ASSESSING THE RESPONSE OF ESTUARINE INTERTIDAL ASSEMBLAGES TO URBANISED CATCHMENT DISCHARGE[1]

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Abstract. Measurement of intertidal rock assemblages was investigated as a potential biological indicator to provide a quantitative estimate of the impact of urbanised catchment discharge on the estuaries of Sydney, Australia, from 1995 to 1999. Based on the presence and characteristics of adjacent human activities, sampling locations were categorised as: Bush; Urban; Urban with Sewer Overflows; and Industry with Sewer Overflows. In Sydney Harbour, variation in assemblage structure was measured between most impact categories, however differences between impact categories were not consistent for each year. Nevertheless, in years of above average rainfall (1998-99), reference assemblages adjacent to national parks and distant from urbanisation were different to all other putatively impacted assemblages. Variability within assemblages was least at reference locations in each year and greatest at locations adjacent to stormwater canals and sewer overflows, particularly in 1998-1999. Variation in assemblage structure in Sydney estuaries was most strongly correlated with chlorophyll-a concentrations. Univariate analysis also identified highly significant differences for a number of factors, however, interactions between year, impact categories and location for numerous analyses, confounded the differentiation between impact categories. The results suggest that intertidal rock assemblages in Sydney Harbour and surrounding estuaries appear to be responding to the quality and quantity of discharge from urbanised catchments and, furthermore, that assemblages are more suitable than individual taxa to indicate the difference between Bush and anthropogenically disturbed estuarine locations.

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1. Introduction

Numerous biological indicators have been proposed to provide better understanding of how estuaries respond to environmental stresses over different temporal and spatial scales (Ward *et al.,* 1998; Deeley and Paling, 1999). Much discussion has focussed on appropriate single indicator organisms for monitoring ecosystem change (Jones and Kaly, 1996). However, since exposure to a contaminant may change any of the distinct processes that govern the dynamics of an assemblage, monitoring of assemblages may provide a better assessment of impact or change (Maher and Norris, 1989). Recently, a number of Australian studies have used multivariate measures to detect disturbance effects as these methods are proving very sensitive for determining spatial and temporal variation in assemblage structure (Roberts *et al*., 1998; Kelaher *et al*., 1998; Glasby, 1999; Lindergarth and Hoskin, 2001).

Measurable changes in biota have been associated with sewage pollution in Australian estuaries. Sewage discharge enhances recruitment of mussels (Hindell and Quinn, 2000), alters the structure of soft bottom macrobenthic assemblages (Poore and Kudenov, 1978; Axelrad *et al*., 1979), degrades seagrass communities (Neverauskas, 1985) and encourages opportunistic epiphytes and benthic macroalgae (Neverauskas, 1985; Fairweather, 1990). Detection of impacts associated with sewage in estuarine environments, however, is typically confounded by the impacts of stormwater and other human activities (Raffaelli and Hawkins, 1999). Nevertheless, despite continued urbanisation along Australia's New South Wales (NSW) coast increasing the potential for human activities to impact on estuarine ecosystems, the effect of sewage-related contamination on estuarine intertidal rock assemblages remains largely undescribed.

Sydney Harbour, for example, is heavily urbanised and has, historically, received high pollutant loadings from stormwater runoff, sewer overflows and other diffuse sources during wet weather (AWT Ensight, 1997). Discharge of sewage effluent and stormwater impacts on

the microbiological quality of the receiving waters and poses potential health problems and risks to aquatic life (Kueh and Grohmann, 1989; Kueh *et al*., 1991; Bickford *et al*., 1999).

Despite areas of fringing mangroves and urban national parks, the major catchments of the Sydney region (Port Jackson with its tributaries and Cooks River) largely incorporate residential housing, commercial and industrial development, and Sydney's three main sewerage transfer systems. In comparison, the catchments at the northern and southern extremities of the Sydney region (Pittwater and Port Hacking, respectively) have largely retained their natural state. These areas, however, are now also experiencing increased urbanisation.

Sydney's three main sewerage transfer systems are potential major pollution sources (AWT Ensight, 1997). Additionally, numerous overflow structures in the Sydney region may discharge into the waterways following a sewer choke or during power failure at a major pumping station or treatment plant, or as a result of stormwater infiltration into the sewerage system following moderate to heavy rainfall (AWT Ensight, 1997). In wet weather, urban stormwater also carries contaminants from a range of industrial and domestic sources, including chemicals contained in gardening products and vehicle exhaust particulate matter (Bickford *et al*., 1999). While undiluted sewage from overflows is toxic to sensitive aquatic species, Bickford *et al*. (1999) estimate that stormwater contributes approximately 85% of the total load of 'chemicals of concern to aquatic life' to Sydney's coastal catchments.

In 1994, in response to a need for a system of measurement which would detect improvements in estuarine ecosystems resulting from Sydney Water Corporation's (SWC) capital works expenditure in the Sydney area, Australian Water Technologies (AWT) proposed the use of aquatic communities as indicators of the effectiveness of impact mitigation (Scammell and Collie, 1994). Between September 1995 and October 1999, five intertidal rock platform assemblage sampling events were conducted in the estuaries of the

Sydney region to assess whether the composition of intertidal rock assemblages is related to anthropogenic disturbance in the form of urbanisation, industrialisation and the presence of sewer overflows, or the absence of such disturbance. Intertidal assemblages at locations in each of the impact categories were compared with assemblages at multiple reference locations. The objectives of the study were to test whether: (1) significant differences existed between the structure of intertidal rock assemblages at locations within impact categories for each year; (2) intertidal rock assemblages were significantly different between impact categories; (3) assemblages at impacted locations were more variable than at reference locations; and (4) significant differences existed between common indices of assemblage structure and abundance or coverage of individual taxa. Additionally, the study aimed to test whether the designation of locations to the impact categories was appropriate.

2. Methods

2.1   STUDY LOCATIONS

Prior to the commencement of sampling, results from previous water quality studies of Sydney estuarine locations were considered to define potentially useful impact categories and to select study locations (AWT Ensight, 1996). Impact categories were classified into: Urban; Urban with Sewer Overflows; and Industry with Sewer Overflows, based on the presence and characteristics of adjacent human activities.

Nineteen intertidal locations in the estuaries of the Sydney region were selected within Iron Cove, Lane Cove River, Cooks River, Port Jackson, Middle Harbour, Port Hacking and Pittwater (Figure 1; Table I). Each location was classified into an appropriate impact category based on available water quality data including levels of faecal coliforms, chlorophyll-a, total phosphorus, total nitrogen, oxidised nitrogen and total uncombined ammonia, relative to

accepted guidelines (AWT Ensight, 1996, 1997; ANZECC, 2002). To negate the confounding influence of salinity from sewage-associated disturbances, only locations with a median salinity greater than 30 ‰ (approximately 46 mS.m^{-1} conductivity, adjusted to 25ºC; UNESCO, 1980), calculated from available water quality data measured at 0.5 m depth, were included in the study. Reference locations were all adjacent to national parks where recorded concentrations of most variables were usually close to lower detection limits (AWT Ensight, 1996).

