

**Inter-relationships between Benthic
Macroinvertebrates and Habitat Types in
Broke Inlet, south-western Australia**



**Report to the Ernest Hodgkin Trust for Estuary
Education and Research**

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1.0 Abstract

The benthic macroinvertebrate fauna of Broke Inlet, a seasonally-open estuary located in the South coast of Western Australia was sampled seasonally between spring 2007 and autumn 2008 at 20 nearshore sites throughout the estuary. These sites were chosen to represent five of the twelve habitat types that were identified quantitatively on the basis of their differences in a suite of enduring environmental criteria that reflected either location within the estuary, exposure to wave activity or the amount of submerged aquatic vegetation. Sampling yielded 5,519 individuals that represented 25 species. Most of which belonged to the class Polychaeta (10 species and 63.5% of the individuals), followed by those representing the classes Amphipoda, Anthurida and Bivalvia contributed 14.8, 8.2 and 6.8% respectively to the total number of individuals. The number of species, density and Shannon diversity of the benthic macroinvertebrate fauna were found to be significantly influenced by both habitat type ($p < 0.001$) and season ($p < 0.001$), with the greatest values in the majority of habitat types occurring in spring and summer. The composition of benthic macroinvertebrate assemblages were significantly influenced both by habitat type ($R = 0.418$) and season ($R = 0.304$), with the greatest differences among habitat types occurring in spring ($R = 0.493$). Furthermore the pattern of spatial differences among habitat types, as exhibited by the benthic macroinvertebrate fauna, statistically matched that among the suite of enduring environmental variables used to distinguish each of those habitat types in both spring 2007 ($Rho = 0.441$) and summer 2008 ($Rho = 0.367$). Possible reasons for the non significant matching between the benthic macroinvertebrate fauna in autumn 2008 and the enduring environmental variables are also discussed.

2.0 Introduction

Benthic macroinvertebrates are those invertebrates greater than 500 μm in size that inhabit the substrate of aquatic environments. These organisms are a vital component of estuarine ecosystems, providing a number of ecosystem functions. For instance, their bioturbation activities such as feeding, tube-building, burrowing, irrigation of burrows, excretion and locomotion, substantially influence the exchange of materials between the sediment and the overlying water column (Rhoads, 1974; Aller & Aller, 1986; Hansen & Kristensen, 1997). Thus, tube-building and burrowing enhance nutrient cycling by increasing the area of the oxic-anoxic interface and the transport of ions through the sediment (Kristensen *et al.*, 1991; Penniford & Davis, 2001), while turbidity levels are reduced through filter feeding and biodeposition, quantities of detrital matter are substantially reduced by grazing deposit feeders (Nielson & Jernakoff, 1996). Furthermore, these benthic fauna represent a major component of food webs within estuarine ecosystems, not only because they consume detrital material and primary food sources (Riisgård, 1991) but provide a major food source to both fish (Hyndes & Potter, 1997; Sá *et al.*, 2006; Chuwen *et al.*, 2007) and birds (Moreira, 1997; Lourenço *et al.*, 2008).

The assemblage structure of benthic macroinvertebrates in estuaries has been related to spatial and temporal differences in a range of individual environmental variables including sediment granulometry and organic matter content, degree of exposure to wave activity, beach slope, length and width, the presence of submerged vegetation and hydrological parameters such as freshwater flow, nutrient levels, salinity and dissolved oxygen content (e.g. McLachlan, 1983, 1990, 2001; Allen & Moore, 1987; Snelgrove & Butman, 1994; Mattila *et al.*, 1999; Kanandjembo *et al.*, 2001). However, the distribution of benthic macroinvertebrates in these environments is most likely better explained by examining the collective influence of a suite of the above environmental variables, *i.e.* their habitat. An essential first step in examining the inter-relationships between benthic macroinvertebrate assemblage structure and their habitats in estuaries is thus to devise an approach for classifying estuarine habitats that is rigorous and quantitative and employs measurements of a suite of environmental variables that are likely to directly influence benthic macroinvertebrates or provide good surrogates for influential variables. Furthermore, the establishment of a good correlation between particular habitat types and their characteristic fauna then provide managers and ecologists with a basis for (i) undertaking thorough habitat type and faunal inventories, (ii) establishing a benchmark against which the

influence and future environmental change can be detected and (iii) predicting the faunal species likely to inhabit any site of interest in those environments.

The ecosystem health of estuaries in south-western Australia is under increasing pressure due to both a number of detrimental anthropogenic influences to these systems and their catchments (e.g. construction of marinas, reclamation of land, catchment clearing, eutrophication and increased fishing pressure) and the influence of climate change. The Australian Catchment, River and Estuary Assessment carried out in 2002 which quantified the level of anthropogenic modification to every Australian estuary identified only one system in south-western Australia that remains 'near pristine' namely Broke Inlet (Commonwealth Government, 2002). Broke Inlet is a large, seasonally-open estuary located within the D'entrecasteaux National Park on the south coast of Western Australia near the town of Walpole. The environmental and ecological knowledge on the estuary is limited (Hodgkin & Clarke, 1989). Furthermore, given its 'near pristine' status Broke Inlet represents an excellent benchmark system against which the characteristics of other seasonally-open estuaries that experience more severe anthropogenic modification can be compared e.g. Wilson Inlet located 100km to the east of Broke Inlet (Commonwealth Government, 2002).

