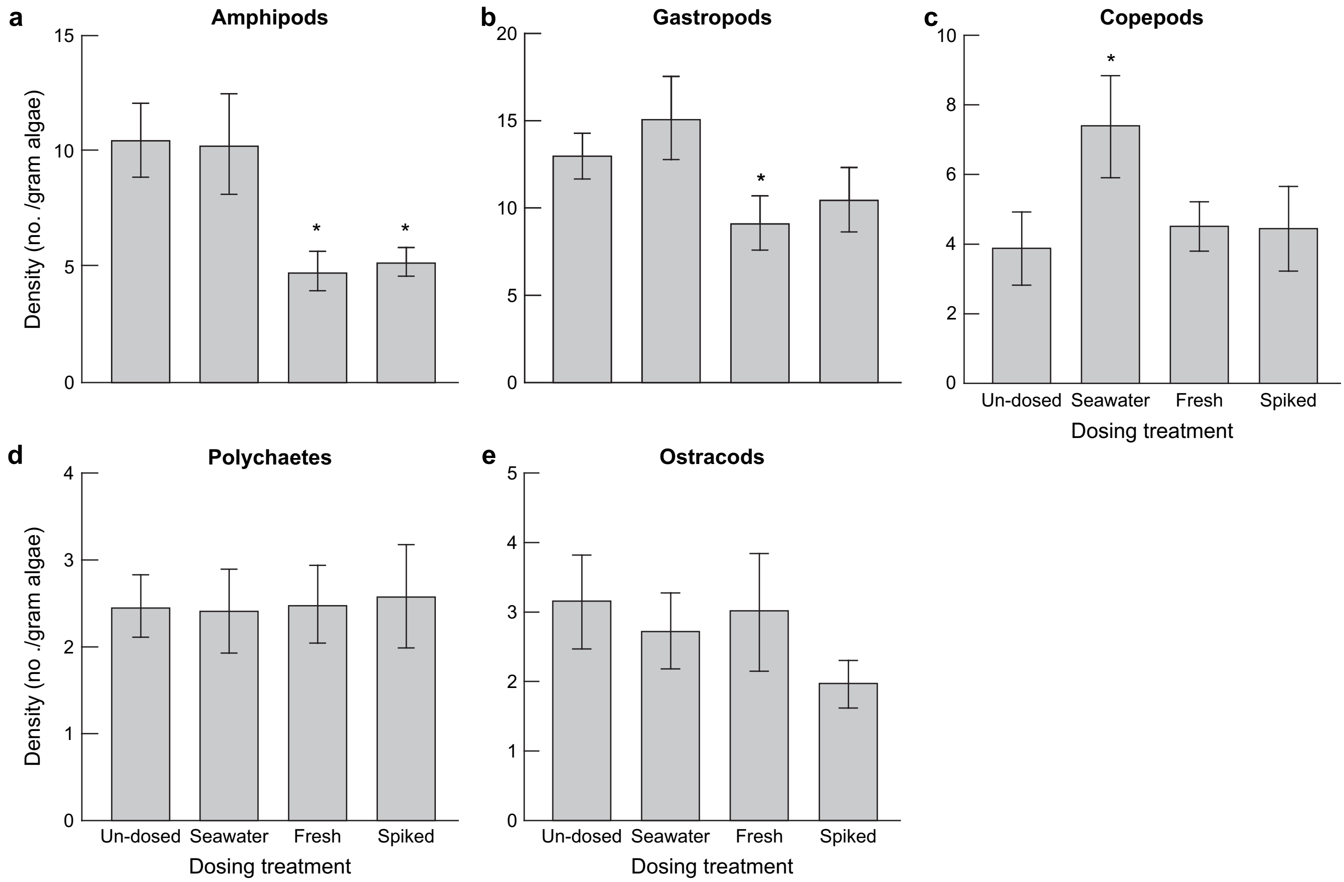

Fig. 2. Abundance of (a) amphipods, (b) gastropods, (c) copepods, (d) polychaetes and (e) ostracods upon *S. linearifolium* following pulsed exposures in the field experiment. Treatments were an un-dosed control ('un-dosed'), seawater control ('seawater'), clean freshwater only ('fresh') and metal spiked freshwater ('spiked'). 'Fresh' and 'spiked' bars marked with an asterisk differ significantly from seawater control treatments in the absence of experimental artifacts. An asterisk over 'seawater' bars indicates a significant artifact of dosing treatment when compared to un-dosed controls. Data are densities (no. organisms per gram algae). Error bars show means ± S.E.

