

Determining positions for control locations in environmental studies of estuarine marinas

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ABSTRACT: Recruitment and development of subtidal epibiota were sampled using settlement plates to identify appropriate control locations for tests of environmental impacts of estuarine marinas. Sampling was designed to determine how far any effects of marinas may extend and how the position of a location within an estuary may affect the recruitment and development of epibiota. Marinas may have had minor impacts on epibiotic assemblages, but certainly none that extended further than 1.5 km; hence, locations 1.5 km from marinas could potentially serve as controls. Assemblages at the inner end of a creek were, however, likely to be very different from those at positions closer to the mouth. Thus, for studies of marinas situated at the ends of creeks, control locations would need to be in corresponding positions in adjacent creeks. Without information of this kind, impacts may be either detected erroneously or masked because inappropriate control locations might be used.

KEY WORDS: Subtidal organisms · Fouling · Recruitment · Environmental impact assessment
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INTRODUCTION

One of the most important aspects of any environmental study is the choice of control locations. This will be particularly important for any 'post-impact' study (i.e. when no data have been collected before the disturbance occurred) because an impact will often not be detected if there is great variability among the controls (Peterson 1993, Glasby 1997). This is not to suggest that the only criterion for choosing control locations is that they be similar to each other. Controls must be chosen randomly from a set of locations which are representative of the disturbed location. Thus, we must outline *a priori* the characteristics of the disturbed location which are important to consider so that a distribution of similar locations (from which controls will be randomly chosen) can be defined. Even though it is not necessary (nor actually possible) for any control locations (even one) to be identical to the disturbed location (Underwood 1994), certain features of both must

be similar. It is difficult to know which characteristics of a location are the most important to duplicate in controls, but the suggested selection criteria fall into 3 broad categories. Control locations must: (1) be unaffected by the disturbance being investigated, (2) have the same types of habitats as the disturbed location and (3) have physical and morphological characteristics similar to those of the disturbed location (Green 1979, Underwood 1992, 1994, Stewart-Oaten 1996). In some situations, this will mean that the closer the controls are to the disturbed location, the better (provided that they are spatially independent of the disturbance in question). This may not be the case, however, if there is some sort of natural environmental gradient away from the disturbed location. If this occurs, controls may need to be in comparable positions within this type of gradient, even if this means that they must be many kilometres away.

When the spatial extent of a disturbance is not known, sampling can be done using a 'gradient' design (see Bayne et al. 1988, Wiens & Parker 1995) or at a variety of spatial scales and the data analysed using a nested design (Underwood 1981, 1992, An-

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drew & Mapstone 1987, Morrissey & Underwood 1992). A combination of these 2 approaches was used here to estimate the spatial extent of effects of marinas on the recruitment of subtidal epibiota growing on hard substrata. Knowledge of spatial scale would facilitate the choice of control locations for future comparisons. This formed part of a larger study to examine the model that marinas in Sydney, Australia, are having a press (sustained) impact (Bender et al. 1984) on these assemblages of epibiota. The test for a press disturbance having an ongoing effect on organisms involves sampling the development of new assemblages (as described here) which can only be affected by current (or future) disturbances (Glasby & Underwood 1996).

Almost no work has been done to determine how far the environmental effects of marinas may extend. The little information available concerns the concentration of contaminants (mainly tributyltin, TBT) in the water and sediments around marinas. These studies were not specifically designed to examine how far contaminants may extend from their source, but the results suggest that large concentrations of TBT (sufficient to cause deformities in marine invertebrates) tend to be found within 1 km of a marina (Batley et al. 1989, Hasan & Juma 1992, Ko et al. 1995, McGee et al. 1995). For most studies, either information about control locations was not provided, or no control was actually used. Baird et al. (1981) had 1 control approximately 1 km from the marina that they studied, while Van Dolah et al. (1992) used control sites situated in an adjacent creek, approximately 5 km from the marina. Given that there is essentially no information about how far ecological effects of marinas may extend (nor about spatial scales of natural variation), it is impossible to predict whether impacts may be small- or large-scale. We investigated the model that marinas have large-scale impacts on sessile epibiota and these impacts extend up to 1.5 km. Thus, it was predicted that if the establishment of subtidal assemblages were sampled in a marina and at a location 1.5 km away, there would be no differences between the 2 locations. Furthermore, the establishment of assemblages at any locations further than 1.5 km from a marina should be different from that at the marina. These patterns should be different from any observed among control locations spaced at similar distances.

The marinas examined were located in estuarine creeks. It was also important, therefore, to consider natural differences along the creeks with regard to the positions of control locations. For this, it was necessary to establish whether there was any sort of environmental gradient within the creeks. There is substantial evidence to suggest that a variety of oceanographic, physical and chemical features of an estuary may vary along its length (e.g. Parsons et al. 1990). Salinity, tem-

perature, turbidity, dissolved oxygen content, water flow and flushing may all vary considerably within an estuary (Barnes 1984, Cosser 1989, Barnes & Hughes 1990, Parsons et al. 1990, Le Bris & Glémarec 1996). A number of these features may alter according to the volume of freshwater entering the estuary (Barnes 1984). Given that the marinas in question were situated at the ends of creeks (see Fig. 1) where freshwater streams discharge, we proposed that a variety of abiotic and biotic features differed between these locations and others closer to the mouths of the creeks and that this differentially affected the establishment of epibiota. We hypothesised that the establishment of subtidal assemblages would differ along a creek and that the greatest difference would be between the mouth and the end of the creek.

The aims of this study, therefore, were to test our hypotheses concerning the spatial extent of the effects of marinas and the prediction that the establishment of subtidal assemblages at the ends of creeks is very different from that closer to the mouths of creeks. It is quite likely that recruitment and/or the effects of marinas may vary over relatively short time periods and that any effects on epibiota may be expressed at different stages of the development of assemblages. It was therefore decided that this study would be done twice during one summer and that different developmental stages would be examined.

MATERIALS AND METHODS

Sandstone settlement plates (15 × 15 cm) were used to study the establishment of subtidal assemblages. All the plates had been used previously (i.e. been under water for at least 1 mo) and had been immersed in 20% HCl, scrubbed clean, soaked in fresh water and dried. Plates were attached to aluminium angle beams (90° angle bracket, 32 mm side width). The beams were attached horizontally to the rocky reef, parallel to the shore, using 2 self-tapping stainless steel screws and rawl plugs that were drilled into the rock. Plates were attached vertically to the beams via a PVC bracket which was placed over and bolted onto 2 stainless steel threaded rods drilled through the side of the aluminium angle that was not attached to the substratum (Glasby 1998). The plates stood vertically in the water column at a depth of 1.5 m below mean low water springs and faced away from the shore.