TABLE I

Sydney estuarine locations where intertidal assemblages were sampled and impact categories.

Waterway	Location (Code)	Impact Category[#]
Port Jackson	Little Sirius Cove (L)	U
	Double Bay (J)	U
	Rushcutters Bay (Q)	US
	Blackwattle Bay (G)	IS
Iron Cove	Hawthorne Canal* (H)	IS
	Iron Cove Creek* (I)	IS
Middle Harbour	Davidson Park (B)	B
	Bantry Bay (A)	B
	Sugarloaf Bay (R)	US
	Tunks Park (S)	US
	Quakers Hat Bay (P)	US
Pittwater	Towlers Bay (F)	B
	McCarrs Creek (D)	B
Lane Cove River	Fig Tree Bridge (N)	US
Cooks River	Cooks River (O)	US
Port Hacking	Gunnamatta Bay (K)	U
	Maianbar (M)	U
	South West Arm (E)	B
	Grays Point (C)	B

[#] Impact categories: Industry/Sewer (IS); Urban/Sewer (US); Urban (U); Bush (B)
* Sampling did not commence at this location until 1996.

While natural, intertidal, sandstone rocky reef was present at most locations, sandstone retaining walls were used at Hawthorne Canal and concrete bridge pylons were used at Fig Tree Bridge. Although variation in assemblages within a location can be attributed to differences in the complexity of the substratum (Minchinton, 1997; Beck, 1998; Glasby, 1999), Downes and Hindell (2000) found spatial variation to be more significant than

substratum texture in accounting for large shifts in faunal composition at the family level. It was therefore assumed that any measurable variation in assemblages would be more attributable to differences in water quality rather than differences in rock surfaces.
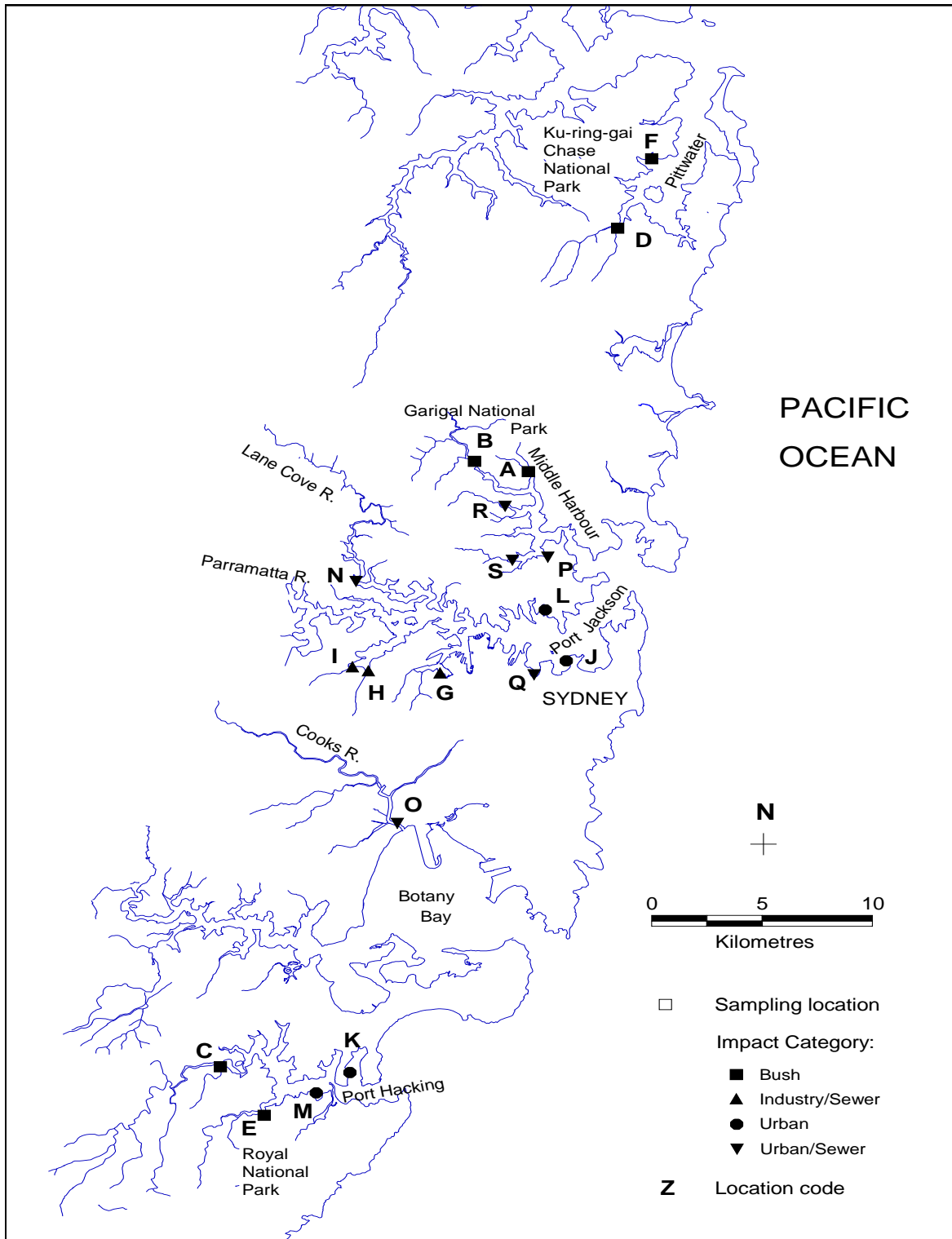

Figure 1. Sydney estuarine intertidal rock platform assemblage sampling locations.

2.2 FIELD SAMPLING

Sampling was conducted within two hours before or after low tide and performed annually in September/October from 1995 to 1999. Measurements were recorded from 7 haphazardly placed $0.25m^2$ quadrats on available rock or retaining wall surfaces at each location. The top of the quadrat was placed 10 to 15 cm below the upper growth line of Sydney Rock Oysters *(Saccostrea glomerata)* or the remains of the shells. Percentage cover of algae, encrusting bryozoans and polychaetes were estimated using grids of $0.01m^2$ within the quadrat. Abundance (per quadrat) was recorded for all other taxa. Pilot studies indicated that 7 quadrats would provide adequate sampling of taxa at each location (AWT Ensight, 1996).

Organisms were identified to a range of recognisable taxonomic levels to allow for rapid assessment and minimal disturbance. Abundance data were aggregated to a relatively low level of taxonomic resolution as this generally results in no loss of information about patterns in assemblage structure (James *et al*., 1995; Vanderklift *et al*., 1996; Chapman, 1998; Thompson *et al*., 2003). Of the relatively abundant taxa, most molluscs and polychaetes were identified to genus, while barnacles were aggregated to class and green algae were identified to phylum. Less abundant taxa including Porifera, Cnidaria, Echinodermata, Platyhelminthes and Bryozoa were generally identified to phylum.