3.4. Colonisation of salinity treated algae

The salinity stress treatments did not influence the rates of colonisation of any taxa to *S. linearifolium* (Fig. 4a–d, Table 4). Epifaunal communities showed highly variable colonisation rates across the three trial dates (Table 4). Amphipod and copepod abundances were particularly variable (Fig. 4a and b). No significant interactions between 'salinity' and 'date' were detected (Table 4).

4. Discussion

Storm water is known to be a major vector of metal transport to marine environments, however, the ecological effects

Table 1
Comparisons for invertebrates inhabiting *Sargassum linearifolium* following experimental pulse exposures in the field

		Comparisons					
		Un-dosed vs. seawater		Seawater vs. fresh		Seawater vs. spiked	
Taxa	MS_{Error}	MS	*P*	MS	*P*	MS	*P*
Amphipods[a]	0.187	0.021	0.740	2.190	**0.002**	1.448	**0.010**
Copepods[a]	0.389	2.155	**0.026**	–	–	–	–
Gastropods	23.737	18.787	0.381	149.813	**0.018**	91.561	0.060
Ostracods	2.689	0.065	0.878	0.001	0.985	0.320	0.733
Polychaetes[a]	0.228	0.020	0.769	0.017	0.787	0.013	0.813

Treatments were 'un-dosed' (un-manipulated algae), 'seawater' (dosed with undiluted seawater), 'fresh' (dosed with clean freshwater only) or 'spiked' (dosed with metal spiked freshwater).
Error terms shown are taken from the full ANOVA, df for each comparison are 1,28.
Data are numbers per gram wet mass algae. Bold values indicate statistically significant results ($P < 0.05$).
[a] Log transformed.