Plates were deployed at 9 locations, 3 in each of 3 creeks in Ku-ring-gai Chase National Park, Sydney (Fig. 1). This area is popular for recreational boating, but there are few other anthropogenic disturbances because locations are in a National Park. The creeks are formed by deeply incised sandstone valleys and

reach a maximum depth of ~25 m. Two creeks had marinas situated near the end. Halvorsen Marina is in Cowan Creek and Akuna Bay Marina is in Coal & Candle Creek (Fig. 1). These marinas have been operating for more than 20 yr (Halvorsen's for 50 yr) and each supports upwards of 200 boats. At Halvorsen's, most boats are on swing moorings, whereas all boats at Akuna Bay Marina are in berths. Both marinas sell fuel and have slipways for repairing boats and applying antifouling paints. Smiths Creek was chosen as a control because it does not contain a marina, it is in between the other creeks and is ostensibly very similar to them, i.e. all 3 creeks are surrounded by National Park, face a similar direction, are steep-sided and have a freshwater stream running into the end of them. Furthermore, Smiths Creek has the same types of subtidal habitats (rocky reef, sandy soft sediments, seagrasses) as do the other 2 creeks.

In each creek, there were 3 locations, one at 'position 1' at the end of the creek, one at 'position 2' a further 1.5 km away, and one at 'position 3', which was 3 km from the end of the creek (Fig. 1). At each location, 2 sites were sampled, one on either side of the creek (i.e. approximately 100 to 150 m apart). There were 4 aluminium beams per site, each approximately 10 m apart, and 1 replicate plate was sampled from each beam ($n = 4$ per site). This sampling regime was determined from studies of natural variability in recruitment of subtidal epibiota (Glasby 1998). In December 1994 (summer 1), 2 plates were attached, one plate distance apart, to each of the 4 beams at every site. One of these

plates was collected from each beam after 5 wk, the other after 12 wk. A second set of plates was deployed in January 1995 (summer 2) in the same manner. Again, 1 set of replicate plates was collected after 5 wk and the other after 12 wk.

When the second set of plates was deployed, 25 × 25 cm areas on vertical faces of rocky reef were cleared next to the plates at 2 locations. The corners of a 15 × 15 cm plot within the cleared area were marked with holes and rawl plugs. A visual comparison of the cleared plots and the adjacent plates was made after 12 wk. The growth on the plates was very similar to that in the plots and both plates and plots appeared similar to the surrounding rock, except for the lack of macro-algae. Macro-algae were, however, not very abundant on natural rocks and generally only occurred in small clumps (Glasby unpubl.).

Plates were suspended and supported in tubs of seawater for transport back to the lab where they were transferred into filtered (10 µm filter) seawater and refrigerated at 5°C until sorted (within 5 d of collection). Percentage cover of sessile organisms on the fronts of plates was estimated under a stereo-microscope using a grid of 64 regularly spaced points which sampled to within 1 cm of the edges of the plates (i.e. a 13 × 13 cm area) and, thus, avoided 'edge effects'.

Replicate measures of physical characteristics of the water at a depth of 1.5 m were taken on 5 occasions during summer (November to January) at the end of and mid-way along each creek. Mean percentage oxygen saturation (\pm SE) at the ends of Cowan, Smiths and

Coal & Candle creeks were 85.3 ± 1.9 , 96.0 ± 1.2 and 100.6 ± 1.1 , respectively. Mid-way along the 3 creeks, percentage oxygen saturation was generally greater, i.e. 101.4 ± 2.7 , 101.1 ± 1.4 and 102.5 ± 1.3 , respectively. Salinity was not significantly different among the positions and was ~31.1. Average water temperature was 22.6°C and did not differ significantly among positions.

Data were analysed using univariate (ANOVA) and non-parametric multivariate (PRIMER software package; Plymouth Marine Laboratory, UK) techniques. For multivariate analyses, data were double square-root transformed and Bray-Curtis similarity matrices (Bray & Curtis 1957) were calculated (Clarke & Green 1988, Clarke 1993). One-way analyses of similarities (ANOSIM; Clarke & Green 1988) and pairwise comparisons tested for differences in the composi-

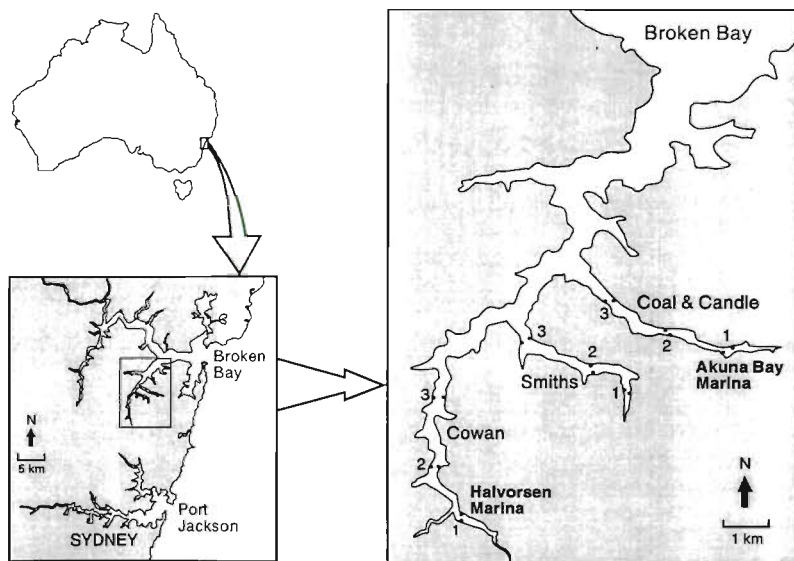


Fig. 1. Study area and the 9 locations in Ku-ring-gai Chase National Park, Australia. There were 3 locations in each creek: 'position 1' at the end of the creek, 'position 2' in the middle (1.5 km away) and 'position 3' at the mouth (3 km from the end). There were 2 sites at each location, one on either side of the creek (black dots)

tion of assemblages. If the Bonferroni procedure is used to control the probability of Type I error for multiple pairwise comparisons between groups of 4 replicates (i.e. comparing sites), α becomes unrealistically small (0.029). So, as a compromise, R values (the test

statistic used in ANOSIM; Clarke 1993) were compared to help determine which samples were likely to have been significantly different. The greater the value of R (closer to 1), the more dissimilar the samples being compared. Only those taxa that were relatively