2.3 WATER QUALITY DATA

Data for total phosphorus, filterable phosphorus, total nitrogen, oxidised nitrogen, total uncombined ammonia, chlorophyll-a and faecal coliforms for each location were obtained from SWC and compared against accepted standards (ANZECC, 2002). Rainfall data collected at stations in the vicinity of the sampling locations were provided by the Bureau of Meteorology. The study commenced in 1994 after a period of prolonged dry weather, however most rainfall stations recorded above average rainfall for 1995. While annual

precipitation was generally below average through 1996 and 1997, above average rainfall was recorded at all stations in 1998 and 1999.

2.4   STATISTICAL ANALYSES

Multivariate and univariate analyses were initially conducted on data collected only from locations within Sydney Harbour and its tributaries (i.e. Bantry Bay, Davidson Park, Blackwattle Bay, Hawthorne Canal, Double Bay, Little Sirius Cove, Rushcutters Bay and Tunks Park). This choice of locations reduced any confounding influence of differences between estuaries. A balanced, orthogonal design of two locations nested within each of the four impact categories was used to test the null hypotheses that: (1) there were no significant differences between the structure of intertidal rock assemblages at locations within impact categories for each year; and (2) intertidal rock assemblages were not significantly different between impact categories. Additionally, we tested the hypothesis that assemblages at impacted locations are more variable than at reference locations (Warwick and Clarke, 1993). Univariate analyses tested the null hypotheses that there were no significant differences between: (1) common indices of assemblage structure (i.e. taxonomic richness, total abundance, heterogeneity, evenness); and (2) abundance or coverage of individual taxa. Analyses were only conducted for the years 1996-1999 as only one Industry/Sewer location was sampled in 1995.

2.4.1   *Multivariate Analysis*

Bray-Curtis similarity matrices, using fourth-root transformed data, were calculated for each year and non-metric Multidimensional Scaling (nMDS) ordinations were used to provide

visual representations of sample similarities (Clark and Warwick, 2001). Analyses were conducted using PRIMER (V.5) software (Plymouth Marine Laboratories, UK).

The statistical significance of differences between assemblages from each of the *a priori* defined impact categories was tested in each year by two-way nested non-parametric multivariate analysis of variance (NPMANOVA; Anderson, 2001).

The similarity percentages (SIMPER) routine in PRIMER was used to determine the identity of taxa contributing to similarity within samples and dissimilarity between samples. To test the hypothesis that assemblage structure is more variable in impacted locations (Warwick and Clarke, 1993), differences in Bray-Curtis similarity values between replicates within each location were examined using the Multivariate Dispersion Indices (MVDISP) routine in PRIMER. MVDISP calculates the relative dispersion among replicates within each category, with larger values corresponding to greater within-category dispersion.

The BIOENV procedure in PRIMER was used to identify correlations between the observed patterns in intertidal assemblages and the following water quality data: mean, median and maximum values for each chemical variable and conductivity; minimum conductivity; geometric mean, median and maximum for faecal coliforms; and the total and maximum monthly rainfall. Most variables were recorded 12-15 times in the 12 months prior to intertidal sampling.

2.4.2   *Univariate Analysis*

Data were analysed using a 3-factor orthogonal model which examined differences between four fixed time periods (Years: 1996, 1997, 1998, 1999), four fixed impact categories (B, IS, U, US) and two random locations nested within each impact category. Prior to analysis of variance (ANOVA) using GMAV5 software (Institute of Marine Ecology, University of Sydney), Cochran's Test was used to test for homogeneity of variances and data were ln(x+1)

transformed to remove heterogeneity. Analysis was performed on untransformed data for variables which remained heterogeneous after transformation. Analysis of variance is relatively robust for most departures from homogeneous variances for balanced designs and sample sizes used in this study (Underwood, 1981, 1997).

While interactions between the factors were also measured, Underwood (1981) suggests that significant higher-order interactions reduce the scope for interpretation of differences between main effects. Student-Newman-Keuls (SNK) multiple comparison procedure was used to investigate patterns of differences. However, where significant interactions occurred, significant results for main effects were not reported.

3. Results

3.1 ASSEMBLAGE STRUCTURE

Twenty four taxa were identified in the Sydney estuaries during this study. Only 12 taxa were present at more than 20% of locations during any sampling occasion (Table II). Of these 12 taxa, 8 were not observed at a number of locations during any of the years sampled. Additionally, for each year, *Austrocochlea* spp., *Hormosira banksii* and chitons were not represented at the location where the minimum conductivity value was recorded in that year (i.e. Cooks River – 1995 and 1997, Fig Tree Bridge – 1996, Davidson Park – 1998, Tunks Park – 1999). Only *Saccostrea glomerata*, *Bembicium* spp., and green and red algae were recorded at all locations during the study. While no taxa were observed at all locations in each year, *S. glomerata* and green algae were observed at every location in 1997 and 1998, respectively.

TABLE II

Taxa identified during the Sydney intertidal assemblage study. Taxa listed in the right-hand side column were identified at less than 20% of locations during any sampling occasion.

TAXON	TAXON
Molluscs	**Molluscs**
Sydney Rock Oyster (Ostreidae) *Saccostrea glomerata*	Mudwhelks (Batillaridae) *Pyrazus* sp., *Batillaria* sp.
Mussel (Mytilidae) *Xenostrobus* sp., *Mytilus* sp.	Murex shell (Muricidae) *Morula* sp.
Periwinkle (Littorinidae) *Bembicium* spp.	Sea Slug Onchidiidae/Aplysiidae
Limpet (Acmaeidae) *Patelloida mimula*	Periwinkle (Littorinidae) *Nodilittorina unifasciata*
Other Limpets & 'false' limpets *Patelloida* spp., *Cellana* spp., *Siphonaria* spp.	Neritidae *Nerita atramentosa*
Top Shell (Trochidae) *Austrocochlea* spp.	
Chiton (Chitonidae) *Rhyssoplax* spp.	
Algae	**Algae**
Green Algae (Chlorophyta) *Enteromorpha* spp., *Ulva lactuca*	
Brown Algae (Phaeophyta) *Hormosira banksii*	Other Brown Algae (Phaeophyta)
Red Algae (Rhodophyta)	Coralline Algae (Corallinaceae) *Amphiroa* sp.
Crustaceans	**Echinoderms**
Barnacle (Cirripedia) *Balanus* spp., *Elminius* spp., *Tesseropera rosea*	Starfish (Asteroidea)
Polychaetes	**Cnidaria**
Encrusting Polychaete *Galeolaria* spp.	Anemone (Anthozoa) Actiniaria
	Porifera (sponges)
	Bryozoa
	Platyhelminths (flatworms)

