Patterns of Spatial Variation in Assemblages of Estuarine Organisms in Brisbane Water Estuary and their Relationship to Environmental Variation



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## **EXECUTIVE SUMMARY**

The objective of this project was to undertake statistical analysis of data collected as part of the Brisbane Water Estuary Process Study. Although detailed analyses had been conducted for all reports additional analyses were requested by Gosford City Council, focussing on testing for relationships between spatial variability in assemblages and spatial variation in measured environmental and habitat features. The latter test was requested to be done by canonical correspondence analysis (CCA).

#### Zooplankton

Zooplankton was sampled in 2 habitats (adjacent to mangrove, middle of bay), in 2 randomly selected sites within each habitat, on 2 phases of the tide (ebb, flood), and over 3 days ( $1^{st}$  day of spring tide,  $2^{nd}$  day of spring tide,  $3^{rd}$  day of spring tide).

Density of crab zoeae was significantly greater on the ebb tide on the  $2^{nd}$  and  $3^{rd}$  days of the spring tide in the two habitats. There was no difference between tidal cycles on the  $1^{st}$  day of the spring tide in both habitats.

The assemblages of planktonic organisms changed over the three days. On the 1<sup>st</sup> day of the spring tide there was no clear separation of assemblages between habitats and tidal stage. On the 2<sup>nd</sup> day of the spring tide there was a clear separation of samples into groups corresponding to the two habitats and the two stages of the tide, suggesting that the zooplankton assemblages were distinctive at each combination of habitat and tide. On the 3<sup>rd</sup> day of the spring tide there were distinct assemblages in each habitat on the flood tide, but not on the ebb tide. Tests of the significance of these apparent differences found that assemblages differed between bay and mangrove habitats on the flood tide on the 2<sup>nd</sup> and 3<sup>rd</sup> days of the spring tide and on the ebb tide on the 2<sup>nd</sup> day of the spring tide.

#### Birds

Birds were sampled in 4 habitats (saltmarsh, mangrove, mudflat adjacent to mangrove, mudflat) and 2 conditions of each habitat were sampled (disturbed, less disturbed). Three locations were sampled in each combination of habitat x condition, and 2 sites were sampled in each location (except less disturbed mudflat where only 1 site was sampled in each location).

Bird assemblages within Brisbane Water estuary were not consistently structured by the level of disturbance or habitat type. Assemblages did not differ significantly between disturbance levels and no significant difference in the bird assemblages of saltmarsh and mangrove or mangrove mudflat and mudflat (all other comparisons of habitats were significantly different).

#### Fishes of Zostera capricorni seagrass beds

Fishes occurring in *Zostera capricorni* seagrass beds were sampled in 6 areas, 2 locations in each area, and 2 sites in each location. In each site the following

characteristics of the seagrass bed were also determined: seagrass shoot density, seagrass leaf length, % cover seagrass, and % cover epiphytes.

Assemblages of fishes occurring within *Zostera capricorni* seagrass beds were not structured by the position of seagrass beds within the estuary. Assemblages from adjacent locations ( $\sim 1 \text{ km}$  apart) or from adjacent sites ( $\sim 500 \text{ m}$  apart) were not more similar to one another than to assemblages from other locations or sites.

Canonical correspondence analysis (CCA) found that spatial variation in fish assemblages was significantly associated with spatial variation in 2 features of seagrass beds: average % cover and average length of *Z. capricorni* leaves. These 2 features together explained 21% of the total spatial variation in the fish assemblages. Therefore 79% of the spatial variation in fish assemblages is not explained by the features of seagrass tested.

The CCA revealed distinct assemblages of fishes associated with combinations of features of seagrass beds. For example, the species assemblage occurring in seagrass with low % cover and intermediate length of seagrass leaves includes *Achoerodus viridis* (blue groper), *Hippocampus whitei* (White's seahorse), *Meuschenia freycineti* (six-spine leatherjacket), and *Parupeneus signatus* (black-spot goatfish).

#### Settlement and juvenile stage fishes

Eight sites were sampled throughout Brisbane Water estuary in August, September, November, and December 2005. Ordinations of species from sampling sites and permutational multivariate analysis of variance provided no evidence that assemblages were spatially or temporally structured in a consistent manner. There was no consistent trend for sites close to one another, or sites sampled in the same month, to have similar assemblages.

#### Macrobenthic organisms of mangroves

Fifteen locations representing mangrove (*Avicennia marina*) habitat throughout Brisbane Water estuary were sampled for macrobenthic organisms and for the features of the mangrove forest. CCA found that the assemblages of macrobenthic organisms occurring in *A. marina* mangrove forests showed considerable spatial variation in assemblage composition. However, despite the existence of significant spatial variation in habitat features none of the measured mangrove habitat features explained a significant amount of the spatial variation in macrobenthic assemblage structure.

#### Foreshore plant species

Foreshore plant species were surveyed (for species presence and relative abundance) at 145 sites that covered the entire foreshore of Brisbane Water estuary. A qualitative assessment of the condition of each site was also undertaken using a Disturbance Index.

Multivariate analyses showed considerable spatial variation in foreshore plant species assemblages. Disturbance Index explained a significant but very small amount (1.4%) of the total variation in foreshore plant species.

Saltmarsh plant species

Species of saltmarsh plants were sampled in meadows from 4 areas representing different tidal flushing regimes, from 3 locations were sampled within each area, and 2 sites within each location. Low and high saltmarsh were sampled separately. Analyses were undertaken to test whether assemblages of saltmarsh plants exhibited spatial variation within the estuary and differed between disturbed and undisturbed meadows.

Multivariate analyses showed considerable variation in species assemblages throughout Brisbane Water estuary in both high and low saltmarsh. Sites in some locations were very similar to one another while sites in other locations were very dissimilar. Saltmarsh species assemblages were not structured by tidal flushing.

There was no consistent difference in the species assemblages of disturbed and undisturbed saltmarsh meadows.

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# **INTRODUCTION: PROJECT OBJECTIVES**

The objective of this project was to undertake statistical analysis of data collected as part of the Brisbane Water Estuary Process Study. Although detailed analyses had been conducted for all reports additional analyses were requested by Gosford City Council, focussing on spatial variability in assemblages and where possible relating this to spatial variation in measured environmental features. The latter test was requested to be done by canonical correspondence analysis (CCA). At the outset of this consultancy it was agreed that this report would provide statistical analysis for the following groups:

Bird assemblages

- spatial patterns in assemblage structure throughout Brisbane Water
- the influence of habitat (saltmarsh, mangrove, mangrove-mudflat, mudflat, water body-mudflat) and habitat condition (disturbed, undisturbed) on assemblage structure

Foreshore vegetation

- spatial patterns in assemblage structure throughout Brisbane Water
- the influence of disturbance (disturbance index 1-5)

Larval fishes

• spatial variation in assemblages of settlement stage and juvenile stage fishes across 7 locations in each of four months

Fishes

- spatial patterns in assemblage structure throughout Brisbane Water from 6 areas, 2 locations within each area, and 2 sites within each location
- the influence of seagrass bed structure (density, length, seagrass cover, epiphyte cover) on spatial patterns in assemblage structure

Mangrove macroinvertebrates

- spatial patterns in assemblage structure throughout Brisbane Water
- the influence of mangrove forest structure (number of trees, canopy height, Specht cover, number of pneumatophores, number of seedlings, number of crab holes) on spatial patterns in assemblage structure

Saltmarsh plants

- spatial patterns in assemblages of saltmarsh plants (high and low shore)
- the influence of disturbance (disturbed, undisturbed) on assemblage structure

Outputs

- ordination plots depicting spatial patterns in assemblage structure throughout Brisbane Water estuary for each data set
- table of results of partial canonical correlation analysis (where applicable) testing for a correlation between the spatial patterns in assemblage structure and the measured environmental variables
- description and interpretation of (1) and (2)

Subsequently, additional analysis of data on variation in zooplankton assemblages was requested. This report provides the results of these analyses. The report provides the results of the analyses but does not attempt to discuss the results in the context of the current understanding and literature relating to each group of organisms. Therefore this report should be read in conjunction with the original reports (references provided herein) from which the data were gathered.