In light of the above, the overall aims of this investigation were as follows.

- 1) Quantitatively classify the nearshore habitat types in Broke Inlet using the habitat classification scheme that has recently been devised by Valesini *et al.*, (in prep) for south-western Australian estuaries.
- 2) Sample the benthic macro-invertebrate fauna at a range of the resultant habitat types in each season between November 2007 and May 2008.
- 3) Ascertain statistically whether the pattern of spatial differences in the characteristics of the invertebrate fauna among habitat types matches that of the environmental variables used to classify those habitat types.

3.0 Materials and Methods

3.1 Study Area

Broke Inlet is a wave-dominated and seasonally-open estuary situated near the town of Walpole on the south coast of Western Australia between 116°22' - 116°32' East and 34°52' - 34°58' South (Figure 1). The estuary, is 15 km long, 3 km wide and has a surface area of 48 km², making it one of the largest on the south coast. Broke Inlet and its catchment are both situated within the Shannon and D'entrecasteaux National Parks, and while the estuary is not protected under the current management plan (CALM, 2005), it has been recommended for protection (CALM, 1994). The wide circular basin of the estuary which is fed by the Shannon, Forth and Inlet rivers comprises three large lagoonal areas *i.e.* Shannon, Middle and Clarke basins which are relatively shallow (average and maximum depths of 1.5 and 4.5 m below sea level, respectively) and homogeneous in depth due to the redistribution of river sediment (Hodgkin & Clarke, 1989). Combined with the sediment derived from the rivers, shoreline erosion and aeolian sand has led to the formation of extensive sand banks and marginal shoals throughout the estuary, which occupy 26.5 km² (56%) of its surface area (Commonwealth Government, 2002). Connection to the sea is via a 3.5 km long and 250 m wide entrance channel with a maximum depth of 7 m (Figure 2). Tidal exchange with the Southern Ocean is seasonal due to the formation of a large sand bar at the estuary mouth through the transport of marine sediment via long-shore drift. This sand bar, which can be up to 500 m thick and 1.8 m high (Hodgkin & Clarke, 1989), has historically opened annually sometime between June and September, and may remain open for up to six months depending on rainfall.



Figure 1: Location of Broke Inlet with south-western Australia and the Shannon Drainage basin

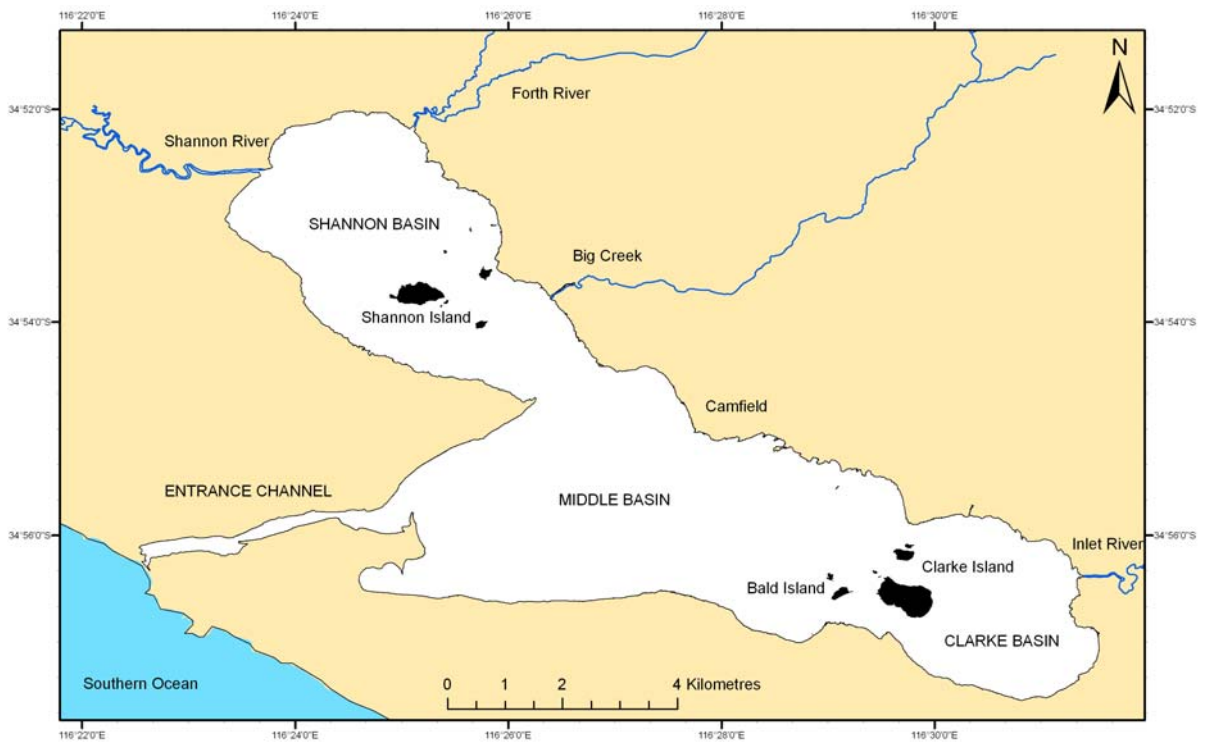


Figure 2: Location of the hydrological features within Broke Inlet.