nMDS ordinations showed a similar separation of impact categories in each year (Figure 2). Although overlap of categories did occur, nMDS ordinations placed Industry/Sewer locations apart from Bush locations on each occasion. Stress values were ≤0.15 for each year suggesting little prospect of misinterpretation.
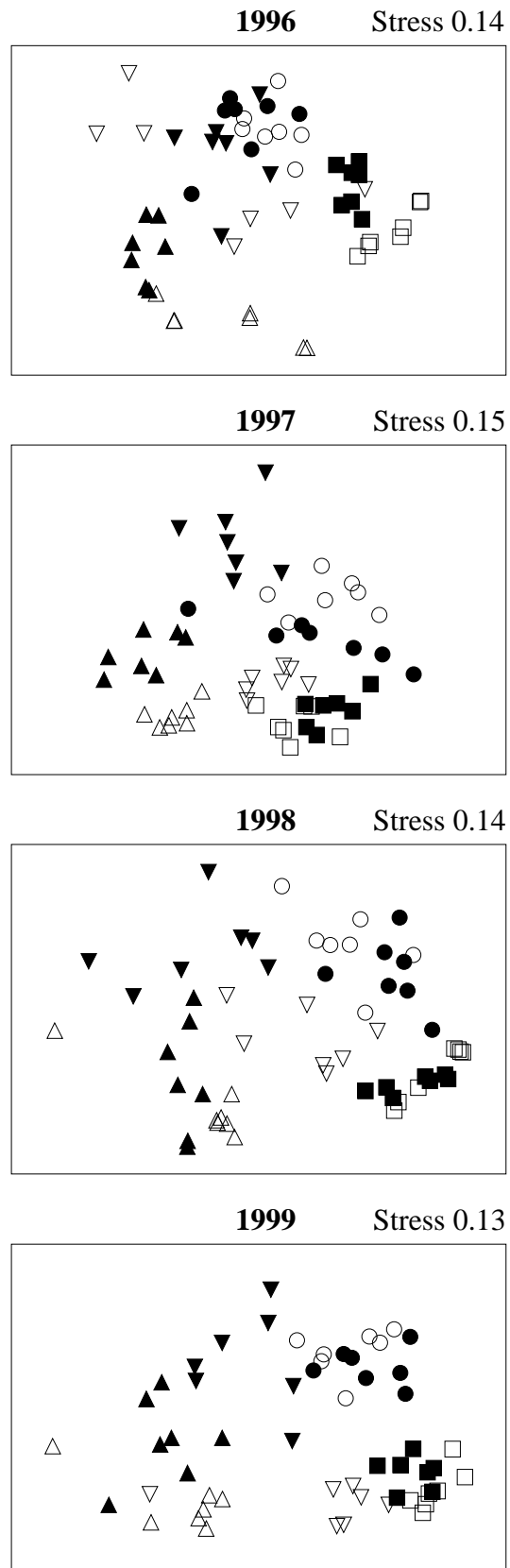
Figure 2. Non-metric multidimensional scaling (nMDS) ordinations comparing Sydney Harbour intertidal rock assemblages for two locations in each impact category for each year, i.e. Industry/Sewer (▲△); Urban/Sewer (▼▽); Urban (●○); Reference/Bush (■□). (*n*=7 replicates per location).

Two-way nested non-parametric multivariate analysis of variance identified consistent, significant differences between impact categories (Table III). Pair-wise *a posteriori* comparisons revealed that the Bush (B) category differed significantly from the Urban (U) category in each year, from the Industry/Sewer (IS) category in all years except 1996 and from the Urban/Sewer category (US) in 1998 and 1999 (Table III). The Urban category was significantly different to the Industry/Sewer category in all years but only to the Urban/Sewer category in 1998. Urban/Sewer and Industry/Sewer categories did not differ in any year.

Assemblages differed between locations in all impact categories (except Urban) in all years.

Relative dispersion values for the Bush locations were consistently below other impact categories (Table IV). In contrast, variability at the Urban/Sewer locations was greatest in all years sampled. The greatest range of dispersion values between the impact categories was measured in 1998 and 1999 when above average rainfall was experienced in the Sydney region.

Barnacles and green algae (Chlorophyta) contributed the greatest amount to assemblage similarity at the Industry/Sewer locations in all years. *S. glomerata*, *P. mimula* and *Bembicium* spp. contributed most to the similarity at Bush locations in all years (Table V). Green algae contributed relatively more to similarity at Urban/Sewer locations in years of above average rainfall (1998-1999). In the same years, the relative contributions of green algae decreased and *Bembicium* spp. increased at Bush locations. Furthermore, the relative contribution of *Galeolaria* sp. to similarity decreased in all impact categories in 1998-1999. The contribution of green algae and barnacles to assemblage similarity was generally lower at Urban locations while the contribution of oysters was generally higher.

TABLE III

Results of two-way nested non-parametric multivariate analysis of variance (NPMANOVA) comparing impact categories and locations within each impact category in Sydney Harbour waterways in each year and results of pair-wise *a posteriori* tests between impact categories in each year.

Year	Source of variation	df	MS	F	p	Impacts	t	p
1996	Impact	3	21284.99	4.69	0.023	Bush & Industry/Sewer	2.52	0.052
	Location (Impact)	4	4536.52	8.48	0.001	Bush & Urban	3.50	0.021
	Residual	48	534.87			Bush & Urban/Sewer	2.28	0.083
						Industry/Sewer & Urban	2.35	0.035
						Industry/Sewer & Urban/Sewer	1.44	0.219
						Urban & Urban/Sewer	1.40	0.191
1997	Impact	3	15439.81	4.37	0.012	Bush & Industry/Sewer	3.35	0.022
	Location (Impact)	4	3535.22	6.85	0.001	Bush & Urban	2.85	0.015
	Residual	48	515.92			Bush & Urban/Sewer	1.55	0.154
						Industry/Sewer & Urban	3.28	0.006
						Industry/Sewer & Urban/Sewer	1.56	0.138
						Urban & Urban/Sewer	1.26	0.256
1998	Impact	3	20063.98	7.94	0.006	Bush & Industry/Sewer	4.26	0.014
	Location (Impact)	4	2526.42	4.65	0.001	Bush & Urban	3.44	0.009
	Residual	48	543.02			Bush & Urban/Sewer	2.65	0.021
						Industry/Sewer & Urban	3.46	0.007
						Industry/Sewer & Urban/Sewer	1.70	0.113
						Urban & Urban/Sewer	2.11	0.039
1999	Impact	3	15826.87	8.17	0.002	Bush & Industry/Sewer	5.29	0.009
	Location (Impact)	4	1937.87	4.69	0.001	Bush & Urban	4.86	0.003
	Residual	48	413.12			Bush & Urban/Sewer	2.04	0.047
						Industry/Sewer & Urban	4.52	0.003
						Industry/Sewer & Urban/Sewer	1.69	0.099
						Urban & Urban/Sewer	1.78	0.067

TABLE IV

Relative dispersion of assemblages within impact categories within Sydney Harbour waterways for each year.

Impact category	1996	1997	1998	1999
Bush	0.646	0.620	0.501	0.457
Industry/Sewer	1.190	0.854	1.034	1.167
Urban	0.811	1.254	1.044	0.888
Urban/Sewer	1.353	1.272	1.421	1.488

TABLE V

Percentage contribution of each taxon to the similarity in assemblage structure between locations in each impact category within Sydney Harbour waterways for each year (to a cumulative percentage of at least 90%).