## **ZOOPLANKTON ASSEMBLAGES**

### Methods

### Sampling

Zooplankton was sampled in 2 habitats (adjacent to mangrove, middle of bay), in 2 randomly selected sites within each habitat, on 2 phases of the tide (ebb, flood), and over 3 days (1<sup>st</sup> day of the spring tide, 2<sup>nd</sup> day of the spring tide, 3<sup>rd</sup> day of the spring tide). Sampling occurred on 26-28 February 2006.

#### Analysis

The null hypothesis that the density of crab zoeae did not differ between days of the spring tide, stages of the tidal cycle, and habitat was tested by four-factor analysis of variance (ANOVA). Day was tested as a fixed orthogonal factor with 3 levels ( $1^{st}$  day of the spring tide,  $2^{nd}$  day of the spring tide,  $3^{rd}$  day of the spring tide). Stage of the tidal cycle was tested as a fixed orthogonal factor with 2 levels (flood tide, ebb tide). Habitat was tested as a fixed orthogonal factor with 2 levels (adjacent to mangroves, middle of bay). A fourth factor, site, was included to test for spatial consistency within each habitat. Two sites (approximately 200 m apart) were sampled within each habitat. The factor site was tested as a random factor nested in the interaction of day x stage of tidal cycle x habitat. This nesting was used because although the position of the site did not change the water body being sampled differed on each sampling occasion.

The data analysed was the mean of the 5 sub-samples taken from each replicate sample (n=3). Prior to analysis data were tested for homogeneity of variances with Cochran's test (Underwood 1981). Significant main effects and interactions were examined post-hoc with SNK test. The analysis was undertaken with GMAV software (Institute of Marine Ecology, University of Sydney).

Assemblages of zooplankton were visualized by non-metric multidimensional scaling (nMDS) ordinations using Primer 6 software (PRIMER-E Ltd). Separate nMDS ordinations were constructed for each day of sampling (day before spring tide, day of spring tide, day after spring tide). Ordinations were based on a Bray-Curtis dissimilarity matrix of square-root transformed data. The raw data used was the mean of the 5 sub-samples taken from each replicate sample (n=3).

Four-factor permutational multivariate analysis of variance was used to test the null hypothesis of no difference in zooplankton assemblages between days, tidal cycle, habitat and site, using the program PERMANOVA (Anderson 2001). Data were square-root transformed prior to analysis and Bray-Curtis dissimilarity was used as the distance measure. Unrestricted permutation of raw data was used (4999 permutations) to determine *P*-values. Significant effects were examined post hoc with

*t*-test and Monte Carlo estimates of *P*-values were used because of the low number of permutations possible.

The similarity percentages routine (SIMPER) in Primer 6 was used to determine the groups of organisms that characterized the zooplankton assemblage of each habitat, and that differentiated the zooplankton assemblages between habitats, on each tidal cycle. Data were square-root transformed prior to analysis and data from the 2 sites in each habitat was pooled for the analysis.

### Results

#### Density of crab zoeae

Density of crab zoeae was significantly greater on the ebb tide on the  $2^{nd}$  and  $3^{rd}$  days of the spring tide in both habitats. However, there was no difference between tidal cycles on the  $1^{st}$  day of the spring tide in both habitats (Figure 1). This is the basis for the significant Day x Tide interaction in Table 1. There was significant variation between Sites in density of crab zoeae, but only on the  $2^{nd}$  day of the spring tide on the ebb tide in the bay habitat, and on the  $3^{rd}$  day of the spring tide on the ebb tide in the range of the spring tide on the ebb tide in the sprine tide.

#### Assemblages of zooplanktonic organisms

The nMDS ordination plots for each day (Figure 2) show a clear shift in the assemblages of zooplanktonic organisms over the three days. On the 1<sup>st</sup> day of the spring tide there was no clear separation of samples between habitats and tidal stage. Samples from the bay habitat on each stage of the tide were clustered together in the centre of the ordination (shown by the clustering of samples in the middle of the ordination plot). Assemblages from the mangrove habitat differed between sites and between stages of the tidal cycle, as shown by the separation of one site of the ebb tide mangrove samples on the right of the ordination and the two sites from the flood sampling on the left of the ordination. On the 2<sup>nd</sup> day of the spring tide there was a clear separation of samples into groups corresponding to the two habitats and the two stages of the tide, suggesting that the zooplankton assemblages were distinctive at each combination of habitat and tide. There appeared to be little difference between sites within each habitat, as shown by the proximity of the replicates from each site within each combination on habitat and tide. On the 3<sup>rd</sup> day of the spring tide there were distinct assemblages in each habitat on the flood tide, but not on the ebb tide. This is shown by the clear separation of the mangrove (top left of ordination) and bay habitats (bottom centre of ordination) on the flood tide, and there overlap on the ebb tide (the cluster of samples in the top right of the ordination).

Assemblages of zooplankton were affected by a significant Day x Tide x Habitat interaction and by significant variation between Sites (Table 2). The significant interaction occurred because assemblages of zooplankton differed between bay and mangrove habitats on the flood tide on the  $2^{nd}$  and  $3^{rd}$  days of the spring tide and on

the ebb tide on the  $2^{nd}$  day of the spring tide. Although there was a significant effect of Site, the only significant difference in zooplankton assemblages between Sites occurred on the  $1^{st}$  day of the spring tide, on the flood tide, in the mangrove habitat.

A consistent suite of organisms differentiated the zooplankton assemblages of mangroves and bay on the flood tide of each tidal cycle: fish eggs, crab zoeae, and copepods (Table 3). On the  $1^{st}$  and  $2^{nd}$  days of the spring tide fish eggs were more abundant in the mangrove habitat and crab zoeae and copepods were more abundant in the bay. On the  $2^{nd}$  day of the spring tide all groups were more abundant in the mangrove habitat.

Different groups of organisms differentiated the zooplankton assemblages of mangroves and bay on the ebb tide over the 3 days of sampling (Table 3). On the 1<sup>st</sup> day of the spring tide crab zoeae, copepods and fish eggs were more abundant in the bay. On the 2<sup>nd</sup> day of the spring tide the assemblages of the 2 habitats differed because fish eggs, obelia, and crab zoeae were more abundant in the mangroves. On the 3<sup>rd</sup> day of the spring tide the assemblages differed because gastopods and crab zoeae were more abundant in the mangroves and polychaetes were more abundant in the bay.



Figure 1. Changes in density of crab zoeae over three days  $(1^{st} day of the spring tide, 2^{nd} day of the spring tide, 3^{rd} day of the spring tide) in two habitats (mangrove, bay) at two stages of the tidal cycle (ebb, flood). Values shown are the mean + standard error of two sites in each habitat (n=3 replicate samples per site).$ 

Source of	DF	MS	F	Р
variation				
Day (Da)	2	26027.66	11.56	0.0016
Tide (Ti)	1	49196.54	21.85	0.0005
Habitat (Ha)	1	79.4142	0.04	0.8542
Site(DaXTiXHa)	12	2251.945	10.26	< 0.001
DaXTi	2	12279.59	5.45	0.0207
DaXHa	2	653.7564	0.29	0.7531
TiXHa	1	1918.182	0.85	0.3742
DaXTiXHa	2	3140.616	1.39	0.2854
Residual	48	219.4722		

Table 1. Summary of results of analysis of variance testing for differences in density of crab zoeae (untransformed data, Cochran's *C*=0.20, *P*>0.05).

Source of	DF	MS	F	P
variation				
Day (Da)	2	9935.82	13.81	0.0002
Tide (Ti)	1	13976.80	19.43	0.0002
Habitat (Ha)	1	15762.63	21.91	0.0002
Site(DaXTiXHa)	12	719.50	1.78	0.003
DaXTi	2	5068.42	7.04	0.0002
DaXHa	2	3269.39	4.54	0.001
TiXHa	1	5042.60	7.01	0.001
DaXTiXHa	2	3728.60	5.18	0.0004
Residual	48			

Table 2. Summary of results of four-factor permutational multivariate analysis of variance testing for differences in assemblages of zooplankton in Cockle Bay.

Post-hoc test of differences in assemblages between habitats in each combination of tide and day.