3.2 Classification of Habitat Types and Selection of Sampling Sites

A recently devised scheme for classifying habitat types within estuaries using measurements for a suite of temporally-enduring environmental variables (Valesini *et al.*, in prep) was applied to Broke Inlet. As the habitat classification methodology is currently unpublished, a short description is provided here. The classification criteria employed in this scheme represent a suite of environmental variables that are (i) enduring, *i.e.* do not exhibit substantial change over time, (ii) directly influential on the distribution of fish and benthic invertebrate fauna or provide good surrogates for influential variables and (iii) were able to be measured in a GIS (Geographical Information System) from a high resolution remotely-sensed georeferenced image of the estuary and a Digital Elevation Model (DEM) depicting the bathymetry of the system. These variables fall into three main categories (Table 1) and were measured at 104 environmentally-diverse nearshore sites throughout Broke Inlet. Each site was defined by a point on the shoreline and all waters within a 100 m radius of that point.

Table 1: The enduring environmental variables (EEVs) used in the habitat classification for Broke Inlet.

| Group | Variable | Units | Transformation | Weighting (%) |
|------------------------------|--|------------------|-----------------------|----------------------|
| <i>Location</i> | | | | |
| | Latitude | UTM co-ordinates | None | 50 |
| | Longitude | UTM co-ordinates | None | 50 |
| <i>Exposure</i> | | | | |
| | Northerly MEF [#] | Metres | Fourth Root | 14.28 |
| | Southerly MEF [#] | Metres | Fourth Root | 14.28 |
| | Easterly MEF [#] | Metres | Fourth Root | 14.28 |
| | Westerly MEF [#] | Metres | Fourth Root | 14.28 |
| | ME direct fetch [#] | Metres | Fourth Root | 14.28 |
| | MEF [#] to the wave shoaling margin | Metres | Fourth Root | 14.28 |
| | Average slope | Degrees | Square Root | 14.28 |
| <i>Substrate/ SAV</i> | | | | |
| | Submerged vegetation cover | Percentage cover | Fourth Root | 100 |

Modified Effective Fetch

The first group of variables, which reflect the location of any site with respect to its vicinity to marine and freshwater sources, was designed to be a surrogate for a range of water quality variables which change spatially throughout an estuary e.g. salinity, temperature, and dissolved oxygen concentration. The second and third groups quantified the exposure of a site to wave activity and the composition of the various substrate/submerged aquatic vegetation types present. All of these EEVs were calculated using a range of GIS software packages namely, ArcGIS 9 (ESRI, California, USA) with the extension XTools Pro 5 (Data East, Novosibirsk, Russia), IDRISI v15.0 Andes (Clarke Labs, Massachusetts, USA) and Surfer 8 (Golden Software, Colorado, USA).

The resultant data matrix containing values for each of the ten EEVs at each of the 104 sites throughout the estuary was then subjected to a range of routines in the multivariate statistics package PRIMER v6 (Clarke & Gorley, 2006). The data were initially pretreated by undertaking transformation, normalisation and weighting procedures (Table 1), so that (i) the distribution of the samples for each variable was not heavily skewed, (ii) all variables were on a common scale and (iii) that each group of variables contributed equally to the classification procedure regardless of the number of variables within that particular group. The pretreated data was then used to create a Manhattan Distance matrix, which was then subjected to a hierarchical agglomerative clustering procedure (CLUSTER) using group average linkage. A Similarity Profile (SIMPROF) permutation test was also carried out in conjunction with this CLUSTER, which enabled identification of those groups of sites in the resultant dendrogram that did not differ significantly in their suite of environmental characteristics and thus represented habitat types. Thus, a SIMPROF test is performed at each successive node of the CLUSTER dendrogram until any particular group of sites being divided fails to exhibit significant internal structure. The null hypothesis that there are no significant environmental differences among sites was rejected if the significance level (p) associated with the test statistic (π) was <1%. Habitat types represented by only one site were considered to be outliers and thus removed from the analyses. CLUSTER-SIMPROF identified 12 habitat types within Broke Inlet (Figure 3), of which five divergent types were chosen for sampling of their benthic macro-invertebrate fauna (Figure 4).