Table 1. ANOVAs for dominant taxa on plates collected after 5 wk. Two sets of plates (summer 1 and summer 2) were sampled 1 mo apart. There was no test for Position except when (1) Site(P) was not significant at $p > 0.25$, in which case Position was tested over $C \times P$, or a combination of $C \times P$ and pooled terms or (2) $C \times P$ or both interaction terms were not significant at $p > 0.25$ in which case Position was tested over Site(P). *Post-hoc* pooling was done at $p > 0.25$ (see footnote). Data were not transformed except for those taxa marked as being $\ln(x + 1)$ transformed. Variances were homogeneous at $p > 0.05$ for all tests except for bare space, summer 2, for which variances were homogeneous at $p > 0.01$. Creek and Position were random factors. NS: $p > 0.05$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Source	df	MS	Summer 1 F	p	MS	Summer 2 F	p
(a) Cladophorales					$\ln(x + 1)$		
Creek	2	9707.17	10.80	*	16.05	6.29	NS
Position	2	818.01	0.91	NS	0.54	0.02	NS
Site(P)	3	92.37 ^a	0.34	>0.25	0.34	0.44	>0.25
$C \times P$	4	898.47	4.27 ^a	*	2.55	3.07 ^a	*
$C \times S(P)$	6	269.37 ^a	3.44	**	0.76 ^a	0.91	>0.25
Residual	54	78.40			0.84 ^a		
(b) Feldmania		$\ln(x + 1)$			$\ln(x + 1)$		
Creek	2	11.10	12.43 ^a	***	0.92	0.87 ^a	NS
Position	2	2.01	2.25 ^a	NS	6.41	6.10	NS
Site(P)	3	0.17 ^a	0.20	>0.25	3.56	4.02	NS
$C \times P$	4	1.48 ^a	1.72	>0.25	1.30 ^a	1.47	>0.25
$C \times S(P)$	6	0.86 ^a	1.67	NS	0.88 ^a	1.36	NS
Residual	54	0.52			0.65		
(c) Oysters					$\ln(x + 1)$		
Creek	2	29.70	1.55	NS	36.83	11.45	*
Position	2	1.93	–		0.71	0.22	NS
Site(P)	3	10.34	1.79	NS	0.55	0.97	>0.25
$C \times P$	4	19.12	3.32	NS	3.22	5.50 ^a	***
$C \times S(P)$	6	5.76	2.37	*	0.57 ^a	0.97	>0.25
Residual	54	2.43			0.59 ^a		
(d) Spirorbids							
Creek	2	731.30	1.47	NS	44.66	2.18	NS
Position	2	47.71	–		29.70	–	
Site(P)	3	30.18	1.40 ^a	NS	13.94	5.71	*
$C \times P$	4	496.88	23.10 ^a	***	20.50	5.91 ^a	***
$C \times S(P)$	6	12.48 ^a	0.55	>0.25	2.44 ^a	0.68	>0.25
Residual	54	22.52 ^a			3.58 ^a		
(e) Serpulids					$\ln(x + 1)$		
Creek	2	23.02	12.74 ^a	***	12.21	9.52	*
Position	2	1.05	0.53	NS	1.14	–	
Site(P)	3	3.97	2.19 ^a	NS	1.02	3.78	NS
$C \times P$	4	1.61 ^a	0.79	>0.25	1.28	2.50 ^a	NS
$C \times S(P)$	6	2.03 ^a	1.13	>0.25	0.27 ^a	0.50	>0.25
Residual	54	1.80 ^a			0.54 ^a		
(f) Bare space							
Creek	2	16842.75	60.12 ^a	***	13125.10	51.00 ^a	***
Position	2	645.85	2.31 ^a	NS	464.27	1.80 ^a	NS
Site(P)	3	159.40 ^a	0.30	>0.25	4.71 ^a	0.02	>0.25
$C \times P$	4	576.38 ^a	1.10	>0.25	378.16 ^a	1.25	>0.25
$C \times S(P)$	6	523.38 ^a	5.67	***	303.07 ^a	1.42	NS
Residual	54	92.36			213.36		

^aMS terms were pooled and the resultant term used as the denominator for the F -ratio

abundant (i.e. covering, on average, at least 5% of the plate) were used in the univariate analyses, whereas all taxa (and 'bare space') were included in the multivariate analyses.

RESULTS

Samples after 5 weeks

There were very distinct patterns among locations for each taxon, and for some taxa these patterns differed between the 2 sets of samples (Table 1, Fig. 2). Differences in the percentage cover of taxa occurred among creeks and among positions within a creek (Table 1). Only for bare space were there any significant differences between the 2 sites at a location (summer 1, Fig. 2); the significant Creek \times Site(Position) interaction for other taxa occurred because of differences among sites from different locations (Table 1, Fig. 2).

The percentage cover of a number of taxa differed among creeks. The overall covers of green filamentous algae (here grouped as Cladophorales) and brown filamentous algae (represented primarily by the genus *Feldmania*) were greater in Cowan Creek than in any other creek for the first samples (Table 1a, b, Fig. 2a, b). Furthermore, the cover of green filamentous algae at the marina (position 1) and position 2 in the middle of Cowan Creek was greater than at corresponding positions in the other creeks for the first set of samples, whereas for the second set of samples the cover at position 3 in Cowan Creek was greater than in the other creeks (Table 1a, Fig. 2a). The cover of oysters *Saccostrea commercialis* in Cowan Creek was greater than in the other 2 creeks for the second set of samples (Table 1c, Fig. 2c), and the cover at the marina in Cowan Creek was greater than at position 1 in Smiths Creek, which was greater than at the marina at Coal & Candle Creek (Table 1c, Fig. 2c). Note the large difference in cover of oysters between the 2 sets of samples (Fig. 2c). Bare space was least common on plates in Cowan Creek for both sets of samples (Fig. 2f, Table 1f).