Tide	Day	Comparison of mangrove and bay habitats		
		t	Р	
Flood	1 <sup>st</sup> day of spring tide	2.86	0.06	
Flood	2 <sup>nd</sup> day of spring tide	3.57	0.03	
Flood	3 <sup>rd</sup> day of spring tide	5.10	0.02	
Ebb	1 <sup>st</sup> day of spring tide	2.10	0.12	
Ebb	2 <sup>nd</sup> day of spring tide	2.94	0.04	
Ebb	3 <sup>rd</sup> day of spring tide	1.45	0.23	



### Second day of spring tide



Figure 2. Non-metric multidimensional scaling ordination plots depicting variation in assemblages of zooplankton in Cockle Bay on three days  $(1^{st} day of the spring tide, 2^{nd} day of the spring tide, 3^{rd} day of the spring tide) in two habitats (mangrove M, bay B), at two stage of the tide (flood F, ebb E), in two sites within each habitat (shown by FM1, FM2 etc.). Three samples were analysed within each combination of habitat, stage of the tide, and site.$ 



Figure 2 cont'd. Non-metric multidimensional scaling ordination plots depicting variation in assemblages of zooplankton in Cockle Bay on three days (1<sup>st</sup> day of the spring tide, 2<sup>nd</sup> day of the spring tide, 3<sup>rd</sup> day of the spring tide) in two habitats (mangrove M, bay B), at two stage of the tide (flood F, ebb E), in two sites within each habitat (shown by FM1, FM2 etc.). Three samples were analysed within each combination of habitat, stage of the tide, and site.

Table 3. Summary of results of SIMPER analysis showing zooplankton groups characterizing and differentiating mangroves and bay habitats on each tidal cycle. Groups are arranged in order of importance (to a maximum of 3 groups).

1 <sup>st</sup> day of spring tide		Flood		Ebb	
		Mangrove	Bay	Mangrove	Bay
	Mangrove	Fish eggs	Fish eggs		
			Crab zoea		
Flood			Copepods		
	Bay		Fish eggs		
			Crab zoea		
			Copepods		
	Mangrove			Fish eggs	Crab zoea
				Crab zoea	Copepods
Ebb					Fish eggs
	Bay				Crab zoea
					Fish eggs
					Copepods

2 <sup>nd</sup> day of spring tide		Flood		Eł	ob
		Mangrove	Bay	Mangrove	Bay
	Mangrove	Fish eggs	Fish eggs		
		Copepods	Copepods		
Flood		Crab zoea	Crab zoea		
	Bay		Copepods		
			Crab zoea		
			Nauplii		
	Mangrove			Fish eggs	Fish eggs
				Crab zoea	Obelia
Ebb				Copepods	Crab zoea
	Bay				Crab zoea
					Copepods
					Fish eggs

3 <sup>rd</sup> day of spring tide		Flood		E	bb
		Mangrove	Bay	Mangrove	Bay
	Mangrove	Fish eggs	Fish eggs		
		Copepods	Copepods		
Flood		Crab zoea	Crab zoea		
	Bay		Copepods		
			Crab zoea		
			Fish eggs		
	Mangrove			Crab zoea	Gastropods
				Gastropods	Crab zoea
Ebb				Copepods	Polychaetes
	Bay				Crab zoea
					Copepods
					Gastropods

## **BIRDS OF BRISBANE WATER ESTUARY**

The results of bird surveys of Brisbane Water estuary are reported in Robinson (2006). Data analysed here were originally reported therein, although multivariate analysis of the results of the bird surveys was not reported in Robinson (2006).

## Methods

### Sampling

Birds were sampled in 4 habitats (saltmarsh, mangrove, mudflat adjacent to mangrove, mudflat) and 2 conditions of each habitat were sampled (disturbed, less disturbed). Three locations were sampled in each combination of habitat x condition, and 2 sites were sampled in each location (except less disturbed mudflat where only 1 site was sampled in each location).

### Analysis

Species assemblages of birds were ordinated by two multivariate techniques: detrended correspondence analysis (DCA) and non-metric multidimensional scaling (nMDS). DCA is an appropriate method when the objective is to examine species variation along an environmental gradient whereas nMDS is appropriate when the objective is to depict variation in species composition between sampling locations (De'Ath 1999). Both approaches are warranted given the uncertainty about the existence of distinct environmental gradients within Brisbane Water estuary (see for example Gladstone 2006).

The null hypothesis of no difference in bird assemblages between disturbance level (disturbed, less disturbed) and habitat (saltmarsh, mangroves, mangrove mudflats, mudflat) was first tested by visualizing the variations in assemblage structure. A Detrended Correspondence Analysis (DCA) was done in Canoco 4.5 (ter Braak and Smilauer, 2002) to display the similarity between locations in their assemblages of bird species. A total of 124 replicate samples were collected, therefore to improve the visual clarity of the DCA ordination plot the data used was the average abundance of each species in 3 locations in each combination of disturbance level/habitat (calculated from the average of 2 site-average abundances per location). Data was untransformed (because of the generally low abundances of all species) and the option to downweight rare species was not selected. An nMDS ordination plot was constructed from a Bray-Curtis similarity matrix based on untransformed data using Primer 6 software (PRIMER-E Ltd).

The null hypothesis was also tested by two-factor permutational multivariate analysis of variance using the program PERMANOVA (Anderson 2001). Data were not transformed prior to analysis and Bray-Curtis dissimilarity was used as the distance measure. Unrestricted permutation of raw data was used (4999 permutations) to

determine *P*-values. Significant main effects were examined post hoc with *t*-test and Monte Carlo estimates of *P*-values were used because of the low number of permutations possible. The 2 factors tested were disturbance level (fixed, orthogonal, 2 levels) and habitat (fixed, orthogonal, 4 levels). The raw data used for the analysis was the average abundance of each species in each location (i.e. n=3 per combination of habitat and disturbance level). Average abundance per location was calculated from the average of the 2 site-average abundances (2 sites were sampled in each location), except for undisturbed mudflat where only 1 site was sampled in each location.

## Results

The nMDS ordination plot (Figure 3) suggests no clear separation of assemblages between the 2 disturbance levels, because of the large degree of overlap between sample points. The 3 locations representing undisturbed mangrove mudflat (MMU1-3) overlap with the 3 locations representing disturbed mangrove mudflat (MMD1-3). The 3 sites representing undisturbed mudflat (MuU1-3) overlap with the 3 locations representing disturbed mudflat (MuU1-3) overlap with the 3 locations representing disturbed mudflat (MuD1-3). The 3 locations representing disturbed mangroves (MD1-3) are distinct from the 3 locations representing undisturbed mangroves (MU1-3). The 3 locations representing undisturbed saltmarsh (SU1-3) are distinct from the 3 locations representing disturbed saltmarsh (SU1-3).

The nMDS ordination plot also suggests no clear separation of assemblages among the different habitats. Assemblages of saltmarsh (beginning with S) and mangroves (beginning with M) are separated from the other habitats in the right corner of the nMDS ordination plot. However, there is overlap in the assemblages of undisturbed saltmarsh (SU1-3) and disturbed mangrove (MD1-3). Assemblages from mangrove mudflat (beginning with MM) and disturbed mudflat (beginning with MuD) overlap in the middle of the ordination plot.

The first 2 axes of the DCA ordination plot (Figure 4) ( $\lambda_1$ =0.77,  $\lambda_2$ =0.33) accounted for 21.5% of the total spatial variation among locations (sum of all eigenvalues=4.96). The third axis explained only a further 5% of variation and was therefore not explored. The DCA was a mirror image of the nMDS ordination plot and therefore supports the results above.

The results of the PERMANOVA supported the interpretation of the ordination plots (Table 2). Assemblages did not differ significantly between disturbance levels but did differ significantly between habitats. Post-hoc pairwise comparisons of habitats found assemblages differed significantly between all habitats except saltmarsh and mangrove and between mangrove mudflat and mudflat.



Figure 3. Non-metric multidimensional scaling ordination plot of assemblages of birds from Brisbane Water estuary in less disturbed and disturbed saltmarsh (SU and SD respectively), less disturbed and disturbed mangroves (MU and MD respectively), less disturbed mudflats adjacent to mangroves (MMU and MMD respectively), and less disturbed and disturbed mudflats (MuU and MuD respectively). Sample points represent 3 locations in each combination of habitat x disturbance (except MuU which represent 3 sites in a single location).

Table 4. Summary of results of permutational multivariate analysis of variance (PERNAMOVA) in bird assemblages of 4 habitats (saltmarsh, mangroves, mangrove mudflats, mudflats) and 2 disturbance levels (disturbed, less disturbed).