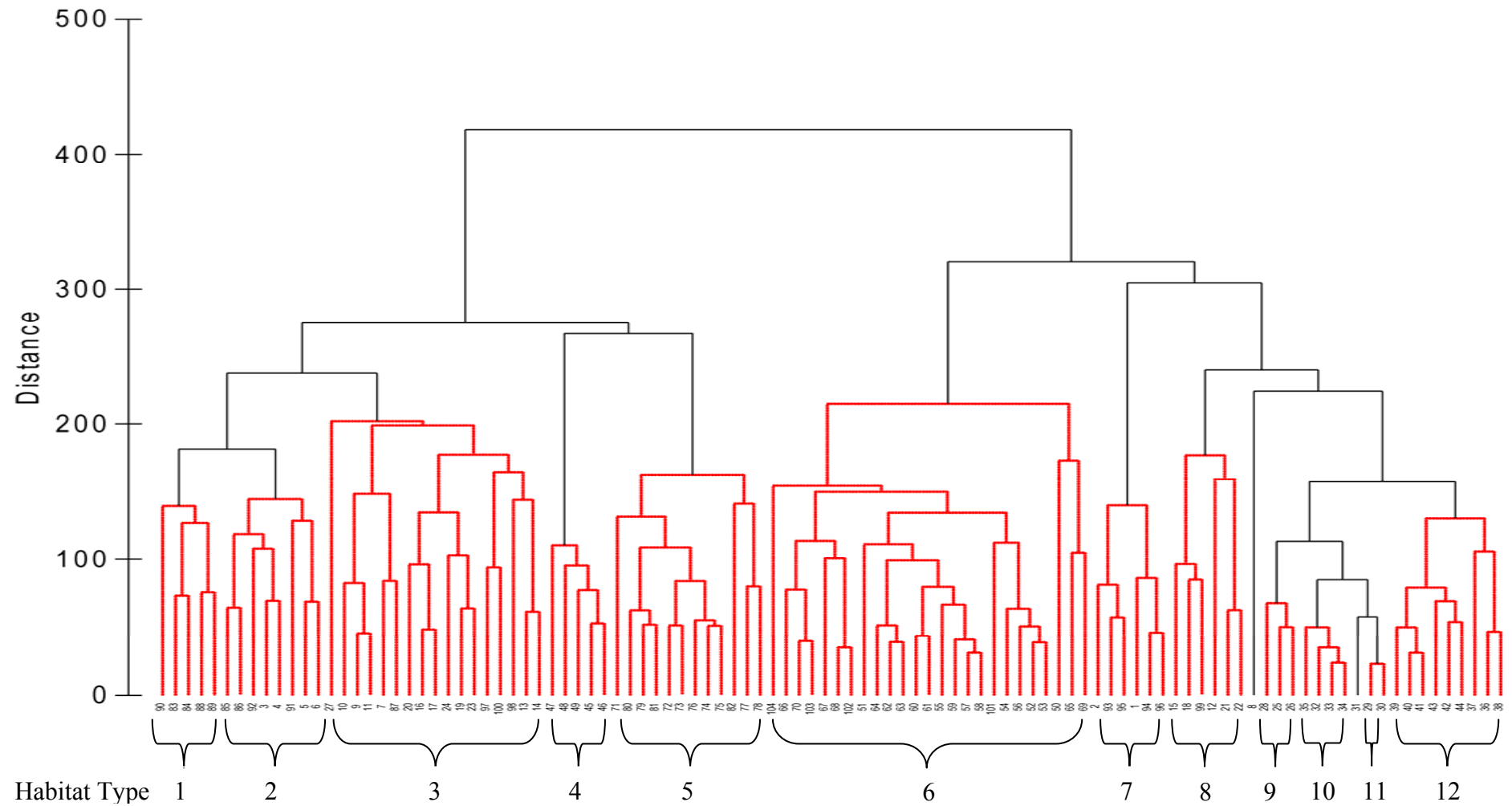


Figure 3: CLUSTER-SIMPROF Dendrogram of the suite of enduring environmental variables recorded at each of the 104 nearshore sites in Broke Inlet. Note: Red lines indicate CLUSTER-SIMPROF found no significant environmental differences among those sites, thus they form a habitat type.