Year	Impact category	Chlorophyta	Rhodophyta	Amphiroa sp.	Galeolaria sp.	Barnacles	Chitonidae	P. mimula	'Other' Limpets	Austrocochlea spp.	Bembicium spp.	Mytilidae	Saccostrea sp.
1996	Industry/Sewer	45				46							
	Urban/Sewer	12	8		28	19			13				11
	Urban	3			14	17	11		19	7			20
	Bush	8						30			15	6	36
1997	Industry/Sewer	30				49					8	8	
	Urban/Sewer	9		6	9	12			20		9	12	15
	Urban				9	9			10	13	15		37
	Bush	8						25			19	12	30
1998	Industry/Sewer	30				43					6	16	
	Urban/Sewer	35	11					9	8			10	17
	Urban					12		6	17	13	14		27
	Bush							29			28		37
1999	Industry/Sewer	25				45					10	11	
	Urban/Sewer	25						4	9		16	16	20
	Urban				9		10	8	13	15	19		20
	Bush							25			30	10	33

TABLE VI

Percentage contribution of each taxon to the dissimilarity in assemblage structure between impact categories within Sydney Harbour waterways for each year (to a cumulative percentage of at least 90%).

Year	Impact categories*	Chlorophyta	Rhodophyta	Amphiroa sp.	Galeolaria sp.	Barnacles	Chitonidae	P. mimula	'Other' Limpets	Austrocochlea spp.	Bembicium spp.	Mytilidae	Saccostrea sp.
1996	B - IS	10				26		16	6		8	9	18
	B - U	5		5	12	15	9	13	11	7	7	6	4
	B - US	7	7		14	14		15	9		9	7	12
	IS - U	11		4	10	17	8		13	7	5	6	13
	IS - US	11	8		15	24			10		5	11	9
	U - US	8	7	6	6	13	10	5	11	8	6	7	10
1997	B - IS	9				26		16	5		7	11	15
	B - U	10	5		9	12		14	8	10	7	12	4
	B - US	8	4	9	9	11	4	10	11		8	8	8
	IS - U	15			7	19		4	7	9	7	10	15
	IS - US	10		8	8	18	4	6	11		8	11	9
	U - US	9	9		7	9	5	7	9	10	9	11	9
1998	B - IS	12				23		18		3	9	10	18
	B - U	4	3		6	12	5	15	16	10	7	5	6
	B - US	15	8	4		5		14	8	4	14	7	14
	IS - U	13			4	16	4	5	10	8	7	10	14
	IS - US	5	8	5		27		8	8		9	12	11
	U - US	15	7	5	5	8		6	9	10	9	7	9
1999	B - IS	13				29		16			11	9	15
	B - U	6			6	13	12	11	15	13		10	6
	B - US	16	4		4	7		16	11	5	11	8	11
	IS - U	10				20	8	7	9	11	7	10	11
	IS - US	6	4		4	32		6	9		9	11	10
	U - US	11			5	10	9	8	8	12	7	11	7

* Industry/Sewer (IS); Urban (U); Urban/Sewer (US); Bush (B).

Although the major taxa contributing to dissimilarity between impact categories varied between impact combinations, the large numbers of barnacles recorded at Industry/Sewer locations each year resulted in this taxa contributing the greatest amount to the dissimilarity between Industry/Sewer and all other impact categories in all years (Table VI). Green algae contributed most to dissimilarity between Bush and Urban/Sewer categories in the years which experienced above average rainfall (1998-1999).

3.2 PATTERNS OF ABUNDANCE OF BIOTA

Significant Year x Impact Category and/or Year x Location (Impact Category) interactions occurred for all variables except total abundance and abundance of barnacles and limpets (Table VII). Therefore, for most variables analysed, the main effects were not examined as the patterns of differences between impact categories varied significantly between years or the magnitude of the differences between locations within each impact category varied between years.

3.2.1 *Diversity Indices*

Differences in taxonomic richness between impact categories in Sydney Harbour varied between years as indicated by the significant Year x Impact interaction (Table VII). Lowest taxonomic richness occurred at an Industry/Sewer location in all years (Figure 3a). Highest taxonomic richness occurred at an Urban location in all years except 1997.

No significant differences in total abundance were measured between years or impact categories. However, SNK tests identified highly significant differences in total abundance between locations within the Industry/Sewer category (Table VII). Differences in total abundance between these locations was greatest (1996) and least (1997) in years when below

TABLE VII

Summaries of results of three factor ANOVA comparing impact categories and locations within each impact category in Sydney Harbour waterways.

Source	df	F-ratio vs	Taxonomic Richness[a]		Abundance[a]		Heterogeneity[b]		Evenness[a]	
			MS	F	MS	F	MS	F	MS	F
Year	3	Yr*Loc(Imp)	2.17×10^8	20.66**	2.42×10^{12}	2.33	9.34×10^7	5.10×10^8**	9.33×10^7	1.54×10^9**
Impact	3	Loc (Imp)	2.02×10^8	12.37*	1.26×10^{13}	1.66	1.34×10^6	936.32**	1.34×10^6	944.49**
Location(Imp)	4	Residual	1.64×10^7	0.44	7.59×10^{12}	8.21**	1.44×10^3	2.03×10^4**	1.42×10^3	4.53×10^4**
Yr*Imp	9	Yr*Loc(Imp)	6.12×10^7	5.84**	1.14×10^{12}	1.10	0.35	1.92	0.09	1.46
Yr*Loc(Imp)	12	Residual	1.05×10^7	0.28	1.04×10^{12}	1.12	0.18	2.59**	0.06	1.93*
Residual	192		3.70×10^7		9.25×10^{11}		0.07		0.03	

Source	df	F-ratio vs	Chlorophyta[a] (% cover)		Barnacles[a]		P.mimula[a]		'Other' Limpets[a]	
			MS	F	MS	F	MS	F	MS	F
Year	3	Yr*Loc(Imp)	2.85×10^9	20.14**	1.21×10^{12}	0.82	1.49×10^{10}	1.30	8.89×10^8	0.43
Impact	3	Loc (Imp)	5.87×10^9	26.74**	1.42×10^{13}	1.82	6.30×10^{10}	2.08	1.69×10^{10}	6.91*
Location(Imp)	4	Residual	2.19×10^8	2.80*	7.82×10^{12}	9.24**	3.07×10^{10}	17.81**	2.45×10^9	1.41
Yr*Imp	9	Yr*Loc(Imp)	7.10×10^8	5.02**	9.99×10^{11}	0.67	1.19×10^{10}	1.04	2.73×10^9	1.32
Yr*Loc(Imp)	12	Residual	1.41×10^8	1.81*	1.48×10^{12}	1.75	1.14×10^{10}	6.61**	2.06×10^9	1.19
Residual	192		7.83×10^7		8.46×10^{11}		1.73×10^9		1.74×10^9	

Source	df	F-ratio vs	Bembicium spp.[c]		S. glomerata[c]	
			MS	F	MS	F
Year	3	Yr*Loc(Imp)	60.95	27.42**	31.13	15.83**
Impact	3	Loc (Imp)	18.09	1.27	120.35	14.78*
Location(Imp)	4	Residual	14.24	18.81**	8.14	8.91**
Yr*Imp	9	Yr*Loc(Imp)	2.45	1.10	1.99	1.01
Yr*Loc(Imp)	12	Residual	2.22	2.94**	1.97	2.15*
Residual	192		0.76		0.91	

(* $p<0.05$, ** $p<0.01$. Unless indicated otherwise, F-values are non-significant $p>0.05$).
[a] untransformed, variances heterogeneous   [b] untransformed, variances homogeneous   [c] ln(x+1) transformed, variances homogeneous

average rainfall was experienced (Figure 3b). These differences were largely attributable to the differences in barnacle numbers between locations in this category.