Source of variation	DF	MS	F	Р		
Habitat Ha	3	9125.65	3.3	7 0.0002		
Disturbance Di	1	2637.82	0.9	7 0.46		
Ha x Di	3	3002.68	1.1	1 0.30		
Residual	16	2711.18				
Pairwise comparisons of habitats $t$ P						
Saltmarsh, mangrove 1.38 0.09						
Saltmarsh, mangrov	e muc	lflat	1.96	0.002		
Saltmarsh, mudflat 2.19 0.002						
Mangrove, mangrove mudflat 1.69 0.01						
Mangrove, mudflat		2.11	0.003			
Mangrove mudflat,	mudfl	lat	1.43	0.08		



Figure 4. Detrended Correspondence Analysis (DCA) ordination plot of locations throughout Brisbane Water estuary showing similarity in their assemblages of bird species. Ordination based on untransformed average abundance of each species at each location. See Figure 3 for an explanation of symbols.

## SPATIAL VARIATION IN ASSEMBLAGES OF FISHES IN ZOSTERA CAPRICORNI SEAGRASS IN BRISBANE WATER ESTUARY AND ITS RELATIONSHIP TO VARIATION IN SEAGRASS CHARACTERISTICS

Data used in the following analyses were originally reported in Boyland (2006) but without any multivariate analyses.

### Methods

#### Sampling

Fishes occurring in *Zostera capricorni* seagrass beds were sampled throughout Brisbane Water estuary in 6 areas, 2 locations in each area, and 2 sites in each location (Figure 5). Fish sampling occurred in July-October 2005 and January-April 2006. In each site the following characteristics of the seagrass bed were also determined: seagrass shoot density, seagrass leaf length, % cover seagrass, and % cover epiphytes. Seagrass sampling occurred in May-June 2004 and February-April 2006.

### Analysis

Spatial patterns in similarity of assemblages of fishes from each sampling location were ordinated by nMDS and DCA for each sampling occasion. Sample data depicted on ordination plots was the average abundance of each species in each site on each sampling occasion (to improve the visual presentation). Prior to analysis data were square-root transformed to reduce the influence of highly abundant species. The nMDS ordination was based on a Bray-Curtis similarity matrix. The option to downweight rare species was selected for the DCA.

The null hypothesis that fish assemblages were unaffected by time (random, orthogonal, 2 levels: time 1, time 2), area (fixed, orthogonal, 6 levels), location (random, nested in area, 2 levels), and site (random, nested in time x area x location, 2 levels) was tested by permutational multivariate analysis of variance using the program PERMANOVA (Anderson 2001). Data were ln(x+1) transformed prior to analysis (to reduce the influence of some very abundant species) and Bray-Curtis dissimilarity was used as the distance measure. Unrestricted permutation of raw data was used (4999 permutations) to determine *P*-values. Significant interactions and main effects were examined post hoc with *t*-test and Monte Carlo estimates of *P*-values were used because of the low number of permutations possible.

Canonical correspondence analysis (CCA) was used to determine the features (if any) of *Z. capricorni* seagrass that explained spatial variation in fish assemblages. CCA was not undertaken for the July-October 2005 fish sampling because seagrass data

had been collected more than 12 months prior in May-June 2004. All analyses were done at the site-level using site-average abundances for each species of fish (based on n=3 replicate samples per site) and site-average values for each of the seagrass features. Seagrass data were tested for normality and, where necessary, transformed prior to analysis. Seagrass data were standardized prior to CCA (by subtracting the mean from each data point then dividing by the standard deviation) because the different seagrass features were quantified in different units.

CCA analysis was done with Canoco 4.5 (ter Braak and Smilauer, 2002). The mean density data for fish species were square-root transformed prior to analysis and the importance of rare species was down-weighted (ter Braak and Smilauer, 2002). A manual forward selection process in Canoco was used to select the subset of seagrass features that best explained the spatial patterns in fish assemblage structure. Seagrass features were ranked according to the proportion of total variance in the species data set they explained. The highest ranking seagrass feature was selected and the remaining features re-ordered according to the proportion of total variance they explained in conjunction with the variable already selected. The statistical significance of the variance explained by each of the seagrass features was tested by a Monte Carlo test (999 permutations) and variables that were significant at P < 0.05 were added to the model.

#### Results

#### Spatial variation in fish assemblages

Assemblages of fishes were not consistently structured according to the location of *Z. capricorni* beds in the estuary: there was no clear separation of assemblages according to the area sampled in the nMDS ordination in Figure 6. In time 1 assemblages from area D were similar to assemblages from area B (based on their proximity near the centre of the nMDS ordination plot) and assemblages from areas A, C, E and F were similar (based on the proximity sample points in the centre of the nMDS ordination plot). In time 2 assemblages from areas D, E and F were similar (based on the proximity of sample points in the centre of the ordination plot) and assemblages from areas C and F were similar (based on the proximity of sample points in the centre of sample points at the top of the ordination plot).

Assemblages from adjacent locations within an area (separated by  $\sim 1$  km) or from adjacent sites within a location (separated by  $\sim 500$  m) were also not more similar to one another than to assemblages from other locations or sites (based on the relative positions of sample points in the nMDS ordinations in Figure 6). In time 1 the assemblage at site B21 appears to be more similar to the assemblages at all sites in area D than to other sites in area B. In time 2 assemblages at sites D12, E22 and F12 appear to be more similar to one another than to assemblages from other sites within the same location and area.

DCA (Figure 7) supports the nMDS analysis and further indicates the absence of a clear gradient in fish assemblages in Brisbane Water estuary. The first 2 axes of the DCA ordination plot for time 1 sampling ( $\lambda_1$ =0.20,  $\lambda_2$ =0.12) accounted for 34.7% of

the total spatial variation among locations (sum of all eigenvalues=0.93). The third axis explained only an additional 5.9% of variation and was therefore not explored. The first 2 axes of the DCA ordination plot for time 2 sampling ( $\lambda_1$ =0.19,  $\lambda_2$ =0.12) accounted for 31.4% of the total spatial variation among locations (sum of all eigenvalues=0.96). The third axis explained only an additional 5.1% of variation.

Results of the PERMANOVA test (Table 3) indicate assemblages of fishes differed between sites (time x area x location), between locations (area), and between times. Post-hoc *t*-tests revealed that assemblages varied between sites in some locations and times, but not all locations and both times. Although the PERMANOVA test indicated significant variation in assemblages between locations (area), post-hoc *t*-tests did not reveal any significant variation.

#### Relationship between seagrass features and fish assemblages

Spatial variation in fish assemblages was significantly associated with spatial variation in 2 features of seagrass beds: average % cover and average length of *Z. capricorni* leaves (Figure 8, Table 4). The first 2 axes of the partial CCA ordination plot (Figure 8) together explained 100% of the species-environment relationship and 21% of the total spatial variation in the fish assemblages. Therefore 79% of the spatial variation in fish assemblages is not explained by the features of seagrass tested. The first CCA axis (the horizontal axis) represents from left to right a gradient of decreasing % cover seagrass. The second CCA axis (the vertical axis) represents from bottom to top of the ordination a gradient of increasing leaf length.

The CCA ordination plot (Figure 8) reveals particular associations between fish species and features of the seagrass bed. The species assemblage occurring in seagrass with low % cover and intermediate length of seagrass leaves (in the lower left quadrant of the CCA ordination plot) includes *Achoerodus viridis* (blue groper), *Hippocampus whitei* (White's seahorse), *Meuschenia freycineti* (six-spine leatherjacket), and *Parupeneus signatus* (black-spot goatfish). The species assemblage occurring in seagrass beds with low % cover and long leaf length includes *Enoplosus armatus* (old wife) and *Myxus elongatus* (sand mullet). The species assemblage occurring in seagrass of intermediate % cover and small leaf length includes *Arenigobius frenatus* (half-bridled goby), *Centropogon australis* (fortescue), and *Rhabdosargus sarba* (tarwhine).



Figure 5. Position of sampling sites for fishes in *Zostera capricorni* seagrass beds in Brisbane Water estuary. Two locations (designated A1, A2 etc) were sampled within each area and 2 sites (designated A11, A12) were sampled in each location. Source: Boyland (2006).



Time 2



Figure 6. nMDS ordination plots depicting patterns of similarity in assemblages of fishes from sites in *Zostera capricorni* seagrass beds in Brisbane Water estuary (site numbering and position of sites are shown in Figure 5).