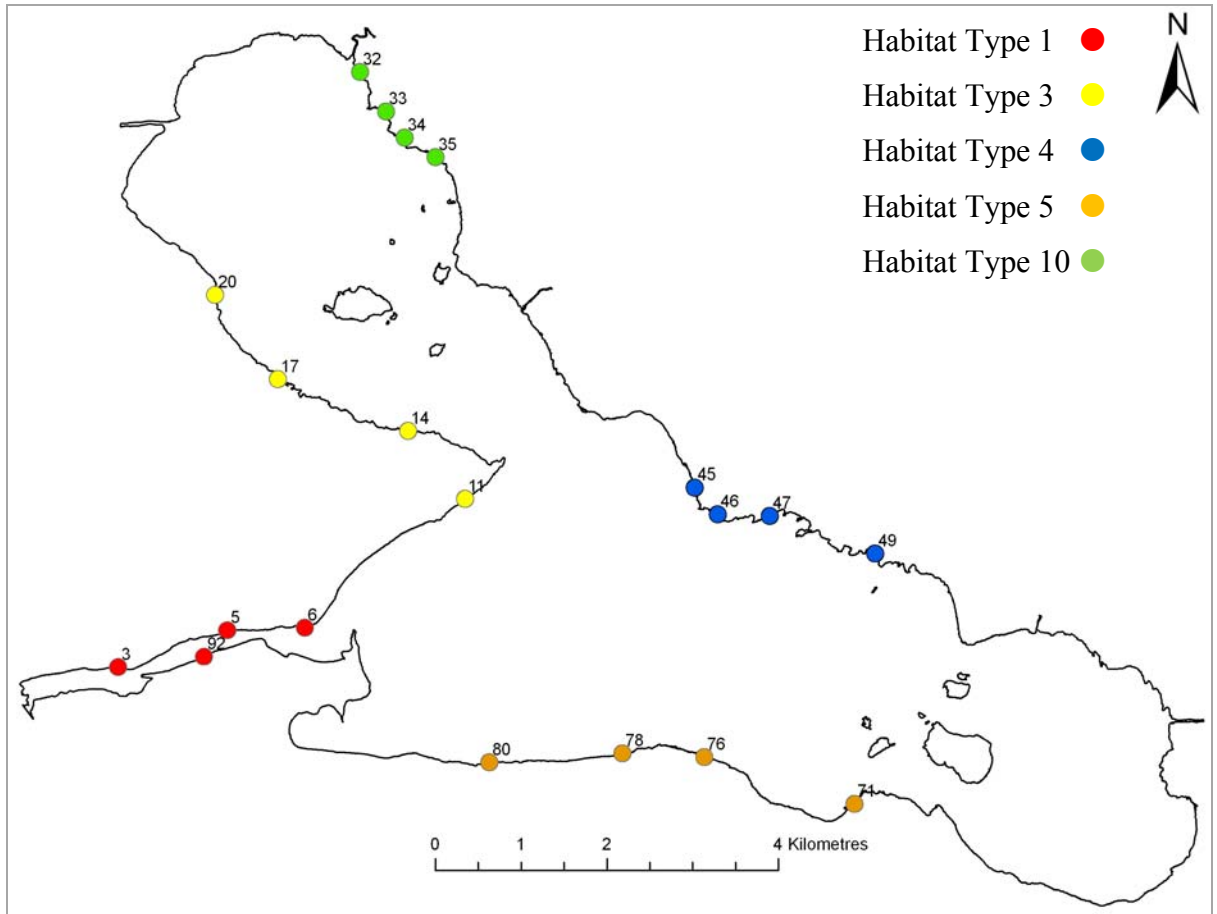


Figure 4: Location of the five habitat types at which benthic macro-invertebrate assemblages were sampled.

3.3 Benthic Macroinvertebrate Collection and Processing

The benthic macroinvertebrate fauna of Broke Inlet were sampled at five nearshore habitat types, each of which were represented by four replicate sites (Figure 4). Three randomly-located cores of sediment were collected subtidally from each site in each season using a cylindrical corer that was 11 cm in diameter, had a surface area of 96 cm² and sampled to a depth of 15 cm. The sediment samples were wet-sieved through a 500 µm mesh and immediately preserved in 5% formalin buffered in estuary water. The invertebrates were removed from the sediment under a dissecting microscope then identified to the lowest possible taxon and counted. All invertebrates were stored in 70% ethanol to provide a reference.

3.4 Statistical Analyses

3.4.1 Univariate Analyses

Two-way Analysis of Variance (ANOVA) was used to ascertain whether the number of species and total number of benthic macroinvertebrates differed significantly among habitat types and seasons. Both of these independent variables were considered to be fixed. The null hypothesis that the values for a dependent variable did not differ significantly among any independent variable was rejected when the significance level was (p) was <0.05 . Prior to undertaking the above analyses, the relationships between the means and the associated standard deviations for each of the dependent variables were investigated to ascertain which type of transformation, if any, was required to satisfy the test assumptions of normality and homogeneity of variance (Clarke & Warwick, 2001). These relationships showed that both variables required a fourth root transformation.

3.4.2 Multivariate Analyses

Prior to undertaking multivariate analyses of the benthic macroinvertebrate species abundance data, all of which were performed using the PRIMER v6 statistical package (Clarke & Gorley, 2006), the replicate data for each site was meaned, rounded to the nearest whole number and the resultant values subjected to dispersion weighting (Clarke *et al.*, 2006). The latter technique employs an index of dispersion for each species, (*i.e.* a variance to mean ratio) which has the effect of downweighting values for erratically occurring species, yet leaves consistently occurring species virtually unchanged. A Bray-Curtis resemblance matrix was then constructed from the pre-treated data, which was subjected to two- or three- dimensional non-metric multidimensional scaling (nMDS) in order to display the relationships among samples. One-way or two-way crossed Analysis of Similarity (ANOSIM) tests (Clarke & Green, 1988) were employed to ascertain whether the composition of the benthic macroinvertebrate assemblages differed significantly among habitat types and/or seasons. The null hypothesis that there were no significant differences in benthic macroinvertebrate assemblage composition among groups was rejected if the significance level (p) was <0.05 . The R-statistic was used to ascertain the extent of any significant differences, with values below 0.2 regarded as negligible. When ANOSIM detected a significant difference among *a priori* groups and the associated R-statistic was >0.2 , Similarity Percentages (SIMPER) (Clarke, 1993) was used to identify which species typified each group and which contributed most to differences between pairs of groups.

The RELATE procedure was employed to determine whether, in each season, the arrangement of the rank orders of similarity among habitat types in the Bray-Curtis faunal matrix was significantly correlated with that in the complementary Manhattan distance matrix constructed from the enduring environmental variable (EEV) matrix, calculated from the values of ten environmental variables that distinguished those habitat types during the habitat classification procedure. The null hypothesis was that there were no significant matching between habitat type averages of the EEV and the benthic macroinvertebrate assemblage composition this hypothesis was rejected if the significance level (p) was <0.05 .

4.0 Results

4.1 Number of species and individuals of benthic macroinvertebrates

Replicate samples from each of the five habitat types in three seasons between November 2007 and May 2008 yielded 5,519 benthic macroinvertebrates. These samples contained 25 species representing seven phyla, namely Annelida, Crustacea, Uniramia, Mollusca, Nemertea, Turbellaria and Cnidaria (Table 2). The Polychaeta, which were the most speciose class, were represented by 10 species, whereas the Polychaeta, Amphipoda, Anthurida and Bivalvia contributed 63.5, 14.8, 8.2 and 6.8% respectively to the total number of individuals.