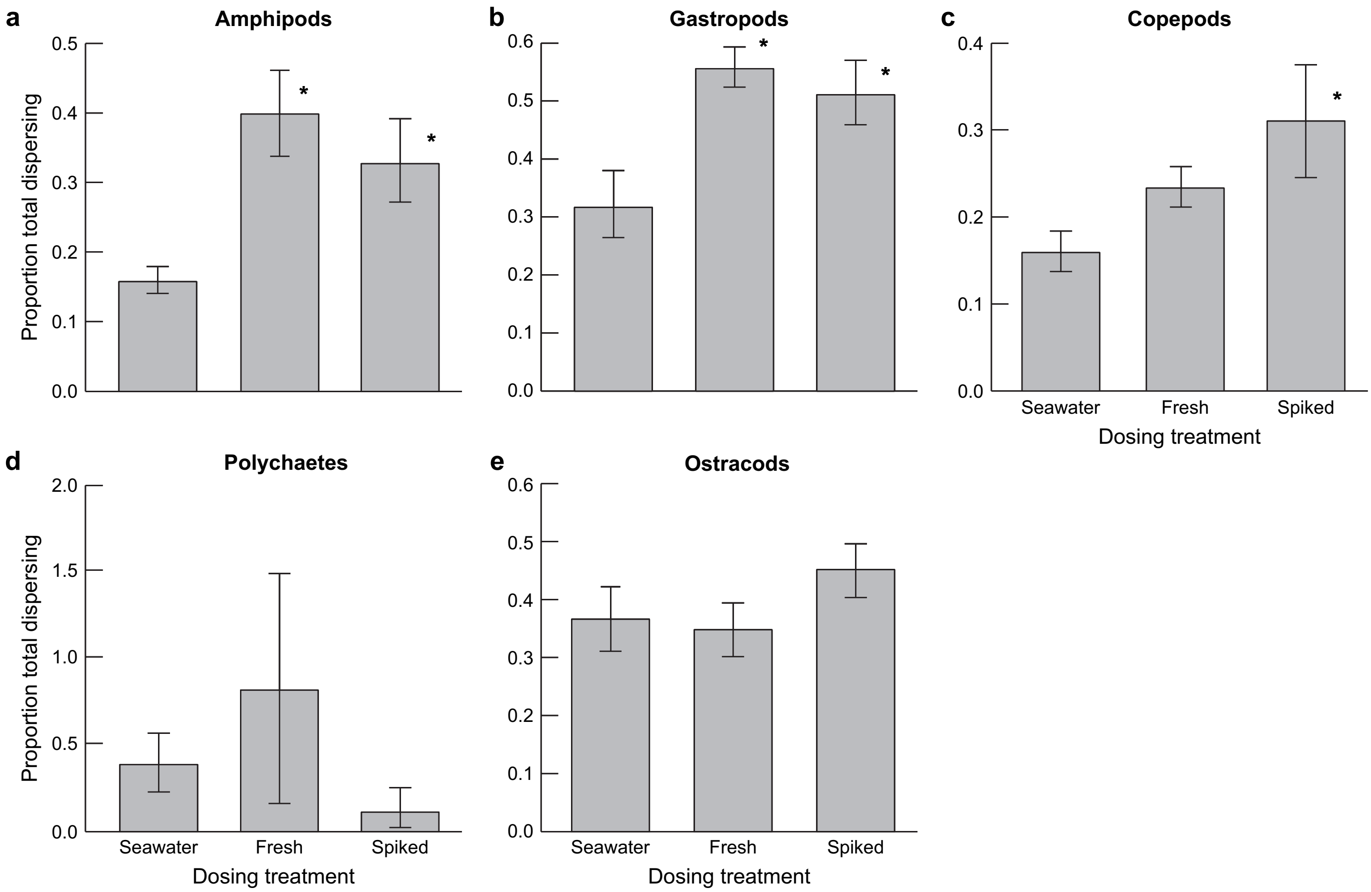

Fig. 3. Proportion of (a) amphipods, (b) gastropods, (c) copepods, (d) polychaetes and (e) ostracods dispersing from *S. linearifolium* following pulsed exposures to storm water in the laboratory. Treatments are a seawater control ('seawater'), clean freshwater only ('fresh') and metal spiked freshwater ('spiked'). Bars marked with an asterisk differ significantly from seawater controls. Data are numbers of individuals found alive in the water column standardized by the abundance of the taxonomic group in the entire assemblage. Error bars show means ± S.E.

of storm water pulses upon marine organisms remain poorly understood. The present study demonstrates that even brief pulses of storm water can have substantial impacts upon assemblages of mobile epifaunal invertebrates. Pulses of mimic storm water of 5 min duration (reducing salinity from ambient 34 ppt to 28 ppt) were sufficient to reduce the abundance of amphipods and gastropods inhabiting *S. linearifolium* by approximately 50%. Salinities of 28 ppt have been measured around storm water drains following periods of heavy rainfall for up to 24 h (Roberts et al., in press).

The reduced abundance of amphipods and gastropods in the field experiment following exposure to mimic storm water could be explained by behavioural avoidance of storm water or direct mortality of fauna when exposed to plumes. The laboratory exposures specifically assessed the escape abilities of epifauna. No direct mortality of epifauna was recorded

Table 2
Comparisons for proportions of invertebrates displaying avoidance behaviours and latent mortality following experimental pulse exposures in the laboratory

	Avoidance					Latent mortality				
	Comparisons					Comparisons				
	Seawater vs. fresh			Seawater vs. spiked		Seawater vs. fresh			Seawater vs. spiked	
Taxa	MS_{Error}	MS	*P*	MS	*P*	MS_{Error}	MS	*P*	MS	*P*
Amphipods[a]	0.166	20.217	**0.002**	11.729	**0.003**	0.007	0.002	0.599	0.002	0.599
Copepods[a]	0.187	3.893	0.062	7.947	**0.010**	0.011	0.001	0.766	0.001	0.766
Gastropods[b]	0.014	15.714	**0.007**	10.429	**0.004**	0.009	0.002	0.642	0.012	0.261
Ostracods[a]	0.017	0.059	0.811	1.706	0.206	–	–	–	–	–
Polychaetes[a]	0.174	0.172	0.682	0.948	0.314	–	–	–	–	–

Treatments were 'seawater' (dosed with undiluted seawater), 'fresh' (dosed with clean freshwater only) or 'spiked' (dosed with metal spiked freshwater). Data for avoidance are proportions of total abundance found in water column, data for latent mortality are proportions of dispersers dead after 24 h. Bold values indicate statistically significant results ($P < 0.05$).