Figure 7. Detrended Correspondence Analysis (DCA) ordination plot showing similarity in species of fishes from *Zostera capricorni* seagrass beds at sites throughout Brisbane Water estuary for time 1 (upper) and time 2 (lower). Site numbering and position of sites are shown in Figure 5.

Source of variation	DF	MS	F	Р
Time Ti	1	16015.50	8.09	0.0002
Area Ar	5	9494.72	No test	
Location Lo(Ar)	6	3361.39	1.70	0.03
Site Si(Ti x Ar x Lo)	24	2555.98	3.60	0.0002
Ti x Ar	5	2453.97	1.24	0.24
Ti x Lo	6	1979.11	0.77	0.87
Residual	96	709.49		

Table 5. Summary of results of permutational multivariate analysis of variance (PERNAMOVA) in fish assemblages of *Zostera capricorni* seagrass beds.



Figure 8. Partial canonical correspondence analysis (CCA) ordination diagram showing associations between seagrass features and spatial patterns in fish assemblages of *Zostera capricorni* seagrass beds in Brisbane Water estuary. Positions of sites (identified as A11, A12 etc.) are shown in Figure 5. The seagrass features (shown by arrows) that explained a significant proportion of the spatial variation in the fish assemblages were selected by manual forward selection (length: length of seagrass blades; cover: % cover of seagrass). Species (indicated by triangles): A fre *Arenigobius frenatus*; A jac *Ambassis jacksoniensis*; A vir *Achoerodus viridis*; B jac *Brachaluteres jacksonianus*; B kre *Bathygobius kreffti*; C aus *Centropogon australis*; E arm *Enoplosus armatus*; G sub *Gerres subfasciatus*; H whi *Hippocampus whitei*; M arg *Monodactylus argenteus*; M elo *Myxus elongatus*; P sig *Parupeneus signatus*; R sar *Rhabdosargus sarba*; S cil *Sillago ciliata*; T gav *Tylosurus gavialoides*; T ham *Tetractenos hamiltoni*.

Table 6. Summary results of partial canonical correspondence analysis (CCA) for assemblages of fishes in *Zostera capricorni* seagrass beds. Abundance data were log(x+1) transformed prior to analysis. Variables included are those selected by manual forward selection to explain a significant amount (at *P*=0.05) of variation in the species data and only significant seagrass features are shown. Conditional effect for each selected variable (in brackets) is the proportion of variation in the species data explained by each of the seagrass features selected in addition to the proportion explained by the first variable selected. The significance of conditional effects was determined by Monte Carlo test (999 unrestricted permutations) (\*\* *P* < 0.01)

Variables	Inter-set correlations Eigenvalues		alues	% variance explained		Total inertia	Canonical inertia	$\mathbb{R}^2$	
included	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2			
Cover (0.10**)	0.53	0.64	0.10	0.08	11.5	9.4	0.89	0.19	21%
Length (0.09**)	-0.81	0.26							

Cover: % cover of Zostera capricorni (untransformed). Length: length of leaves of Z. capricorni (untransformed).

## SETTLEMENT AND JUVENILE STAGE FISHES

The data analysed here was collected as part of a study by University of NSW and the results of that study are reported in Ford et al (2006). Multivariate analyses of the data were not reported in Ford et al (2006).

## Methods

#### Sampling

"Eight sampling sites were selected in seagrass within the tidal dominated region of Brisbane Water (Figure 9). Two replicate beach seine hauls were done over seagrass at each site with a 2 mm square mesh beach seine so that approximately 25 m<sup>2</sup> was sampled. Fish larger than 20 mm in length were identified, counted and returned to the water along with sygnathids, gobiids, molluscs, and crustaceans. All other fish were euthanized with 1% benzocaine solution and preserved with 5% formalin. Fish were sampled during the day on a low tide for two separate days during the new moon. Samples were taken on 5th and 8th August, 5th and 7th September, 2nd and 4th November and 5th and 7<sup>th</sup> December 2005" (Ford et al. 2006 p 8).

### Analysis

Following Ford et al. (2006) samples from the 2 days within each month were pooled to give a sample size of n=4 per month. Data for the Port Jackson glassfish (*Ambassis jacksoniensis*) was removed prior to analysis because its schooling behaviour led to very large variations in abundance between replicate samples (Ford et al. 2006). Spatial and temporal structuring of fish assemblages was visually investigated by nMDS and DCA ordinations, based on average abundance of each species in each site in each month. Data were square-root transformed fro both analyses, rare species were downweighted for DCA and Bray-Curtis similarity was used as the measure of similarity between samples for nMDS. Sites were grouped at 40% similarity in the nMDS ordinations to further investigate relationships between sites. Analyses were done with Primer 6 (nMDS) and Canoco (DCA) software. Separate ordinations were constructed for settlement and juvenile stage fishes for all species and for the subset of coastal spawning species (listed in Table 1 in Ford et al. 2006).

One-factor permutational multivariate analysis of variance (using PERMANOVA software, Anderson 2001) was used to test the null hypothesis that assemblages of settlement stage and juvenile stage fishes did not differ between sites. Sampling months were analyzed separately because site BD was not able to be sampled in August, which resulted in an unbalanced design. Separate analyses were done for all species and species of coastal spawning fishes. Data were square-root transformed prior to analysis and Bray-Curtis dissimilarity was used as the distance measure. Unrestricted permutation of raw data was used (4999 permutations) to determine *P*-

values. Significant effects were examined post hoc with *t*-test and Monte Carlo estimates of *P*-values were used because of the low number of permutations possible.

### Results

nMDS ordinations provided no evidence that assemblages were spatially or temporally structured in a consistent manner (Figure 10). There was no consistent trend for sites close to one another, or sites sampled in the same month, to have similar assemblages. For example, in the group of settlement stage fishes (all species) sites that were grouped with similar assemblages included: (1) BB-S, BB-N, BC-N, BA-N; (2) BA-S, BD-S, BE-S, BF-S, BG-S, BH-S; and (3) BC-A, BC-S, BD-N, BE-N, BF-N, BE-D, BF-D. Group 2 included sites from the extreme southern (BA) and northern (BH) ends of the estuary. Group 3 included sites from the southern end of the estuary (BC), mid-estuary (BD), and northern end of the estuary (BF). A similar absence of consistent structuring occurred in the other groups of species.

DCA ordinations of patterns of similarity among sites supported the conclusions from the nMDS ordinations (Figures 11 and 12). Furthermore, although sampling sites were located through the centre of the estuary from north-south there was no evidence of a gradient in the similarity of species assemblages.

Results of the 1-factor PERMANOVA test confirmed the patterns of the nMDS ordination (Table 7). Assemblages of settlement stage fishes (all species and coastal spawning species) did not differ significantly between sites in August and September 2005, but did differ significantly between sites in November and December 2005. Assemblages of juvenile stage fishes differed between sites in most months. In months when assemblages did differ significantly between sites, there was no consistent pattern of significant pairwise differences between sites. For example, in September for juvenile stage fishes (all species) the assemblage at site BC was significantly different from the assemblage at BD (its nearest neighbouring site) but not significantly different from BH (the site furthest from BC). This further supports the conclusion that assemblages were not spatially structured throughout Brisbane Water estuary.



Figure 9. Location of collecting sites (BA-BH) for settlement and juvenile stage fishes in Brisbane Water estuary (copied from Ford et al. 2006 p 29).





Settlement stage fishes (coastal spawning)



Figure 10. Non-metric multidimensional scaling ordination plots depicting similarity in assemblages of settlement stage (all species and coastal spawning species) and juvenile stage fishes (all species and coastal spawning species) in Brisbane Water estuary. Ordinations are based on the average abundance of each species in each site (see site codes in Figure 9) in each month (BA-A site BA in August, BA-S site BA in September, BA-N site BA in November, BA-D site BA in December etc.). Ellipses (at 10% slack) enclose sites that are 40% similar in assemblage structure.



Juvenile stage fishes (coastal spawning)



Figure 10 cont'd. Non-metric multidimensional scaling ordination plots depicting similarity in assemblages of settlement stage (all species and coastal spawning species) and juvenile stage fishes (all species and coastal spawning species) in Brisbane Water estuary. Ordinations are based on the average abundance of each species in each site (see site codes in Figure 9) in each month (BA-A site BA in August, BA-S site BA in September, BA-N site BA in November, BA-D site BA in December etc.). Ellipses (at 10% slack) enclose sites that are 40% similar in assemblage structure.