The differences in heterogeneity and evenness between locations within each impact category varied significantly between years as indicated by the significant Year x Location (Impact) interaction (Table VII, Figures 3c-d).

3.2.2   *Algae*

The significant Year x Impact interaction for Chlorophyta indicates that differences in green algal cover between impact categories changed significantly between years (Table VII, Figure 3e). While no significant differences in algal cover were measured between impact categories in 1996, algal cover in the Industry/Sewer category was significantly greater than all other categories in 1997. In 1998 and 1999 when above average rainfall was recorded, algal cover in the Industry/Sewer and Urban/Sewer categories was significantly greater than Bush and Urban categories but not significantly different to each other. Differences in Chlorophyta cover between locations varied significantly within each impact category between years as indicated by the significant Year x Location (Impact) interaction. No differences in algal cover were measured between locations in any impact category in 1996 however, significant differences were measured between Urban/Sewer locations in 1997 and between Industry/Sewer locations in 1998 and 1999.

3.2.3   *Fauna*

Density of barnacles only differed significantly between locations within the Industry/Sewer category (Figure 3f). Abundance of barnacles was significantly higher at Blackwattle Bay (IS) than at Hawthorne Canal (IS) in 1996 and 1999.
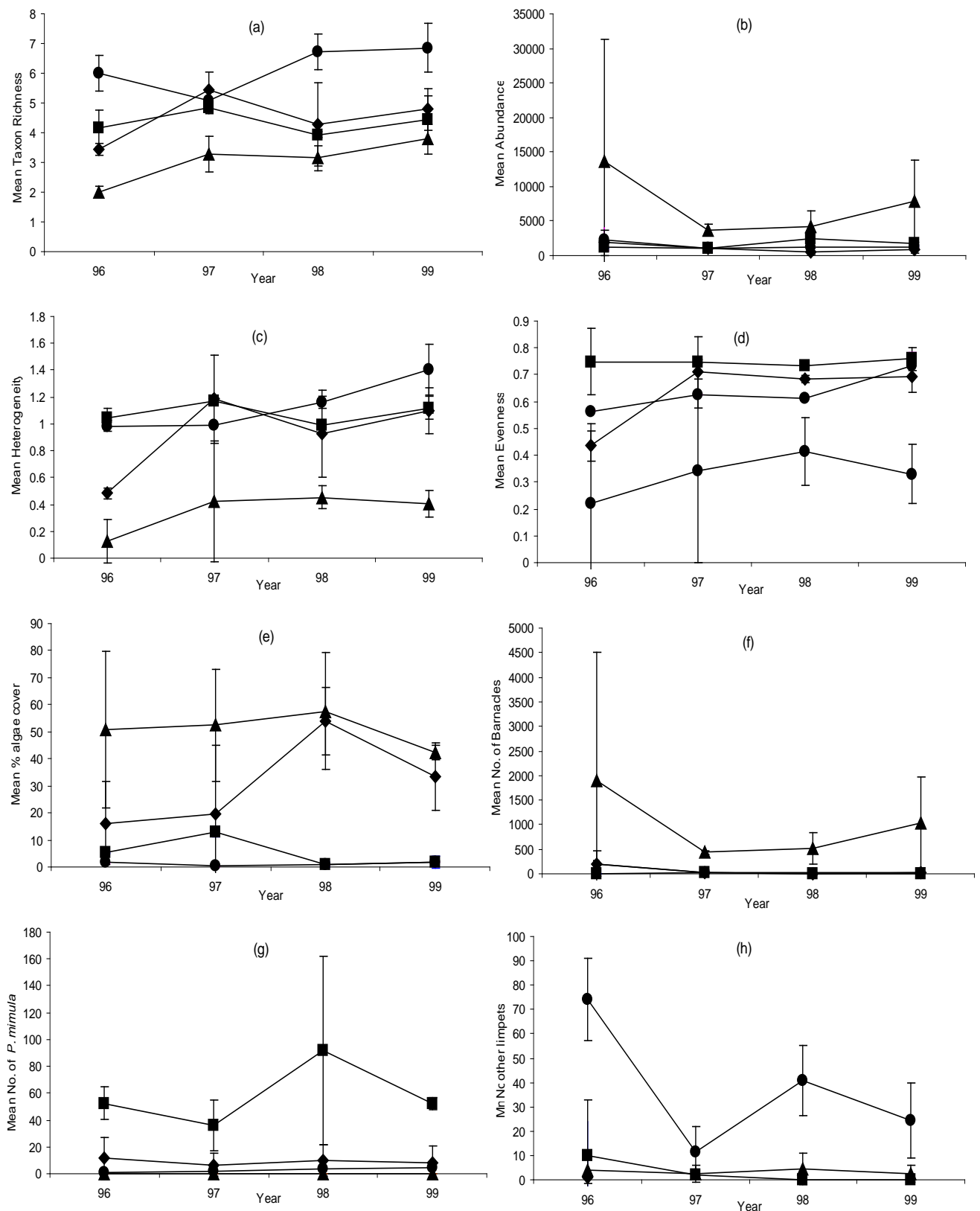
Figure 3. Means (± SE; *n*=2) of (a) taxonomic richness, (b) total abundance, (c) heterogeneity and (d) evenness at each Sydney Harbour location in each impact category for 1996 – 1999. Means (± SE; *n*=2) of abundance of (e) Chlorophyta, (f) barnacles, (g) *Patelloida mimula* and (h) 'other limpets' at each Sydney Harbour location in each impact category for 1996 - 1999. Impact categories: Industry/Sewer (▲); Urban/Sewer (♦); Urban (•); Bush (■).
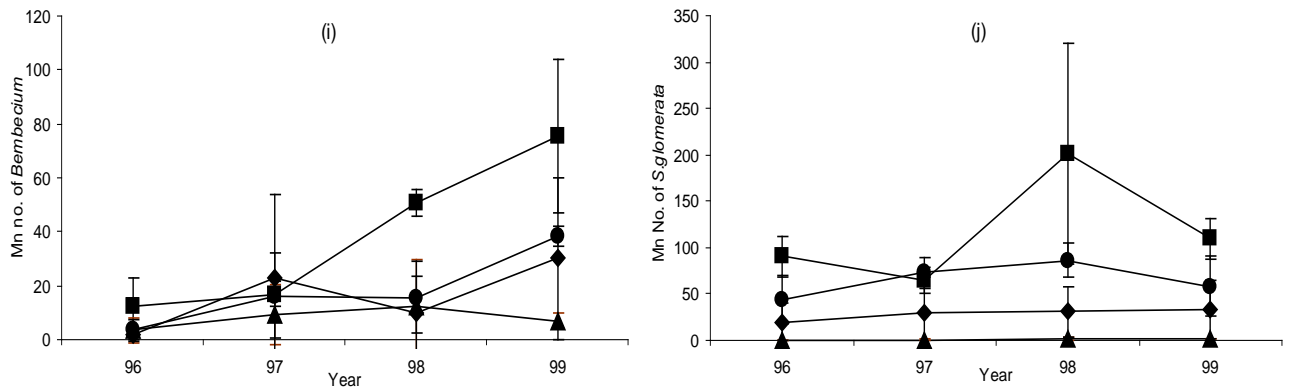
Figure 3. (continued) Means (± SE; *n*=2) of abundance of (i) *Bembecium* spp. and (j) *Saccostrea glomerata* at each Sydney Harbour location in each impact category for 1996 - 1999. Impact categories: Industry/Sewer (▲); Urban/Sewer (♦); Urban (•); Bush (■).