Similar patterns in the cover of spirorbid polychaetes (species of *Neodexiospira*, *Janua* and *Pileolaria*) occurred for the 2 sets of samples, but far more spirorbids occurred in the summer 1 samples (Fig. 2d). Comparing only the locations at the ends of creeks (position 1), there was a significantly greater percentage cover of spirorbids at Akuna Bay Marina (in Coal & Candle Creek) than at the other locations (Table 1d, Fig. 2d). The cover of spirorbids at the end (position 3) of Smiths Creek was significantly less than that at position 3 in the other creeks (Table 1d, Fig. 2d). Serpulid polychaetes (species of *Hydroides* and *Spirobranchus*)

were relatively uncommon and patchily distributed among plates for summer 1, but there were significantly more in Coal & Candle Creek than in the other 2 creeks (Fig. 2e, Table 1e). Serpulids were far more abundant in the second set of samples (Fig. 2e), when percentage covers were greater in Cowan and Coal & Candle creeks than in Smiths Creek (Table 1e).

Significant differences in the cover of taxa also occurred within creeks. For the first set of samples in Cowan Creek, the cover of green filamentous algae at the marina and 1.5 km away (position 2) was greater than at position 3 closer to the mouth of the creek (Table 1a, Fig. 2a). Conversely, the cover of spirorbid polychaetes decreased from the marina to position 2 and position 3 along Cowan Creek (Table 1d, Fig. 2d). No differences in the cover of spirorbids occurred among positions in Smiths Creek, but, in Coal & Candle Creek, the cover of spirorbids at the marina was significantly greater than at the other 2 positions (Table 1d).

Multivariate analyses indicated that, for each set of samples, the sites at most locations were generally not significantly different ($p > 0.05$). For both samples, however, the significance level of the comparison between sites at Halvorsen Marina was minimal ($p = 0.029$). The relatively large R value for the comparison for the first set of samples ($R = 0.604$) suggests that the sites may in fact have been different, but this may not have been the case for the second set of samples ($R = 0.281$). Despite the possible difference between sites at Halvorsen's, comparisons were made between all locations by pooling replicates between sites (so that $n = 8$ at each location). ANOSIM indicated that, for the first set of samples, all 3 positions in Cowan Creek were different from each other (Table 2, Fig. 3) and different from the positions in Smiths and Coal & Candle creeks (Table 3, Fig. 3). For the second set of samples, position 1 in Cowan Creek was significantly different from positions 2 and 3 (Table 2, Fig. 3), and again all 3 positions in Cowan were different from positions in other creeks (Table 3, Fig. 3). The composition of assemblages at all 3 positions within Smiths Creek were similar to each other and the same pattern occurred among positions in Coal & Candle Creek (Table 2, Fig. 3). All 3 locations at the end of the creeks (position 1) were, however, significantly different (Fig. 3a, b, Table 3).

Samples after 12 weeks

As for samples after 5 wk, differences in the cover of taxa occurred among creeks and among positions within creeks after 12 wk (Table 4, Fig. 4). Abundances of most taxa were very different by this later stage, but

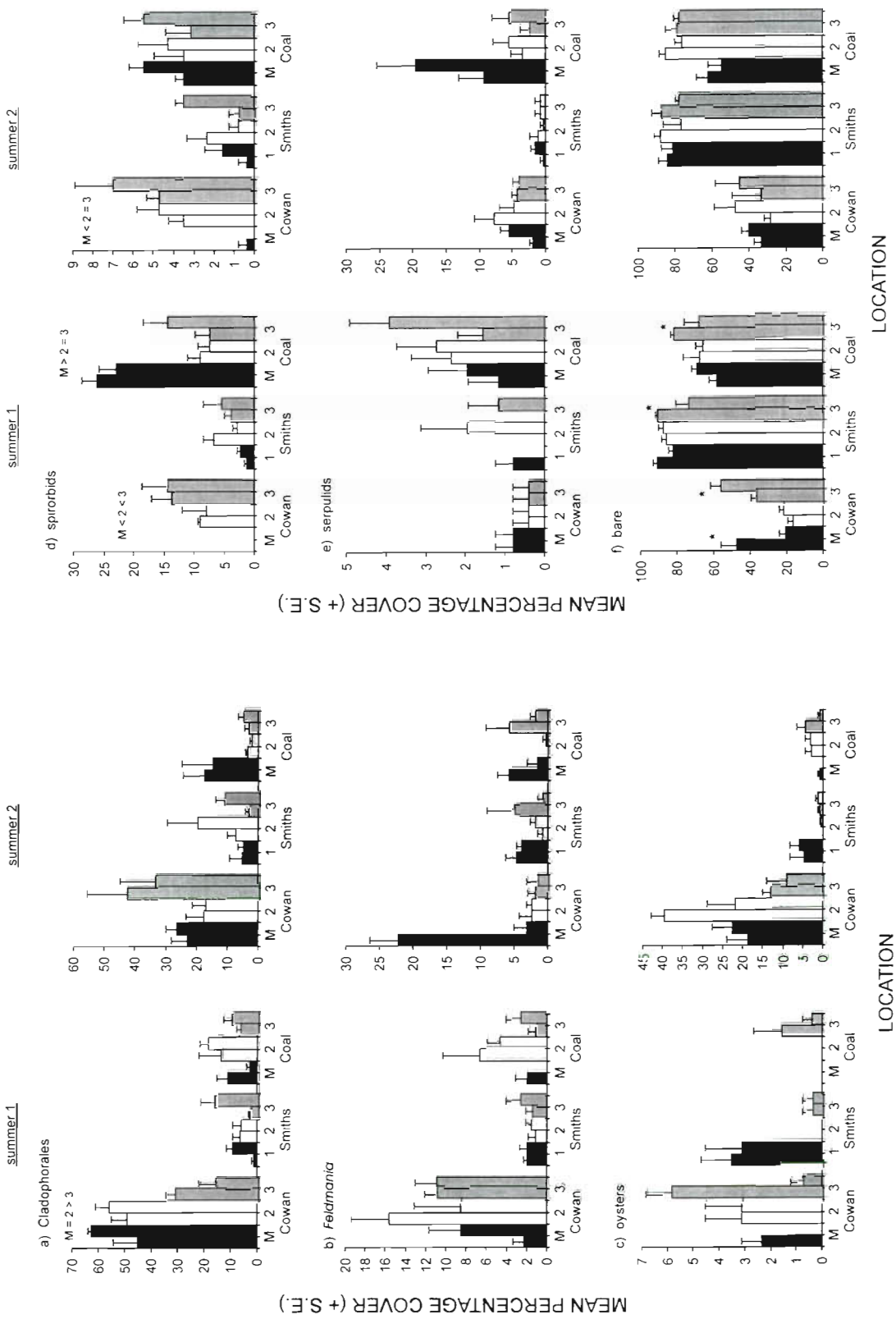


Fig. 2. Percentage cover estimates of dominant taxa on plates after 5 wk. Two sites (bars of the same shading) at each of 3 positions were sampled in 3 creeks. Two sets of samples (summer 1 and summer 2) were submerged 1 mo apart. M = marina. Results of SNK tests comparing positions within creeks are written above graphs. *Significant difference between the 2 sites at that location