Figure 11. Detrended Correspondence Analysis (DCA) ordination plot showing similarity in species of settlement stage fishes from sites throughout Brisbane Water estuary. The upper graph is for all species ( $\lambda_1$ =0.48 and  $\lambda_2$ =0.32 accounted for 36.6% of the total spatial variation among locations, sum of all eigenvalues=2.18). The lower graph is for coastal spawning species ( $\lambda_1$ =0.53 and  $\lambda_2$ =0.31 accounted for 37.4% of the total spatial variation among locations, sum of all eigenvalues=2.26). Symbols as for Figure 10.



Figure 12. Detrended Correspondence Analysis (DCA) ordination plot showing similarity in species of juvenile stage fishes from sites throughout Brisbane Water estuary. The upper graph is for all species ( $\lambda_1$ =0.22 and  $\lambda_2$ =0.12 accounted for 28.3% of the total spatial variation among locations, sum of all eigenvalues=1.19). The lower graph is for coastal spawning species ( $\lambda_1$ =0.54 and  $\lambda_2$ =0.26 accounted for 40.6% of the total spatial variation among locations, sum of all eigenvalues=1.97). Symbols as for Figure 10.

Table 7. Summary of results of one-factor PERMANOVA to test for differences in assemblage structure of settlement (all species and coastal spawning species) and juvenile stage fishes (all species and coastal spawning species) between sites in Brisbane Water estuary. Significant post-hoc pairwise *t*-tests are also shown (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001, P-values were not corrected for multiple comparisons).

August, settlement stage, all species

Source of variation	df	MS	F	Р
Site	6	3465.65	1.17	0.32
Residual	21	2969.52		

August, settlement stage, coastal spawning species

Source of variation	df	MS	F	Р
Site	6	3186.54	1.23	0.29
Residual	21	2591.80		

August, juvenile stage, all species

Source of variation	df	MS	F	Р
Site	6	4643.37	2.64	0.001
Residual	21	1759.74		

	BB	BC	BE	BF	BG	BH
BA			*	*		
BB			*	*		
BC			*	*		
BE						
BF						
BG						
BH						

August, juvenile stage, coastal spawning species

Source of variation	df	MS	F	Р
Site	6	7297.84	3.65	0.0008
Residual	21	1997.17		

	BB	BC	BE	BF	BG	BH
BA			*	*		
BB			*	*		
BC			**	**		
BE					**	*
BF					**	*
BG						
BH						

September	settlement	stage	all	species
September,	settlement	stage,	an	species

Source of variation	df	MS	F	Р
Site	7	3788.82	1.30	0.15
Residual	24	2919.79		

a , 1			. 1	•	•
Sontombor	cottlomont	cto no	constal	cnowning	CHACIAC
SUBULIER.	SULLIUHUHU	stage.	CUastar	SDawming	SUCCIUS
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		~			

Source of variation	df	MS	F	P
Site	7	3931.80	1.41	0.12
Residual	24	2792.72		

September,	juvenile s	stage, al	l species	
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Source of variation	df	MS	F	Р
Site	7	5598.69	2.96	0.0004
Residual	24	1890.42		

	BB	BC	BD	BE	BF	BG	BH
BA			*		*		*
BB							*
BC			*		*		
BD				*	*	*	*
BE							
BF							
BG							
BH							

September,	juvenile stage.	coastal s	pawning	species
,,	J		$\Gamma$	- <u>r</u>

Source of variation	df	MS	F	P
Site	7	9816.15	6.29	0.0002
Residual	24	1561.08		

	BB	BC	BD	BE	BF	BG	BH
BA		**	**	*	**	**	*
BB			*		*		
BC			***	*	***	*	*
BD				**	**	**	**
BE							
BF							
BG							
BH							

November, settlement stage, all species								
Source of variation			ion (	df N	ЛS	F	1	0
Site			,	7 7	983.1	0 3.	57 (	0.0002
Resi	dual			24 2	236.5	3		
	BB	BC	BD	BE	BF	BG	BH	_
BA			*				***	
BB			*				*	
BC			**	*	**	**	***	
BD							***	
BE							**	
BF							**	
BG								
BH								

November, settlement stage, all species

November, settlement stage, coastal spawning species

Source of variation	df	MS	F	Р
Site	7	8052.60	3.66	0.0002
Residual	24	2202.53		

	BB	BC	BD	BE	BF	BG	BH
BA			**		*		***
BB			*				
BC			*		*	*	***
BD						*	***
BE							**
BF							**
BG							
BH							

November, juvenile stage, all species

i to veniever, ju venine stuge, un species									
Source of variation	df	MS	F	Р					
Site	7	5184.80	2.89	0.0004					
Residual	24	1793.86							

	BB	BC	BD	BE	BF	BG	BH
BA			*				*
BB							
BC			*				*
BD				*	*		*
BE					*		*
BF							*
BG							
BH							

November, juvenile stage, coastal spawning species

Source of variation	df	MS	F	Р
Site	7	4431.92	1.52	0.12
Residual	24	2906.50		

December, settlement stage, an species								
Sour	ce of	variat	ion o	df N	ЛS	F		Р
Site				77	652.2	9 4.	09	0.0002
Resi	dual		2	24 1	863.8	6		
	BB	BC	BD	BE	BF	BG	BH	
BA			*			**		_
BB			**	*	*			
BC						**	*	
BD						**	**	
BE						**	*	
BF						**	*	
BG								
BH								

December, settlement stage, all species

December, settlement stage, coastal spawning species

Source of variation	df	MS	F	Р
Site	7	8027.64	4.95	0.0002
Residual	24	1620.00		

	BB	BC	BD	BE	BF	BG	BH
BA			*	*	*	*	*
BB			**	**	**		
BC					*	**	*
BD						**	**
BE						**	**
BF							**
BG							
BH							

December, juvenile stage, all species

December, Juvenne stage, an species									
Sour	ce of	variati	on	df	M	IS	F	7	Р
Site				7	50	660.3	5 3	.46	0.0002
Resi	dual			24	10	635.6	9		
	DD	DC	DD	D	Г	DE	DC	DU	r

	BB	BC	BD	BE	$\mathbf{BF}$	BG	BH
BA		*	**	*	*	*	**
BB			*				*
BC			*				*
BD						*	*
BE							
BF							
BG							
BH							

Sour	Source of variation			df N	/IS	F	P	)
Site			,	7 7	417.6	50 3.8	86 0	.0002
Resi	dual			24 1	922.1	4		
	BB	BC	BD	BE	BF	BG	BH	
BA			**	*	**	***		
BB			*	*	*	*		
BC						*		
BD						***		
BE						***		
BF						***		
BG							*	
BH								_

December, juvenile stage, coastal spawning species

## SPATIAL VARIATION IN ASSEMBLAGES OF MACROBENTHIC ORGANSIMS WITHIN MANGROVE FORESTS AND ITS RELATIONSHIP TO ENVIRONMENTAL FEATURES OF MANGROVE FORESTS

Roberts (2006) and Roberts and Sainty (2006) sampled, respectively, the macrobenthic organisms inhabiting *Avicennia marina* mangrove forests and the habitat features of these mangrove forests throughout Brisbane Water estuary. Multivariate analysis of the assemblages of macrobenthic organisms indicated significant differences in assemblage structure among the 15 locations sampled (Roberts 2006). Univariate analysis of the habitat features of these mangrove forests found significant differences in all variables (at several nested spatial scales). The objective of the following analysis was to test for a relationship between spatial variation in macrobenthic organisms and spatial variation in mangrove forest habitat features. Analysis was done at the scale of locations because this was the scale at which spatial variation in assemblage structure was reported by Roberts (2006).

#### **Methods**

#### Sampling

Fifteen locations were sampled in Brisbane Water estuary for macrobenthic invertebrates (Figure 13). Two randomly nested sites  $(50 \times 30 \text{ m})$  were sampled at each location. Within each site, three  $10 \text{ m}^2$  plots were randomly selected. Within each plot the number of adult mangrove trees (*Avicennia marina* and *Aegiceras corniculatum*) were counted, and the height (m) of the forest canopy and its percentage cover (Specht Classification) were estimated. Five randomly placed 0.25 m<sup>2</sup> quadrats in each plot were used to estimate the number of mangrove seedlings, pneumatophores and crab holes. Macrobenthic organisms were collected with a benthic sediment core (10 cm diameter and 10 cm deep) (n=3 replicates) from each site (Roberts 2006, Roberts and Sainty 2006).