Two-way ANOVA showed that the mean number of species, density and Shannon diversity differed significantly among seasons and habitat types, and that there were significant interactions between these two main effects for each of these variables (Table 3). The mean number of species remained fairly consistent between spring and summer at all habitat types except 4, in which it exhibited a pronounced increase. However, the number of species declined between summer and autumn at all habitat types and particularly at 3, 4 and 5. Habitat type 1 was the most speciose, followed closely by habitat type 5 in spring and summer, while the least number of species were found at habitat types 4 and 10 in spring, 10 in summer and, by far, at habitat type 1 in autumn.

Mean density of benthic macroinvertebrates exhibited a similar trend at all habitat types except 13, with the highest densities occurring in summer and the lowest in autumn. Densities at the latter habitat type were greatest in spring and approximately similar in summer and autumn (Figure 5b). Mean densities were greatest at habitat type 1, followed by those at habitat type 3 in spring and summer, while habitat types 4 and 13 harboured the lowest in these seasons. The lowest densities in autumn were recorded at habitat type 1, which exhibited a precipitous decrease between summer and autumn.

Shannon diversity remained fairly consistent between spring and summer at habitat types 1, 3 and 13, but exhibited a pronounced increase between these seasons at habitat types 4 and 10. However, a marked decline in diversity was recorded at all habitat types between summer and autumn. Habitat types 1 and 5 generally contained the most diverse assemblages, while, habitat type 10 and in particular seasons, habitat types 3 and 4, generally contained the least (Figure 5c).

Table 2: Mean density per 0.1m² (M), standard deviation (Sd) of the benthic macroinvertebrate taxa in samples collected at each of the 5 habitat types during spring 2007, summer 2008 and autumn 2008. Each taxon has been assigned to its respective phyla (Ph) (A- Annelida, Cn- Cnidaria, C- Crustacea, M- Mollusca, N- Nemertea, T-Turbellaria and U- Uniramia).

| | Ph | Habitat Type 1 | | | | | | Habitat Type 3 | | | | | | Habitat Type 4 | | | | | |
|---------------------------------|----|----------------|------|--------|------|--------|------|----------------|-----|--------|------|--------|------|----------------|-----|--------|-----|--------|-----|
| | | Spring | | Summer | | Autumn | | Spring | | Summer | | Autumn | | Spring | | Summer | | Autumn | |
| | | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd |
| <i>Armandia intermedia</i> | A | | | 0.1 | 0.3 | 1.6 | 3.1 | | | 2.1 | 3.2 | 5.3 | 4.3 | | | 4.3 | 5.6 | 1.7 | 2.6 |
| Capitellid spp. | A | 3.9 | 5.4 | 24.8 | 18.9 | 7.5 | 14.6 | 17.5 | 6.7 | 13.5 | 16.2 | 14.1 | 18.2 | 0.4 | 0.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| Hydroides sp. | A | 0.6 | 1.2 | | | | | | | | | | | | | 0.1 | 0.3 | | |
| <i>Leitoscoloplos bilobatus</i> | A | 0.3 | 0.9 | | | | | 0.5 | 0.7 | 0.1 | 0.3 | | | 0.4 | 0.9 | 0.2 | 0.4 | 0.1 | 0.3 |
| Nereidid spp. | A | 24.2 | 18.9 | 21.8 | 20.7 | 4.1 | 6.0 | 5.1 | 4.8 | 7.7 | 7.0 | 0.3 | 0.7 | 5.1 | 8.1 | 10.5 | 6.4 | 8.6 | 6.0 |
| <i>Prionospio</i> sp. | A | 0.2 | 0.4 | 7.6 | 13.8 | | | | | 2.3 | 5.2 | | | | | | | | |
| Sabellid sp. | A | | | 0.2 | 0.6 | | | 0.3 | 0.8 | 0.1 | 0.3 | 0.2 | 0.4 | | | 0.1 | 0.3 | | |
| <i>Scoloplos normalis</i> | A | | | 0.3 | 0.6 | 0.2 | 0.4 | 0.8 | 1.2 | 0.7 | 1.0 | 0.8 | 1.0 | 1.1 | 1.4 | 0.8 | 1.2 | 1.6 | 1.2 |
| <i>Scoloplos simplex</i> | A | | | | | | | | | | | | | | | | | | |
| Syllid sp. | A | 3.0 | 4.6 | 0.3 | 1.2 | 0.5 | 1.2 | 1.5 | 3.1 | | | | | | | | | | |
| Cirolandiae sp. | C | | | | | 0.1 | 0.3 | 1.2 | 1.9 | 1.9 | 2.2 | 0.7 | 1.8 | | | | | | |
| <i>Corophium minor</i> | C | 2.6 | 5.2 | 0.1 | 0.3 | 2.3 | 7.2 | 3.1 | 2.4 | 17.0 | 23.9 | 14.2 | 21.0 | 0.1 | 0.3 | 0.2 | 0.4 | | |
| Eusirid sp. | C | | | | | 0.9 | 2.9 | 0.1 | 0.3 | 0.5 | 0.8 | 3.8 | 8.6 | 0.2 | 0.4 | 1.6 | 3.2 | 0.8 | 2.0 |
| Gasterosaccinae sp. | C | | | | | | | | | | | 0.1 | 0.3 | | | | | | |
| Mesanthura sp. | C | 2.3 | 4.2 | 1.4 | 1.8 | 0.3 | 0.6 | 1.7 | 2.2 | 2.6 | 5.6 | 0.7 | 0.9 | 1.2 | 2.0 | 5.0 | 5.1 | 4.6 | 4.3 |
| <i>Palaemonetes australis</i> | C | 0.4 | 0.7 | 0.9 | 1.4 | 0.1 | 0.3 | | | | | | | | | | | | |
| Scyphozoa sp. | Cn | | | | | | | | | 0.1 | 0.3 | | | | | | | | |
| <i>Arthritica semen</i> | M | 1.3 | 1.4 | 2.0 | 4.5 | 1.3 | 4.6 | 1.3 | 1.8 | 1.6 | 2.6 | 0.2 | 0.6 | | | | | | |
| <i>Fluviolatus suborta</i> | M | 4.3 | 4.7 | 6.8 | 7.0 | | | 2.1 | 4.0 | 2.1 | 2.9 | 0.8 | 1.5 | 0.4 | 0.7 | 3.8 | 5.0 | 0.1 | 0.3 |
| <i>Sanguinolaria biradiata</i> | M | | | 0.1 | 0.3 | 0.1 | 0.3 | | | | | | | | | | | | |
| Nemertean sp. | N | | | 0.3 | 0.9 | 0.4 | 1.2 | | | 0.8 | 1.9 | 0.9 | 1.5 | | | | | | |
| Turbellarian sp. | T | 0.7 | 1.7 | | | | | 0.1 | 0.3 | | | | | | | | | | |
| Chironomidae sp. | U | | | | | | | | | | | | | | | | | | |
| Leptoceridae sp. | U | | | | | | | 0.1 | 0.3 | | | | | | | | | | |
| <i>Paratanytarus grimmii</i> | U | 0.4 | 0.7 | 0.4 | 0.7 | 0.9 | 2.1 | 0.7 | 2.0 | 1.4 | 4.6 | 1.2 | 1.9 | 2.7 | 4.2 | 2.0 | 2.0 | 0.5 | 1.4 |