[a] Avoidance data log transformed.

[b] Latent mortality data log transformed.

Table 3
Percentage of dispersing organisms exhibiting latent mortality following a 5 min exposure to mimic storm water pulses in the laboratory (24 h latent period)

	Seawater	Fresh	Spiked
Amphipods	8.6 ± 2.7	10.6 ± 3.1	10.5 ± 3.2
Copepods	11.3 ± 4.4	12.7 ± 3.5	12.7 ± 2.8
Gastropods	0.7 ± 0.7	0.4 ± 0.2	n/a
Polychaetes	n/a	n/a	n/a
Ostracods	1.0 ± 1.0	4.2 ± 2.2	n/a

Treatments were 'seawater' (dosed with undiluted seawater), 'fresh' (dosed with clean freshwater only) or 'spiked' (dosed with metal spiked freshwater). Data are proportions of dispersers dead after 24 h (means ± S.E., $n = 8$ per treatment).
n/a Indicates that no mortality was recorded.

following laboratory exposures, indicating that the exposure scenario created in the field experiment was unlikely to have led to direct mortality. Instead, amphipods, gastropods and copepods all rapidly vacated algae in the presence of freshwater plumes, indicative of behavioural avoidance. Avoidance of water-borne contaminants is an important mechanism by which many organisms regulate their exposure to chemical contaminants. For example, avoidance of metal contaminated water has been noted in the rainbow trout *Oncorhynchus mykiss* (Hansen et al., 1999), the freshwater amphipod *Gammarus lacustris* (de March, 1983) and the water flea *Daphnia longispina* (Lopes et al., 2004) in laboratory exposures. Enhanced drift of macro-invertebrates has also been noted in streams within minutes of experimental pulses of the insecticide Permethrin® (Sibley et al., 1991). Whilst less is known about the responses of marine invertebrates to freshwater plumes, avoidance of low salinities has been noted in some organisms including the American lobster *Homarus americanus* (Jury et al., 1994).

Contrary to expectations, most faunal groups did not demonstrate increased avoidance of plumes which contained trace metals compared to freshwater alone. In fact copepods were the only group to show enhanced emigration from algal habitats in the presence of metals, and they only did this in the laboratory. Many species of marine copepods are highly sensitive to elevated concentrations of water-borne metals and can be amongst the most sensitive benthic invertebrates in marine