Table 2. *R* values from multivariate pairwise comparisons of assemblages between positions within the same creek. Two independent sets of samples each were taken after 5 wk and 12 wk. There were 3 positions (1: end, 2: middle, 1.5 km from end and 3: mouth, 3 km from end) in each creek; the marinas (M) were at 'position 1' in Cowan and Coal & Candle creeks. Significant differences are marked (*), but different levels of significance are not distinguished

Creek	Comparison	5 weeks		12 weeks	
		Summer 1	Summer 2	Summer 1	Summer 2
Cowan	M vs 2	0.480*	0.426*	0.535*	0.539*
	2 vs 3	0.419*	0.141	-0.008	0.001
	M vs 3	0.739*	0.532*	0.529*	0.651*
Smiths	1 vs 2	0.249	0.102	0.579*	0.323*
	2 vs 3	-0.060	0.096	-0.009	0.431*
	1 vs 3	0.008	0.235	0.876*	0.532*
Coal & Candle	M vs 2	0.265	0.313	0.123	-0.110
	2 vs 3	0.065	0.066	-0.001	-0.068
	M vs 3	0.201	0.136	0.161	-0.021

Table 3. *R* values from multivariate pairwise comparisons of assemblages between positions in different creeks. Significant differences are marked (*), but different levels of significance are not distinguished

Position	Comparison	5 weeks		12 weeks	
		Summer 1	Summer 2	Summer 1	Summer 2
1	Cowan vs Smiths	0.702*	0.400*	0.425*	0.558*
	Smiths vs Coal	0.652*	0.379*	0.811*	0.484*
	Cowan vs Coal	0.946*	0.667*	0.802*	0.749*
2	Cowan vs Smiths	0.863*	0.677*	0.642*	0.403*
	Smiths vs Coal	0.249	0.331	0.162	0.436*
	Cowan vs Coal	0.810*	0.501*	0.855*	0.271
3	Cowan vs Smiths	0.332*	0.683*	0.876*	0.200
	Smiths vs Coal	0.090	0.075	0.115	0.155
	Cowan vs Coal	0.539*	0.480*	0.826*	0.257*

there were, nevertheless, similar patterns among locations for certain taxa (Figs. 2 & 4). Differences between sites were uncommon and occurred only at some locations for serpulid polychaetes and green and brown filamentous algae (Fig. 4).

The percentage cover of green filamentous algae (Cladophorales) was significantly greater in Cowan Creek for the first set of samples (Fig. 4a, Table 4a). For the second samples, the cover in Cowan was not different from that in Smiths and was significantly greater than that in Coal & Candle Creek (Fig. 4a, Table 4a). There was a similar trend for the cover of the brown filamentous alga *Feldmanina* sp. to decrease from Cowan Creek to Coal & Candle Creek (i.e. moving seawards, Fig. 4b). The cover of oysters at the marina in Coal & Candle Creek was significantly less than at the ends of the other 2 creeks (Table 3d). Furthermore, there were significantly more oysters in the middle (position 2) and mouth (position 3) of Cowan Creek than at corresponding positions in Smiths and Coal & Candle creeks. None of these differences was apparent in the second set of samples (Fig. 4c, Table 4c).

There tended to be far more spirorbid polychaetes at position 1 in Coal & Candle Creek (Akuna Bay Marina) than at position 1 in the other 2 creeks (Fig. 4d). Serpulid polychaetes were significantly more abundant in Coal &

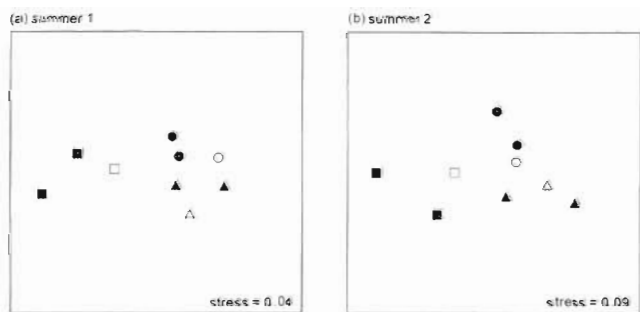


Fig. 3. nMDS ordinations for the 2 sets of 5 wk samples (see Fig. 2), comparing the composition of assemblages at 3 positions (1: black, 2: grey, 3: white) within 3 creeks: Cowan (squares), Smiths (circles) and Coal & Candle (triangles). The 2 marinas are the black square and the black triangle