Table 2: Continued

| | Ph | Habitat Type 5 | | | | | | Habitat Type 10 | | | | | |
|---------------------------------|----|----------------|------|--------|-----|--------|------|-----------------|------|--------|-----|--------|-----|
| | | Spring | | Summer | | Autumn | | Spring | | Summer | | Autumn | |
| | | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd | M | Sd |
| <i>Armandia intermedia</i> | A | 0.4 | 0.9 | 6.8 | 4.2 | 4.5 | 4.8 | | | 4.3 | 3.4 | 6.0 | 4.1 |
| Capitellid spp. | A | 11.0 | 13.4 | 0.8 | 1.9 | 0.1 | 0.3 | 17.8 | 10.3 | 0.3 | 0.7 | 0.1 | 0.3 |
| Hydroides sp. | A | | | | | 0.1 | 0.3 | | | | | | |
| <i>Leitoscoloplos bilobatus</i> | A | 0.3 | 0.9 | 0.3 | 0.6 | | | | | | | 0.1 | 0.3 |
| Nereidid spp. | A | 4.4 | 4.5 | 9.4 | 8.6 | 7.3 | 10.4 | 1.4 | 1.4 | | | 0.3 | 0.9 |
| <i>Prionospio</i> sp. | A | | | | | | | 0.1 | 0.3 | | | | |
| Sabellid sp. | A | | | | | | | | | 0.3 | 0.9 | | |
| <i>Scoloplos normalis</i> | A | 1.4 | 1.4 | 1.7 | 1.2 | 0.1 | 0.3 | | | 0.8 | 1.1 | 0.3 | 0.5 |
| <i>Scoloplos simplex</i> | A | | | 0.1 | 0.3 | | | | | | | | |
| Syllid sp. | A | 0.3 | 0.7 | | | | | | | | | | |
| Cirolandiae sp. | C | 0.4 | 0.9 | 0.1 | 0.3 | | | 0.1 | 0.3 | 0.5 | 1.7 | 0.1 | 0.3 |
| <i>Corophium minor</i> | C | 3.3 | 4.6 | 2.9 | 7.5 | 0.4 | 1.4 | 0.2 | 0.4 | 4.8 | 7.1 | 5.4 | 7.0 |
| Eusirid sp. | C | 0.2 | 0.4 | 0.4 | 0.8 | 1.7 | 5.8 | | | 0.2 | 0.4 | 1.7 | 4.8 |
| Gasterosaccinae sp. | C | | | | | | | 0.2 | 0.4 | | | | |
| Mesanthura sp. | C | 4.6 | 6.4 | 4.5 | 4.3 | 8.6 | 9.2 | | | | | 0.5 | 0.7 |
| <i>Palaemonetes australis</i> | C | | | 0.1 | 0.3 | | | | | | | 0.1 | 0.3 |
| Scyphozoa sp. | Cn | | | | | | | | | | | | |
| <i>Arthritica semen</i> | M | 0.8 | 1.0 | 0.3 | 0.6 | 0.1 | 0.3 | 1.7 | 1.2 | | | | |
| <i>Fluviolatus suborta</i> | M | 0.1 | 0.3 | 0.2 | 0.6 | | | | | 0.2 | 0.4 | | |
| <i>Sanguinolaria biradiata</i> | M | | | | | 0.1 | 0.3 | | | | | | |
| Nemertean sp. | N | | | | | | | | | 0.3 | 0.5 | | |
| Turbellarian sp. | T | 0.1 | 0.3 | | | | | | | | | | |
| Chironomidae sp. | U | | | 0.1 | 0.3 | 0.6 | 1.5 | | | | | | |
| Leptoceridae sp. | U | | | | | | | | | | | | |
| <i>Paratanytarus grimmii</i> | U | 1.3 | 1.9 | 8.7 | 8.2 | 0.2 | 0.4 | 0.2 | 0.6 | 1.0 | 1.6 | 0.3 | 1.2 |