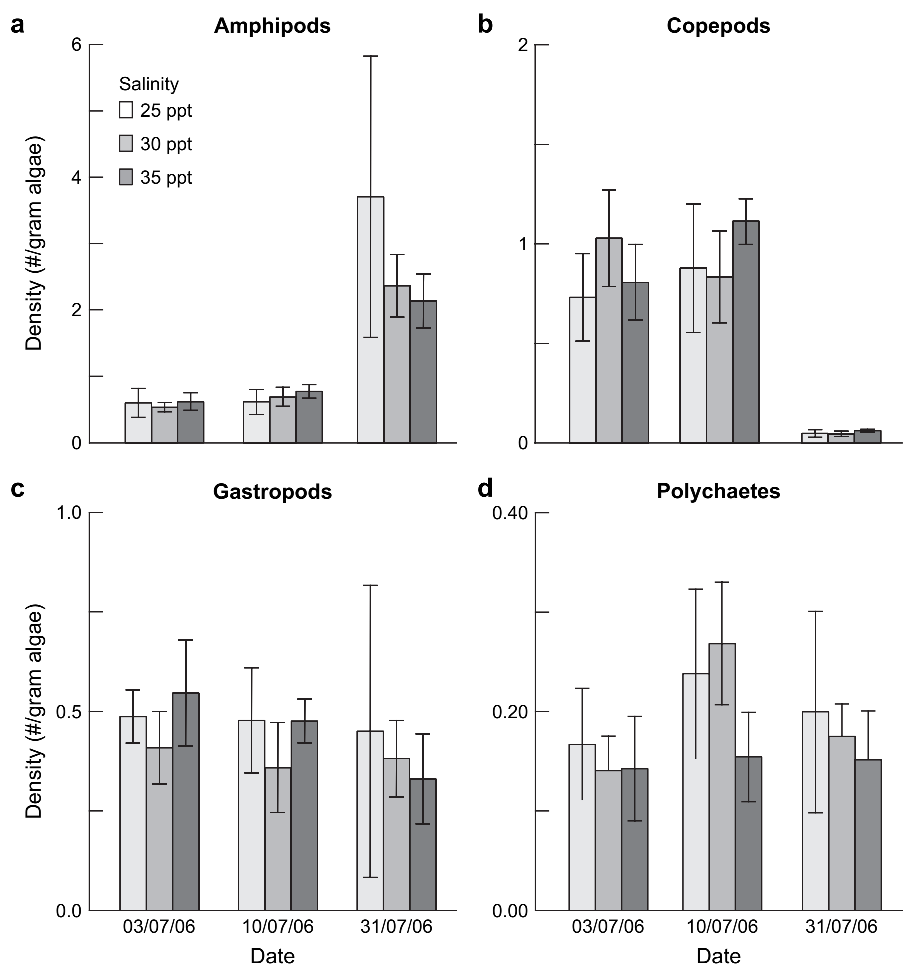

Fig. 4. Densities of (a) amphipods, (b) copepods, (c) gastropods and (d) polychaetes colonising *S. linearifolium* in the field following salinity stress of algal habitats. Treatments were trial date ('date') and salinity stress ('salinity'). There were three levels of the salinity stress treatment (25 ppt, 30 ppt and seawater control). Data are densities (no. colonists per gram algae). Error bars show means ± S.E.

Table 4
Analyses of variance (ANOVA) for invertebrates recolonising salinity stressed *S. linearifolium* in the field

Effect	Amphipods[a]			Copepods		Gastropods[a]		Polychaetes[a]	
	df	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Date	2	11.121	**0.001**	4.825	**0.001**	1.367	**0.007**	0.241	0.300
Salinity	2	0.065	0.625	0.055	0.644	0.264	0.591	0.159	0.129
Date × salinity	4	0.122	0.800	0.112	0.612	0.439	0.147	0.045	0.921
Error	45	0.298		0.166		0.244		0.195	

Treatments were 'date' (experimental date) and 'salinity' (salinity stressed and unstressed algae).
Data are numbers of colonists per gram algae. Bold values indicate statistically significant results ($P < 0.05$).
[a] Data log transformed.

systems (Barka et al., 2001; Forget et al., 1998; Hutchinson et al., 1994). This may explain their enhanced avoidance of freshwater plumes containing metals in the present study.

In the field experiment copepods were found in greater numbers in seawater controls than in un-manipulated controls, suggesting an artifact of the field dosing procedure for that group. It is unlikely that copepods responded positively to increased water flows by migrating towards areas dosed with seawater as copepods prefer sheltered, low flow environments (Gibbons, 1988). Due to their small size, copepods may have been transferred along with seawater which was pumped in the seawater control treatments resulting in greater abundances on algae receiving this treatment (seawater was not filtered prior to use in field-based exposures).

Rapid reductions in abundance and relatively rapid recovery have been previously demonstrated in epifaunal communities in the Sydney region following storm water exposure across larger spatial scales. The abundances of polychaetes and gastropods inhabiting *S. linearifolium* adjacent to storm water drains are substantially reduced within 24 h of large rain events, with recovery of assemblages within 4 days while algal beds located several hundred metres away from storm drains are unaffected (Roberts et al., in press). The localized impacts of runoff and the rapidity of faunal response and recovery suggest behavioural avoidance, rather than direct mortality as explanatory factors. The present study further supports this conclusion. In a field monitoring study amphipods appeared to respond to factors such as storm-related turbulence, with no obvious effects of storm water *per se* (Roberts et al., in press). In the present study pulses were created in the absence of environmental changes which co-occur with storm events. Under these conditions amphipods appear to be amongst the most sensitive inhabitants of algal epifaunal assemblages, demonstrating the strongest response to freshwater pulses.

The ability of organisms to actively avoid contaminants in marine systems has implications for the way in which the ecological effects of pulsed contamination events are assessed. Field studies are generally used within a toxicological framework as a means by which laboratory toxicity data can be confirmed in the field. As a consequence, there has been a tendency to focus upon endpoints such as survivorship which are most often measured in the laboratory. Due to the inherent difficulties associated with monitoring these endpoints in mobile organisms within their natural environments, *in situ* experiments have generally involved caging test organisms to facilitate measurements (Burton et al., 2005). It is suggested that this approach provides greater ecological relevance than laboratory based exposures as exposures occur under realistic environmental conditions (Burton et al., 2005; Maltby, 1999). However, this approach considers organisms to be passively exposed to water-borne contaminants and fails to allow for behavioural responses which, in part, determine the degree to which an organism is exposed to contaminants.