Table 4. ANOVA for dominant taxa on plates collected after 12 wk. Two sets of plates (summer 1 and summer 2) were sampled 1 mo apart. See Table 1 for details of tests. Variances were homogeneous at $p > 0.05$ for all tests. NS: $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Source	df	MS	Summer 1 <i>F</i>	<i>p</i>	MS	Summer 2 <i>F</i>	<i>p</i>
(a) Cladophorales					ln(<i>x</i> + 1)		
Creek	2	1193.95	12.72 ^a	***	15.66	20.88 ^a	***
Position	2	34.28	0.37 ^a	NS	0.09	0.12 ^a	NS
Site(P)	3	113.59 ^a	1.49	>0.25	1.01 ^a	1.34	>0.25
C × P	4	105.64 ^a	1.39	>0.25	0.93 ^a	1.24	>0.25
C × S(P)	6	76.16 ^a	2.44	*	0.75 ^a	1.04	>0.25
Residual	54	31.26			0.72 ^a		
(b) <i>Feldmania</i>		ln(<i>x</i> + 1)					
Creek	2	12.78	3.60	NS	528.56	2.00	NS
Position	2	3.14	0.89	NS	574.44	2.18	NS
Site(P)	3	0.50	0.27	>0.25	88.77	1.07	>0.25
C × P	4	3.55	1.94	NS	264.08	3.19	NS
C × S(P)	6	1.83	3.91	**	82.87	2.14	NS
Residual	54	0.47			38.81		
(c) Oysters		ln(<i>x</i> + 1)			ln(<i>x</i> + 1)		
Creek	2	29.51	4.35	NS	2.69	2.02 ^a	NS
Position	2	10.77	–		6.57	2.55	NS
Site(P)	3	1.85	2.07	NS	2.57	3.25	NS
C × P	4	6.78	7.59	*	1.24 ^a	1.57	>0.25
C × S(P)	6	0.89	1.66	NS	0.79 ^a	0.57	>0.25
Residual	54	0.54			1.40 ^a		
(d) Spirorbids		ln(<i>x</i> + 1)					
Creek	2	1.73	0.22	NS	117.73	1.94	NS
Position	2	5.40	–		360.75	5.95	NS
Site(P)	3	1.57	2.11	NS	33.03	1.44	>0.25
C × P	4	7.72	10.36	**	60.61	2.64	NS
C × S(P)	6	0.75	2.35	*	22.96	0.98	>0.25
Residual	54	0.32			23.31		
(e) Serpulids		ln(<i>x</i> + 1)					
Creek	2	15.28	47.58	**	520.87	10.19	*
Position	2	0.74	–		48.05	–	
Site(P)	3	2.63	2.98	NS	45.47	0.55	NS
C × P	4	0.32	0.36	>0.25	51.10	0.61	NS
C × S(P)	6	0.88	2.47	*	83.11	4.97	***
Residual	54	0.36			16.72		
(f) Bryozoans							
Creek	2	908.64	2.48	NS	14.36	15.92 ^a	***
Position	2	31.57	–		6.45	2.28	NS
Site(P)	3	301.46	4.58	NS	2.82	2.80	NS
C × P	4	366.55	3.03 ^a	*	1.69 ^a	1.67	>0.25
C × S(P)	6	65.75	0.52	>0.25	1.01 ^a	1.21	>0.25
Residual	54	127.00 ^a			0.83 ^a		

^aMS terms were pooled and the resultant term used as the denominator for the *F*-ratio

Candle Creek than in the other creeks (Table 4e). Similarly, the percentage cover of encrusting bryozoans tended to increase from Cowan Creek to Coal & Candle Creek, and there were significant differences among all 3 creeks for the second set of samples (Fig. 4f, Table 4f). For the summer 1 samples, there was a significantly smaller percentage cover of bryozoans in the middle of Cowan Creek compared to the corresponding positions in Smiths and Coal & Candle creeks (Table 4f).

Differences occurred among positions within creeks for only a few taxa. There was a trend for the cover of green filamentous (Cladophorales) and brown filamentous (*Feldmania* sp.) algae to increase towards the end (position 1) of Smiths Creek for the summer 1 samples (Fig. 4a, b). A similar pattern occurred for oysters within Smiths Creek. For the first set of samples, the percentage cover of oysters at the end of Smiths Creek was significantly greater than that at the other 2 posi-

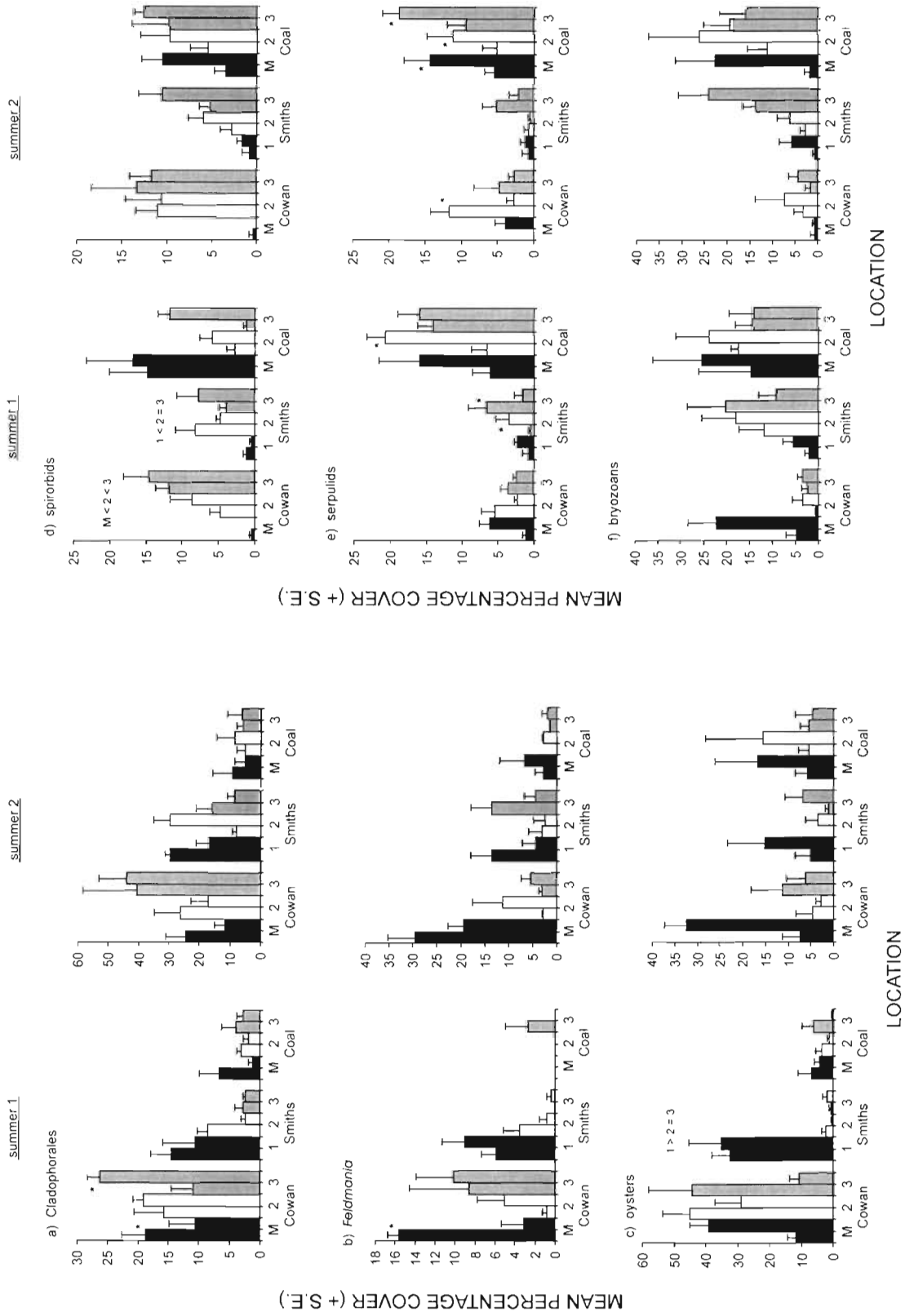


Fig. 4. Percentage cover estimates of dominant taxa on plates after 12 wk. Two sites (bars of the same shading) at each of 3 positions were sampled in 3 creeks. Two sets of samples (summer 1 and summer 2) were submerged 1 mo apart. M = marina. Results of SNK tests comparing positions within creeks are written above graphs. *Significant difference between the 2 sites at that location