The significant Year x Location (Impact) interaction for density of *P. mimula* occurred because density varied significantly between the two Bush locations in 1998 and 1999 (Table VII, Figure 3g).

Density of 'other' limpets differed significantly between Urban and Industry/Sewer impact categories. Specifically, SNK tests identified significantly greater numbers of 'other' limpets within the Urban category than within the Industry/Sewer category (Figure 3h).

The significant Year x Location (Impact) interactions for *Bembicium* spp. and *S. glomerata* indicate that density of these taxa differed significantly at locations in some impact categories in some years but not all years (Table VII). SNK tests identified significant differences in density of *Bembicium* spp. between Bush locations in 1996, between locations within all impact categories in 1997, between locations in Industry/Sewer, Urban and Urban/Sewer locations in 1998, and between locations in Bush and Urban/Sewer locations in 1999 (Figure 3i). Density of *S. glomerata* varied significantly between Bush locations in 1996 and between Urban/Sewer locations in 1997, 1998 and 1999 (Figure 3j).

3.3   WATER QUALITY

Water quality variables were measured at only 6, 10 and 12 of the 19 Sydney locations for biota sampling in 1995, 1996 and 1997-1999 respectively. Available data collected prior to the commencement of the biota study indicate elevated levels (relative to ANZECC guidelines) of faecal coliforms, chlorophyll-a and/or nutrients at Industry/Sewer, Urban/Sewer and Urban impact category locations, as well as at Davidson Park, a Bush location. During the study period, median levels of these variables were generally highest at the three Industry/Sewer locations and a number of Urban/Sewer locations (Cooks River, Fig Tree Bridge, Tunks Park and Rushcutters Bay).   Concentrations at Davidson Park (B), however, were often similar in magnitude.

   When median values for chlorophyll-a, total phosphorus and total nitrogen measured during the study were compared to low-risk guideline default trigger values applicable to New South Wales' estuaries (ANZECC, 2002), trigger guidelines were exceeded by at least one variable at all locations sampled except Gunnamatta Bay (U) and Maianbar (U). Median total phosphorus was consistently high at other locations except Little Sirius Cove (U). Median total nitrogen exceeded trigger values each year at Figtree Bridge (US) and Cooks River (US). High median chlorophyll-a values only occurred in conjunction with high total phosphorus concentrations.

3.4   ENVIRONMENTAL INFLUENCES ON BIOTIC PATTERNS

Chlorophyll-a was the best single correlating environmental variable in the years 1997-1999 (Table VIII). In 1996, when water quality data were not available for two of the three Industry/Sewer locations, chlorophyll-a ($\rho = 0.418$) ranked second after median conductivity ($\rho = 0.481$). In 1995, when water quality data were not available for any of the

industry/Sewer locations, chlorophyll-a was not identified as a major contributing factor to any correlation.

TABLE VIII

Best combinations linking biotic patterns to available environmental variables.

Period	Best single variable	Best combination of variables
Oct 94 - Sep 95[a]	TUA median (**0.826**)	TUA median, FC median, Maximum Rainfall, Conductivity median, Conductivity maximum, TP median (**0.993**)
Oct 95 - Sep 96[b]	Conductivity median (**0.481**)	Chlorophyll-a mean, Conductivity median, Total Rainfall (**0.502**)
Oct 96 - Sep 97	Chlorophyll-a median (**0.758**)	Chlorophyll-a median, (**0.758**)
Oct 97 - Sep 98	Chlorophyll-a mean (**0.596**)	Chlorophyll-a mean, Conductivity mean (**0.654**)
Oct 98 - Sep 99	Chlorophyll-a mean (**0.684**)	Chlorophyll-a mean, Chlorophyll-a median, Conductivity maximum (**0.750**)

[a] Water quality data not available for Blackwattle Bay, Iron Cove, Little Sirius Cove, Rushcutters Bay.
[b] Water quality data not available for Iron Cove.
(Figures in **bold** represent Spearman rank correlation coefficients).

4. Discussion

Prior to the commencement of Sydney Water's major 20-year sewerage infrastructure remediation program (SewerFix) in 1997, Sydney's estuaries were receiving high pollutant loadings from stormwater runoff, sewer overflows and other diffuse sources during wet weather (AWT Ensight, 1997). Although a number of recent studies have identified changes in diversity or variability of assemblages attributable to various anthropogenic disturbances in Sydney estuaries (Kelaher *et al.*, 1998; Glasby, 1999; Lindegarth and Hoskin, 2001), sewage and stormwater-related impact on estuarine organisms have received little attention.

The results of the present study suggest that intertidal rock assemblages are more suitable than individual taxa to assert the difference between Bush (reference) and anthropogenically disturbed estuarine locations. Although green algal cover (predominantly *Enteromorpha* spp. and *Ulva lactuca*), for example, was highest at sewage impacted locations in all years, analysis of any individual taxa was unable to reveal any differences among impact categories.

Despite a high level of spatial and temporal variability in assemblages between locations within arbitrary impact categories, significant differences were consistently detected between the Bush category of locations, adjacent to national parks and distant from urbanisation, and those locations considered to be impacted due to their proximity to sewer overflows, and industrial and urban catchments. The differences appear to be exemplified in wet weather since Bush assemblages were significantly different to those of all other impact categories in years of above average rainfall (1998-1999). When annual rainfall was below average, Bush assemblages were not significantly different to the Urban/Sewer (in 1996-1997) or Industry/Sewer (in 1996) assemblages. Urban/Sewer assemblages and Industry/Sewer assemblages were not significantly different in any year.

Bush locations were dominated by *Bembicium auratum*, *Saccostrea glomerata* and *Patelloida mimula* with the latter two taxa contributing highly to differences between the Bush category and sewer impacted categories in all years. *P. mimula* were usually only found on oyster shells alongside newly settled *Bembicium auratum*. Furthermore, abundances of *P. mimula* and *Bembicium* spp. generally increased with numbers of oysters present. Similar associations between *S. glomerata*, and *P. mimula* and *Bembicium* spp. have previously been observed by Underwood and Chapman (1995) and Minchinton and Ross (1999), respectively.