Many organisms are not passive in their exposure to contaminants and the ability to detect and avoid toxic chemicals is common (Kravitz et al., 1999; Lefcort et al., 2004; Lopes et al., 2004). As in the present study, the initial response of many mobile organisms in the early stages of a contamination event will be to actively avoid contaminants. Thus, the likelihood of acute toxicity may be determined by the spatial and temporal distribution of the contaminant in the environment (and subsequently the potential for refuge), and the ability of the test organism to avoid the contaminant across those spatial, and temporal scales (Chapman, 1995b; Lefcort et al., 2004). Caging experiments may over-estimate exposure durations and current toxicological protocols may place undue emphasis on acute endpoints (i.e. mortality) at the expense of more ecologically relevant, behaviourally mediated endpoints (Doving, 1991). An alternative to the prevailing approach is to use field-based research as a means to determine appropriate endpoints such as in the present study, where the results of the field experiment governed the design of the subsequent laboratory assay. Studies which allow for behavioural avoidance to occur may prove particularly useful (Maund et al., 2001).

Whilst previous research has identified substantial latent mortality following brief toxicant exposures (Reynaldi and Liess, 2005; Schill et al., 2003), in the present study latent mortality was negligible and unaffected by experimental treatments. Exposure duration in mobile invertebrates will be tightly coupled to the ability of an organism to escape storm water plumes. Thus, both acute and latent mortality may be more important factors following exposures to storm water pulses of greater temporal and spatial extent than were tested in the present study. There is clearly a great need to characterize the spatial and temporal extent of environmental contamination and relate this to the scale of dispersal associated with mobile organisms.

The direct ecological effects of contamination events upon exposed organisms have been the historical focus of pollution research, however, there is an increasing awareness that contaminant effects in marine systems may be mediated through biological interactions (Johnston and Keough, 2002). In the present study we examined the potential indirect effects of storm water pulses in algal beds which may arise through stress of algal habitats following the inundation of freshwater runoff. Environmental stressors such as desiccation and UV irradiation may increase the susceptibility of marine seaweeds to herbivory (Cronin and Hay, 1996; Heaven and Scrosati, 2004). In the present study, however, the colonisation of epifauna to *S. linearifolium* was not affected by salinity treatments to that alga. Whilst the period of dosing that could be created in the field dosing experiment was constrained by logistics, algal stress treatments could be maintained in the laboratory for longer periods, designed to mimic the duration of a storm water pulse which might occur in the field. The use of longer exposures and lower salinities in the salinity stress experiment strengthens our confidence that indirect effects did not contribute to the impacts of freshwater observed in our field and laboratory experiments.

Epifaunal communities are highly dynamic and generally display rapid colonisation of empty habitats, high daily turnover, and rapid recovery from disturbances (Martin-Smith, 1994; Taylor, 1998b). The naturally dynamic nature of epifaunal assemblages implies that these communities may bear some resilience against pulse contamination and other transient disturbances. However, it is likely that multiple components of storm water pose ecological threats to marine life, and that these impacts may be realized across differing temporal scales. These will range from the immediate effects of individual pulses of storm water (primarily attributable to the inundation of freshwater), through to longer-term effects of repeated exposures which are likely to be mediated indirectly through the accumulation of chemical contaminants in benthic habitats (Roberts et al., 2006).

Efficient management of storm water runoff will require strategies that reduce the potential for short-term impacts of freshwater inundation whilst decreasing the transport of chemical contaminants to marine systems in the longer term. On-site detention of rain water, re-cycling of runoff and vegetation swales designed to enhance infiltration of runoff are management strategies which aim to reduce runoff volumes (Marsalek and Chocat, 2002; Niemczynowicz, 1999). These strategies both reduce the likelihood of short-term impacts of freshwater pulses in marine environments and ultimately reduce the longer-term transport and accumulation of chemical contaminants in benthic habitats (Marsalek and Chocat, 2002).

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