#### Analysis

Analysis was done at the scale of locations. Location-average values were calculated for all habitat variables and for the abundance of each macrobenthic taxon. A Detrended Correspondence Analysis (DCA) was done in Canoco 4.5 (ter Braak and Smilauer, 2002) to display the similarity between foreshore sites in their assemblages of foreshore plant species. Canonical correspondence analysis (CCA) in Canoco 4.5 was used to determine the habitat features (if any) of *A. marina* mangroves that explained significant amounts of the spatial variation in macrobenthic assemblages. Habitat variables were initially checked for normality and, where necessary, transformed (this was only necessary for density of crab holes which was transformed

to log(x+1)). Habitat variables were measured in different units and were therefore standardized (by subtracting the mean and dividing by standard deviation) prior to analysis. The mean density data for macrobenthic taxa were log(x+1) transformed prior to analysis and the importance of rare species was down-weighted (ter Braak and Smilauer, 2002). A manual selection process in Canoco was used to select the subset of mangrove habitat features that best explained the spatial patterns in macrobenthic assemblages. Mangrove habitat features were ranked according to the proportion of total variance in the macrobenthic data set they explained. The statistical significance of the variance explained by each of the habitat features was tested by a Monte Carlo test (1000 permutations).

### Results

DCA showed that locations of *A. marina* mangrove forest varied considerably in their assemblages of macrobenthic organisms (Figure 14). The first 2 axes of the DCA ordination plot (Figure 10) ( $\lambda_1$ =0.40,  $\lambda_2$ =0.18) accounted for 29.0% of the total spatial variation among locations (sum of all eigenvalues=1.97). The third axis explained only a further 3.7% of variation. There appeared to be no relationship between distance between mangrove forests and similarity in their macrobenthic assemblages. For example, locations that were very similar in terms of their macrobenthic assemblages (5 and 11; 8 and 10; 13 and 15) were separated by other locations. Location 12 (Cockle Bay Wetland) had the most distinctive macrobenthic assemblage. Interestingly, this was quite dissimilar to another location in Cockle Bay (11 Cockle Bay Nature Reserve).

Patterns of similarity among locations revealed by the DCA are similar to the patterns revealed by nMDS ordinations in Roberts (2006). For example, the similarity between locations 13 and 15, locations 5 and 11, and the group of locations at the centre of the DCA ordination plot (locations 3, 7, 9, 14).

CCA of macrobenthic taxa and locations (Figure 15) found that locations 2 and 7 were characterized by Anthuridae (isopod), *Laternula tasmanica* (bivalve mollusc), Hymenosomatidae (decapod crustacean), Neredidae (polychaete), amphipod sp. 2, and insect larvae. Locations 13 and 14 were characterized by *Victorioposia cf. australiensis* (amphipod), *Assiminea* sp. (gastropod mollusc), and *Sesarma erythrodactyla* (decapod crustacean).

Despite the spatial variation in macrobenthic assemblage structure and the spatial variation in habitat features (Roberts and Sainty 2006), none of the measured mangrove habitat features explained a significant amount of the spatial variation in macrobenthic assemblage structure (Table 8). The habitat variables are ranked in Table 8 in order of the variance they explain singly ( $\lambda_1$ ). Also shown is the statistical significance of this fit between each habitat variable and the species data set (*F* and *P* values). None of the habitat variables are significantly related to the species data set (all *P*-values >0.05).



Figure 13. Positions of mangrove sampling locations 1-15 in Brisbane Water estuary (source: Roberts and Sainty 2006).



Figure 14. Detrended Correspondence Analysis (DCA) ordination plot of 15 locations of *Avicennia marina* mangrove forest in Brisbane Water estuary showing similarity in their assemblages of macrobenthic organisms. Ordination based on average abundance of each taxon (n=2 sites per location, 3 replicates per site).



Figure 15. Canonical correspondence analysis (CCA) ordination diagram showing assemblages of macrobenthic species characterizing locations of *Avicennia marina* mangrove forests in Brisbane Water estuary. Positions of locations (identified as 1, 2 etc.) are shown in Figure 9. Species with a fit of at least 10% to the two axes are shown (indicated by triangles): *A sp. Assiminea* sp., A sp. 2 amphipod sp. 2; Ant Anthuridae; *B aur Bembicium auratum; B aus Batillaria australis*; Cap Capitellidae; Eun Eunicidae; *G pla Glauconome plankta; H cor Heloecius cordiformis; H has Helograpsus haswellianus*; Hym Hymenosomatidae; Ins insect larvae; *L ta Laternula tasmanica*; Mos Mosquito pupae; Nep Nephtyidae; Ner Neredidae; Onc Onchidiidae; *O sul Ophicardelus sulcatus; P lae Paragrapsus laevis; P qua Paragrapsus quadridentatus; S ery Sesarma erythrodactyla; S sol Salinator solida; T del Tellina deltoidalis; T huo Tatea huonensis; V cf. aus Victorioposia cf. australiensis.* 

Table 8. Summary results of canonical correspondence analysis (CCA) for assemblages of macrobenthic organisms in *Avicennia marina* mangrove forests at the scale of locations (see Roberts 2006). Values shown are the marginal effects for each habitat variable. Abundance data were log(x+1) transformed prior to analysis. The significance of marginal effects was determined by Monte Carlo test (1000 unrestricted permutations) and the resulting *P*-values and *F*-values are shown.

Habitat feature	$\lambda_1$	F	Р
No. crab holes	0.18	1.32	0.11
Canopy height	0.16	1.15	0.30
No. seedlings	0.12	0.87	0.60
Specht cover	0.12	0.86	0.66
No.	0.1	0.67	0.86
pneumatophores			
No. trees	0.09	0.61	0.94

## FORESHORE VEGETATION

Results of an analysis of variation in foreshore vegetation and condition (measured as a Disturbance Index) of Brisbane Water estuary were originally presented in Sainty and Roberts (2004). Data from this was report was provided for the following analyses.

## Methods

### Sampling

Sainty and Roberts (2004) surveyed foreshore vegetation at 145 sites that covered the entire foreshore of Brisbane Water estuary. At each site plant species present were noted and the relative abundance of each species present assigned to an abundance scale (range 1-3). A qualitative assessment of the condition of each site was also undertaken using a Disturbance Index (Table 9).

### Analysis

A Detrended Correspondence Analysis (DCA) was done in Canoco 4.5 (ter Braak and Smilauer, 2002) to display the similarity between foreshore sites in their assemblages of foreshore plant species. Species abundance data were untransformed and the option to downweight rare species was not selected. For comparison a non metric multiscaling ordination ordination plot was also produced based on a Bray Curtis similarity matrix of untransformed data.

A Canonical Correspondence Analysis (CCA) was done on the single environmental variable, Disturbance Index, to determine if it was significantly related to variation in foreshore plant species and to determine the amount of variation it explained. This was done by a manual selection process with the significance determined by Monte Carlo randomization (n=1000).

## **Results**

The first 2 axes of the DCA ordination plot (Figure 16) ( $\lambda_1$ =0.67,  $\lambda_2$ =0.43) accounted for only 9.5% of the total spatial variation in foreshore species (sum of all eigenvalues=11.60). The ordination plot is dominated by a large group of sites at the centre (with similar species assemblages) and sites of increasingly dissimilar species to the right. Sites to the right of the ordination plot represent increasing values of the Disturbance Index (less disturbed) e.g. sites 109 and 124 (DI=5) and sites 106, 123, 138 (DI=3).

The nMDS ordination plot (with Disturbance Index overlaid) (Figure 17) shows a similar pattern of little separation among a large number of sites at the centre of the

ordination plot and greater dispersion among sites towards the edges of the ordination plot. The latter sites represent smaller values of the Disturbance Index (highly disturbed).

The single environmental variable, Disturbance Index, explained a significant but very small amount of the spatial variation in foreshore species ( $\lambda$ =0.16, *F*=1.63, *P*=0.008)). The amount of variation explained by Disturbance Index represented 1.4% of the total variation in the species data set.