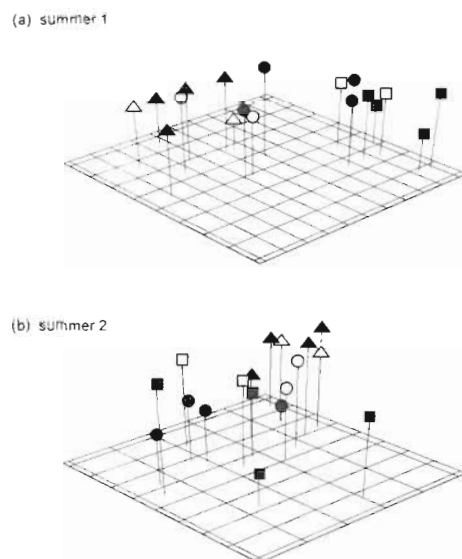


Fig. 5. Three-dimensional nMDS ordinations for the 2 sets of 12 wk samples (see Fig. 4), comparing the composition of assemblages at 2 sites (duplicated symbols) within locations in 3 creeks: Cowan (squares), Smiths (circles) and Coal & Candle (triangles). The differently shaded symbols represent the 3 positions in each creek: position 1 (black), position 2 (grey) and position 3 (white). The 2 marinas on the black squares and the black triangles. Stress values are (a) 0.06, (b) 0.07

tions (Table 4c, Fig. 4c). Spirorbid polychaetes were significantly less abundant at the end of Cowan and Smiths creeks than at the other 2 positions within the creeks for the summer 1 samples (Table 4d, Fig. 4d). The same pattern occurred for the summer 2 samples, but the difference was not significant (Table 4d, Fig. 4d).

Multivariate analyses indicated that there were quite probably differences among sites at some locations (i.e. $p = 0.029$). The greatest R values for the comparison between sites occurred at the 2 marina locations. Furthermore, the only difference between sites that may have occurred at both times of sampling was at Halvorsen Marina (position 1 in Cowan Creek) and

this is clearly shown by the nMDS ordinations (Fig. 5). Ordinations were presented as 3-dimensional plots to provide the most accurate representation of all the sites. ANOSIM demonstrated that, for both samples, the assemblages at position 1 in Cowan Creek were quite unlike those at any other position (Tables 2 & 3). Moreover, locations at the ends of the 3 creeks were all dissimilar at each time of sampling (Table 3). Other patterns differed slightly between samples. For the summer 1 samples, the composition of assemblages at the end of Smiths Creek (position 1) was unlike that at the other 2 positions in the creek (Table 2). Whereas in Coal & Candle Creek, all 3 positions were similar to each other (Table 2) and similar to positions 2 and 3 in Smiths Creek. Likewise, the summer 2 samples from Coal & Candle Creek were all similar to each other (Table 2) and not different from those in position 3 in Smiths Creek (Table 3). All 3 of the positions in Smiths Creek were, however, different from each other in the second set of samples (Table 2).

Using the results of multivariate comparisons among assemblages at different positions within creeks (R values in Table 3), 2-factor ANOVAs were constructed to compare the dissimilarity between positions for each creek for the 5 wk and 12 wk samples. If marinas had effects that extended to 1.5 km, the dissimilarity between assemblages at positions 1 and 2 in the creeks containing marinas should be less than that between the corresponding positions in the control (Smiths) creek. The results showed that there were no differences in the patterns among positions in the marina and control creeks (i.e. no $C \times P$ interaction) and, therefore, no indication that the marinas were having any effects on assemblages 1.5 km away (Table 5).

DISCUSSION

Patterns among locations were generally similar for the 2 sets of samples, although for some taxa there were large differences in percentage covers between

Table 5. ANOVAs comparing the dissimilarity between positions 1 (end), 2 (middle, 1.5 km from end) and 3 (3 km from end) in each of 3 creeks (Cowan, Smiths, Coal & Candle). Data used are R values from multivariate comparisons of the assemblages among positions within each creek (see Table 3) for 2 sets of samples after 5 wk and 12 wk; $n = 2$. The test for overall differences among creeks was irrelevant to our hypotheses. Data were untransformed and variances were homogeneous. NS: not significant

Source	df	MS	5 weeks F	p	MS	12 weeks F	p
Creek	2	0.206	16.04	—	0.332	12.28	—
Position	2	0.069	4.46	NS	0.248	5.21	NS
$C \times P$	4	0.015	1.20	NS	0.048	1.76	NS
Residual	9	0.013			0.027		

samples. Spirorbids, serpulids and oysters were common on plates in only 1 set of samples after 5 wk, but, by 12 wk, the percentage covers of these taxa were similar for the 2 sets of samples. Thus, recruitment of these taxa occurred at different stages of development of the assemblages for the 2 sets of samples. Percentage covers of green and brown filamentous algae (*Cladophorales* and *Feldmania* sp.) differed consistently between sets of samples. These discrepancies between samples resulted in less bare space on many plates for the second set of samples after 12 wk. Thus, over a period of 12 wk, the composition of assemblages on the plates differed according to the week that the plates were put into the water. This supports previous findings that the development of epibiotic assemblages can differ considerably within as well as among seasons (e.g. Keough 1983, Anderson & Underwood 1994). It is not clear whether differences that occur over short time periods may be maintained for longer periods (Greene & Schoener 1982, Anderson & Underwood 1994), but there is substantial evidence that established organisms can influence subsequent colonization (see review by Downes & Keough 1998).

Spatial extent of the effects of marinas

Large differences in the cover of many taxa occurred between the 2 marinas. Clearly, if the marinas are having any effects on the establishment of epibiota, they are not the same at the 2 locations. Few previous studies have compared multiple marinas, but those that have also identified significant differences in the cover and biomass of certain sessile organisms among marinas (Lenihan et al. 1990, Van Dolah et al. 1992, Glasby 1997, Turner et al. 1997). Patterns in the percentage cover of spirorbid and serpulid polychaetes within Coal & Candle Creek were clearly different from those in the other 2 creeks. Polychaetes were generally uncommon on plates at the ends of creeks, but at Akuna Bay Marina they were very abundant. This suggests that the marina may somehow enhance the local abundance/settlement of tubicolous polychaetes, a result consistent with the findings of Lenihan et al. (1990).