In contrast, barnacles, mussels and green algae generally contributed most to similarity at Industry/Sewer locations each year. Since eutrophication usually results in reduced species diversity (Valiela, 1995), barnacles and mussels may thrive if gastropod predators such as whelks are not able to survive adverse conditions (Boaden and Seed, 1992). Increased recruitment and abundance of *Mytilus edulis* (one of two species of mussels recorded during this study) and *Brachidontes rostratus* have been observed at locations where sewage is present (Dakin, 1987; Hindell and Quinn, 2000). Furthermore, barnacles are rapid colonisers

and may delay succession of grazers (Sutherland and Karlson, 1977; Farrell, 1991; Mann, 2000).

Reduced grazing pressure, in addition to organic enrichment, may result in colonisation by *Ulva* and *Enteromorpha* species (Underwood, 1980; Valiela, 1995; Raffaelli and Hawkins, 1999). These opportunistic algae have been associated with locations experiencing sewage discharge (Fairweather, 1990; Bellgrove *et al*., 1997; Panayotidis *et al*., 1999) and were relatively more abundant in all years at locations impacted by sewage during this study. Furthermore, cover of green algae at Urban/Sewer locations increased markedly when above average rainfall was recorded.

Hormosira banksii was not observed at any locations in Sydney Harbour. The commencement of sewage effluent discharge has been observed to greatly diminish the abundance of *H. banksii* at discharge locations (Bellgrove *et al.* 1997). Bellgrove *et al.* (1997) suggested that absence of *H. banksii* in locations experiencing environmental pressure may be a result of competitive interaction with more opportunistic species such as *Ulva* and *Enteromorpha*. In addition, inhibited germination of *H. banksii* zygotes at locations receiving sewage effluent was demonstrated by Burridge *et al*. (1996) with greater sensitivity of the species being displayed to poorer quality discharge. The absence of *H. banksii* throughout Sydney Harbour may be associated with sporadic contaminated freshwater input or be due to competition with other species.

Warwick and Clarke's (1993) suggestion that assemblage variability increases with anthropogenic disturbance appears to be supported by these results. Although studies by Chapman *et al*. (1995) and Kelaher *et al*. (1998) found no relationship between anthropogenic disturbance and increased assemblage variability, relative dispersion values for this study showed that variability in assemblages measured at impacted locations was consistently greater than assemblages recorded at Bush locations. Variability at the

Urban/Sewer locations was greatest in all years sampled but particularly high in 1998 and 1999 when above average precipitation was experienced in the Sydney region.

Overlapping of replicates of different impact categories was commonly observed on nMDS ordinations. Similarity of assemblages across impact categories and differences between locations within each impact category confound the appropriateness of these impact categories. When the assemblages of all study locations are considered, it becomes apparent that some locations may have been incorrectly categorised. Since contaminants may be assimilated in different ways at different locations, the criteria for categorising impacted locations for future studies will therefore need careful consideration.

For example, assemblages at Davidson Park (B) were sampled approximately 500 metres upstream from a sewer overflow but adjacent to Garigal National Park. During 1995-1999, Davidson Park experienced relatively high levels of nutrients and chlorophyll-a yet exhibited assemblages characteristic of Bush locations. The contamination was probably due to tidal inflow transporting sewage upstream from the overflow. The contaminants would likely have been flushed with the next tidal cycle thus limiting exposure of biota to the discharge. The contaminants would likely have been flushed with the next tidal cycle thus limiting exposure of biota to the discharge. The lack of apparent impact at other locations across the greater Sydney region may possibly be due to the small size, steepness and rapid flushing of the catchment (Quakers Hat Bay) or the proximity to national parks (Sugarloaf Bay, Maianbar).

Water quality data indicated that highest contamination in Sydney Harbour occurred adjacent to stormwater canals and sewer overflows at Industry/Sewer locations (Blackwattle Bay, Hawthorne Canal and Iron Cove Creek) and a number of the Urban/Sewer locations (Fig Tree Bridge and to a lesser extent Rushcutters Bay and Tunks Park). Biological response to nutrient-enriched freshwater inflow is complex and it is difficult to draw causal links between inflow volumes, their characteristics and the resultant estuarine water quality (Chan

and Hamilton, 2001; Pierson *et al*., 2002). Nevertheless, analyses of the assemblages at Industry/Sewer and Urban/Sewer locations in Sydney Harbour confirmed significant differences when compared to reference locations.

Dependent on a number of factors including residence time of contaminated water and depth of estuary, point source effluent discharges have been shown to cause increased primary production of phytoplankton, measured as chlorophyll-a concentration (Day *et al*., 1989; Hargrave, 1991; Valiela, 1991; Kennish, 1992; Mann, 2000; Pierson *et al*., 2002). This study appears to confirm that variation in chlorophyll-a is associated with variation in assemblages. Chlorophyll-a was the best single environmental variable correlating with intertidal assemblage patterns in 1997-1999. Furthermore, it is likely that the inclusion of 1995-1996 water quality data for Industry/Sewer locations would have resulted in chlorophyll-a featuring more prominently in the best combination of correlating variables in those years.

As nutrient loading increases, opportunistic pioneer flora overcome control by grazers and occupy much of the available space (Valiela, 1991; 1995). Since secondary production is ultimately dependent on primary production, sessile suspension feeders, such as barnacles in this study, flourish on decomposed phytoplankton and detrital organic matter associated with the sewage input (Boaden and Seed, 1992; Mann, 2000). Modifications to the algal community due to organic enrichment may also alter the food source for oysters and result in a decline in oyster abundance (Day *et al*., 1989).

5. Summary

Underwood (1991) suggests that an impact on biota should be deemed to have occurred when there is a significant departure in any direction from appropriate controls. It is impractical and unnecessary (and clearly difficult) to find reference locations which display identical

characteristics. However, reference locations should be of the same general habitat and should be compared with locations in other estuaries along the coast (Underwood, 1996). Despite individual differences, the Bush locations studied in the Sydney region are relatively free of urbanisation and sewage contamination, and are arguably "the healthiest assemblage(s) that we can expect in this place(s)" (Fairweather, 1999).

In this study, significant spatial and temporal variation in assemblages was measured between locations within most impact categories in every year and between impact categories in most years. Nevertheless, variability within the Bush category assemblages was below that of the other impact categories in all years.

Although ecological responses to water quality changes within estuarine systems are difficult to predict, intertidal assemblages in Sydney Harbour and surrounding estuaries appear to be responding to the quality and quantity of discharge from urbanised catchments, particularly in years of above average rainfall. While measurement of estuarine intertidal rock assemblages appears to satisfy many of the basic criteria that an indicator should meet (Ward *et al.,* 1998; Deeley and Paling, 1999) and provides a quantitative estimate of the environmental conditions, refinement of the impact categorisation scheme should be undertaken before future studies are conducted on a wider scale. Assuming that intertidal rock assemblages recover if a disturbance is removed, the impact of improving sewerage and stormwater infrastructure should be measurable.

Acknowledgements

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