Figure 16. Detrended Correspondence Analysis (DCA) ordination plot of 145 foreshore sites throughout Brisbane Water estuary showing similarity in their assemblages of plant species. Ordination based on relative abundance of each species at each site.



Figure 17. Non-metric multidimensional scaling ordination plots depicting similarity in assemblages of foreshore plant species at 145 sites (numbered 1-145) in Brisbane Water estuary.

Table 9. Disturbance Index developed by Sainty and Roberts (2004) to assess condition of shoreline of Brisbane Water estuary (source: Sainty and Roberts 2004 p 6).

INDEX	DESCRIPTION
1	Highly disturbed/modified foreshore. Includes seawalls with limited
	ecological niches e.g. vertical concrete or stone. Includes buildings in
	close proximity to the seawall, often with jetties and stormwater inlets.
	Catchment substantially developed.
2	Disturbed/modified foreshore. Seawall with limited ecological niches.
	Includes foreshore with scattered mangroves. Saltmarsh limited to
	narrow discontinuous strip. Catchment substantially developed.
3	Modified foreshore. Seawall absent. Includes irregular saltmarsh strip
	or natural rock platform associated with a variable width forest,
	contiguous to waters edge. Catchment partly/variably developed.
4	Unmodified foreshore. Seagrass, mangrove/saltmarsh/forest on waters
	edge. Catchment partially or wholly developed.
5	Unmodified foreshore. Seagrass, mangrove/saltmarsh/forest on waters
	edge. Catchment with no development.

## SALTMARSH PLANTS

This analysis is based on data originally collected and analysed by Roberts and Sainty (2005). The following should be read in conjunction with that report.

#### **Methods**

#### Sampling

Saltmarsh meadows were sampled from 4 areas representing different tidal flushing regimes within Brisbane Water estuary. Three locations were sampled within each area (Table 10) and locations were stratified into low and high saltmarsh. As a test of the hypothesis that disturbed saltmarshes would differ from undisturbed saltmarshes sampling was done by randomly selecting 4 locations representing undisturbed saltmarsh and 4 locations representing disturbed saltmarsh. Two randomly selected sites were sampled within each location by estimating the percentage cover of saltmarsh species in 10 randomly placed 0.25 m<sup>2</sup> quadrats (Roberts and Sainty 2005).

#### Analysis

A Detrended Correspondence Analysis (DCA) was done in Canoco 4.5 (ter Braak and Smilauer, 2002) to display the similarity between locations in their assemblages of saltmarsh plant species for both high and low saltmarsh. DCA was based on average coverage of each species in each site (n=10 replicate samples per site). Species coverage data was square-root transformed prior to analysis and the option to downweight rare species was selected.

Following Roberts and Sainty (2005) 4 disturbed and 4 undisturbed locations of high and low saltmarsh were randomly selected to test the hypothesis that saltamarsh plant assemblages differed between disturbed and undisturbed locations. The test was done by DCA on the average coverage of each species in each site (n=10 replicate samples per site). Species coverage data was square-root transformed prior to analysis and the option to downweight rare species was selected.

#### **Results**

The first 2 axes of the DCA ordination plot (Figure 18) ( $\lambda_1$ =0.31,  $\lambda_2$ =0.18) accounted for 36.1% of the total spatial variation in high saltmarsh foreshore species (sum of all eigenvalues=1.35). The third axis explained only a further 6.6% of spatial variation. The DCA ordination plot showed considerable variation in high saltmarsh species assemblages throughout Brisbane Water estuary. Sites in some locations were very similar to one another (e.g. 31+31, 61+62, 91+92) while sites in other locations were very dissimilar (e.g. 41+42, 71+72, 81+82, 121+122). Locations and sites within locations did not group according to tidal flushing regime e.g. locations 1-3 (within Brisbane Water) overlapped with locations and sites from Cockle Bay (71, 72, 82) and Kincumber Broadwater (122).

The first 2 axes of the DCA ordination plot (Figure 19) ( $\lambda_1$ =0.20,  $\lambda_2$ =0.04) accounted for 62.0% of the total spatial variation in low saltmarsh foreshore species (sum of all eigenvalues=0.39). The third axis explained only a further 6.3% of spatial variation. The DCA ordination plot shows that assemblages from sites were very similar to one another in some locations (e.g. 71+72, 81+82) but were very dissimilar to one another in other locations (11+12, 41+42, 101/102, 111/112). Locations and sites within locations were not grouped according to tidal flushing regime e.g. assemblages from the 3 locations within Brisbane Water (locations 1-3) overlapped with sites from Cockle Channel (41, 62), Cockle Bay (91), and Kincumber Broadwater (102).

These results support those reported by Roberts and Sainty (2005) based on nMDS ordinations of the same data set.

The first 2 axes of the DCA ordination plot of disturbed and undisturbed high saltmarsh meadows ( $\lambda_1$ =0.32,  $\lambda_2$ =0.24) accounted for 45.4% of the total spatial variation in high saltmarsh foreshore species (sum of all eigenvalues=1.23) (Figure 20). The third axis explained only an additional 7.6% of variation. The DCA ordination shows sites and locations do not form discrete groups according to their disturbance status.

The first 2 axes of the DCA ordination plot of disturbed and undisturbed low saltmarsh meadows ( $\lambda_1$ =0.27,  $\lambda_2$ =0.03) accounted for 68.2% of the total spatial variation in high saltmarsh foreshore species (sum of all eigenvalues=0.45) (Figure 21). The third axis explained only an additional 1.3% of variation. The DCA ordination shows that 3 undisturbed sites formed a distinct group at the left of the ordination plot (U11, U12, U22). However, there was considerable overlap in species assemblages between the remaining disturbed and undisturbed sites.

Table 10. Saltmarsh locations sampled in Brisbane Water estuary (source: Roberts and Sainty 2005 p 5). Locations were allocated to 4 groups according to hypothesized similarity in tidal flushing regime: Brisbane Water (locations 1-3); Cockle Channel (locations 4-6); Cockle Bay (locations 7-9); Kincumber Broadwater (locations 10-12).

Saltmarsh	Location	Comments
Erina Creek Wetland	1	Undisturbed
Egan Creek Saltmarsh	2	Disturbed
Saratoga Wetland	3	Disturbed
Rileys Island	4	Undisturbed
Lintern Saltmarsh	5	Disturbed
Empire Bay Wetland	6	Disturbed
Cockle Bay Nature Reserve	7	Undisturbed
Cockle Bay Wetland	8	Undisturbed
Bensville Saltmarsh	9	Relatively undisturbed, but disturbed in places
Davistown Wetland	10	Disturbed
Saratoga Saltmarsh	11	Disturbed
Kincumber Creek	12	Undisturbed
Pelican Island	13	Not used in formal analyses (Undisturbed however
		had very little low marsh habitat)
Davistown Saltmarsh	14	Not used in formal analyses (directly connected to
		Lintern Saltmarsh, i.e. not independent)



Figure 18. Detrended Correspondence Analysis (DCA) ordination plot of 28 sites (2 sites within each of 14 locations) throughout Brisbane Water estuary showing similarity in their assemblages of high saltmarsh plant species. Sites are numbered as 11 (site 1 within location 1) etc. Relative positions of locations are listed in Table 10 and in Roberts and Sainty (2005). Ordination based on average coverage of species at each site.



Figure 19. Detrended Correspondence Analysis (DCA) ordination plot of 28 sites (2 sites within each of 14 locations) throughout Brisbane Water estuary showing similarity in their assemblages of low saltmarsh plant species. Sites are numbered as 11 (site 1 within location 1) etc. Relative positions of locations are listed in Table 10 and in Roberts and Sainty (2005). Ordination based on average coverage of species at each site.



Figure 20. Detrended Correspondence Analysis (DCA) ordination plot of 16 sites (2 sites within each of 8 locations) throughout Brisbane Water estuary showing similarity in their assemblages of high saltmarsh plant species. Locations and sites within locations are classified as undisturbed (U11, U12 etc) and disturbed (D11, D12 etc). Ordination based on average coverage of species at each site.



Figure 21. Detrended Correspondence Analysis (DCA) ordination plot of 16 sites (2 sites within each of 8 locations) throughout Brisbane Water estuary showing similarity in their assemblages of low saltmarsh plant species. Locations and sites within locations are classified as undisturbed (U11, U12 etc) and disturbed (D11, D12 etc). Ordination based on average coverage of species at each site.

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