The great variability among positions within creeks makes it difficult to determine whether the marinas are having effects which extend along the creeks. We hypothesised that large-scale (>1.5 km) effects of marinas would be implied if recruitment at the marinas was similar to that at other positions within the creeks and different from that in the control creek. The only univariate result indicative of a large-scale effect was for *Cladophorales* in the first 5 wk sample. The percentage cover of these green algae in Cowan Creek was

significantly greater at Halvorsen Marina (position 1) and position 2 than at position 3. This result was, however, not consistent and, in fact, for the second set of samples the opposite pattern occurred.

Multivariate analyses did not indicate that Halvorsen Marina had any large-scale effect on the composition of newly developed assemblages. Assemblages at the marina were always different from those at other positions within Cowan Creek. The pattern within Coal & Candle Creek (containing Akuna Bay Marina) was, however, different in that the composition of assemblages at all 3 positions was always similar. This result, on its own, could suggest that the marina affected the entire creek. There were, however, rarely any significant differences between positions 2 and 3 in Coal & Candle Creek and the corresponding positions in the control creek (Smiths Creek). Furthermore, position 1 at the end of Smiths Creek was different from the other positions in the creek for the 12 wk samples and different from Akuna Bay Marina. Thus, it is perhaps more likely that Akuna Bay Marina either had only a localized effect or did not have any effect on the composition of the subtidal assemblages of epibiota. This conclusion is supported by the comparison of dissimilarities between assemblages in different positions within the creeks (Table 5). The patterns among positions were similar for each of the 3 creeks. Thus, there was no suggestion that the marinas were affecting assemblages 1.5 km or 3 km away.

The composition of assemblages at Halvorsen Marina was very different from all other locations. This may be a consequence of the marina (which has been in operation for 50 yr) or may be because the location is at the end of the entire estuary and therefore different from other locations. The end of Smiths Creek is ostensibly very similar to the end of Cowan Creek, but movement of water, tidal flushing, input of fresh water, etc. could still differ between the 2 locations. Certainly there were differences in concentrations of dissolved oxygen (see 'Materials and methods'). Abiotic differences, together with natural patchiness in recruitment, could quite conceivably explain the observed differences in assemblages among locations. Large spatial variability has been documented in many studies of subtidal epibiota (e.g. Kay & Keough 1981, Butler 1986, Kennelly & Underwood 1992, Glasby 1998), but this is often not estimated in environmental studies.

Natural differences within creeks

Natural differences in recruitment and the development of sessile assemblages occurred along the creeks. There was often a gradient from the end to the mouth of Smiths Creek in the percentage cover of taxa.

Spirorbids and bryozoans tended to be less common at the end of the creek (position 1) and their percentage covers increased towards the mouth. Conversely, the percentage cover of oysters was often greater at the end than at the other 2 positions in Smiths Creek. Furthermore, green and brown filamentous algae (*Cladophorales* and *Feldmania* sp.) tended to be most common at the end of this creek.

Multivariate analyses indicated that, after 12 wk, assemblages at the end of Smiths Creek were significantly different from those at the other 2 positions. This was not due to certain taxa being absent from the end of the creek, but, rather, because different taxa dominated assemblages at this position. This suggests that conditions at the end of the creek may be different from those near the mouth and that the dominant taxa may either recruit in greater densities or be better able to survive and grow in the conditions at the end of the creek.

Considerations for environmental studies of estuarine epibiota

This study has highlighted the importance of large-scale variability (over 1000s of metres) in epibiotic assemblages. Significant differences occurred among newly developed assemblages of epibiota at control locations that appeared to be very similar. Many of these differences were correlated with natural gradients within creeks. This has important ramifications for selecting control locations. Unless controls were in positions within creeks that corresponded to the position of a marina, it is likely that an impact could either be detected erroneously or masked (because different habitats were actually being sampled, see Morrissey & Underwood 1992, Morrissey et al. 1992). Moreover, multiple control locations will obviously be necessary in order to obtain an accurate estimate of the composition of 'natural' assemblages of epibiota at undisturbed locations (Underwood 1989, 1992).

Unfortunately, most studies of marinas have forgone detailed investigations of natural variation and it is rare for sufficient justification to be given for the choice of control locations (e.g. Baird et al. 1981, Voudrias & Smith 1986, Lenihan et al. 1990, Wendt et al. 1990, Van Dolah et al. 1992, McGee et al. 1995, Turner et al. 1997). The specific criteria used for the selection of control locations must be given for any environmental study (e.g. Kennicutt et al. 1996) in addition to full details about the locations used. Our results demonstrate how seemingly appropriate control locations may, in fact, be totally inadequate because of environmental variables that were not considered because there was no prior reason to include them.

The choice of control locations will be particularly important for any 'post-impact' study (i.e. when no data are available from before the disturbance) because the detection of an impact often relies on spatial differences between the putatively impacted location and the controls (Underwood 1992, Peterson 1993, Glasby 1997, Keough & Mapstone 1997). Thus, it is surprising that detailed descriptions of control locations are rarely presented as part of investigations of environmental impacts. Most ecologists accept the need for control locations, but it seems adequate merely to state that controls were used and present a map showing their positions. To anybody who is not extremely familiar with the study area, such non-specific information is of little use for assessing the validity of the study. The rationale for choosing controls is as important as any other aspect of an environmental study.

This leads to the question of how controls should be selected. Some of the common (and fairly non-specific) considerations for choosing control locations were outlined in the 'Introduction'. Results from this study have indicated that certain physical factors may play a very important part in determining the composition of assemblages of epibiota. Clearly, therefore, these factors need to be identified and accounted for when choosing control locations for post-impact studies. In essence, this means trying to identify the factors that most determine natural variability among seemingly similar locations (Underwood 1994). Certainly this is no easy task, but we suggest that it will become increasingly necessary to enable comprehensive tests for environmental impacts when no 'before' data are available. Data collected from investigations of natural variability could also provide important baseline data (i.e. 'before' data) if done in habitats that were often subjected to anthropogenic disturbances (Lewis 1976, Hilborn & Walters 1981, Fairweather 1993, Underwood 1994).

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