Table 3: Mean squares and their significance levels for ANOVA on the number of species, total density and Shannon diversity of the benthic macroinvertebrate communities in each of the five habitat types sampled seasonally between November 2007 and May 2008. Df, degrees of freedom. *** $p < 0.001$.

| | Df | No Species | Density (0.1m ²) | Shannon Diversity |
|-----------------------|-----|------------|------------------------------|-------------------|
| Season | 2 | 0.5285*** | 2.151*** | 1.976*** |
| Habitat Type | 4 | 0.2934*** | 1.635*** | 1.516*** |
| Season x Habitat Type | 8 | 0.1494*** | 0.975*** | 0.721*** |
| Total | 180 | | | |

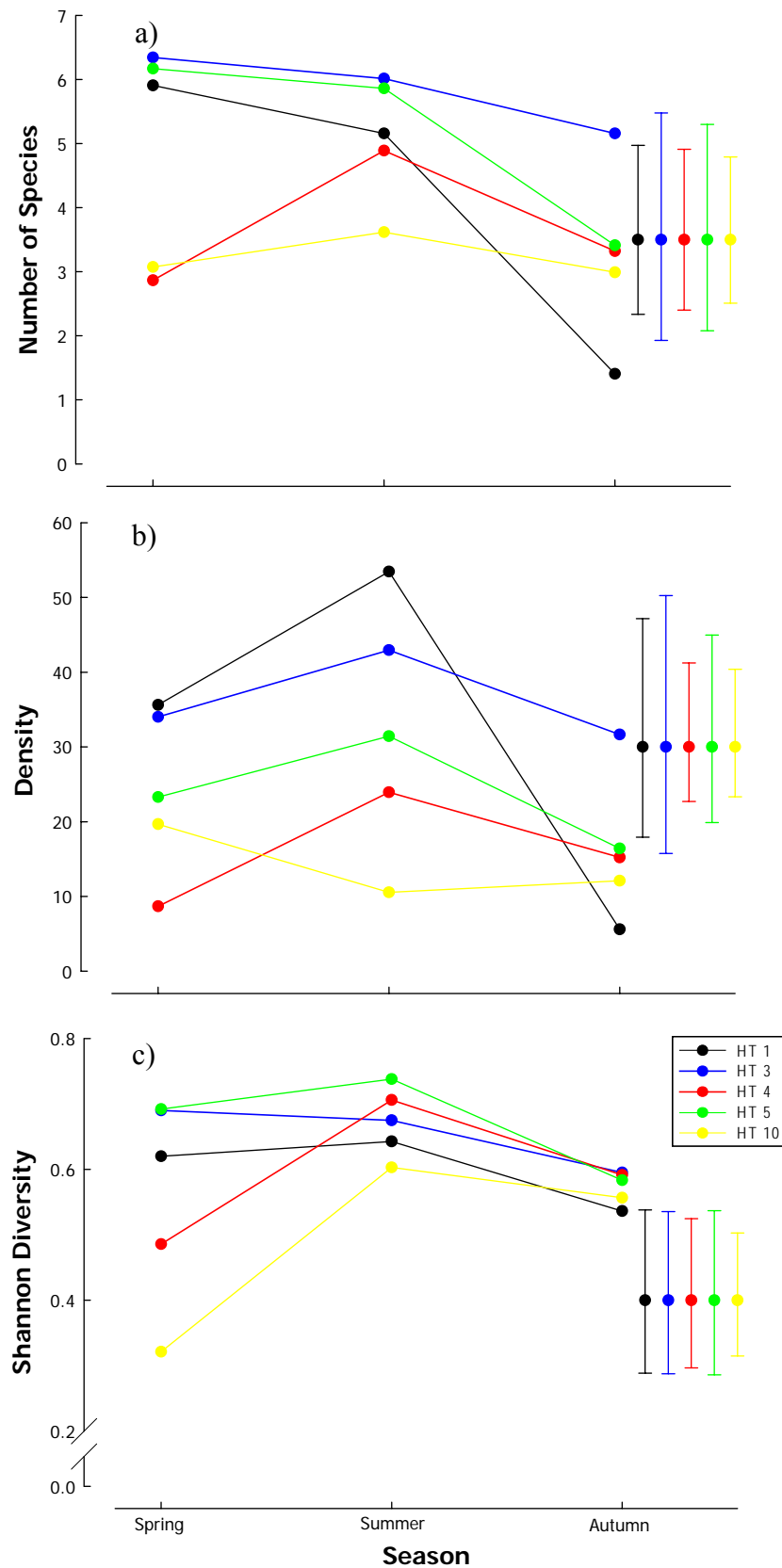


Figure 5: Mean ($\pm 95\%$ confidence intervals) for the a) number of species, b) density and c) Shannon diversity of benthic macroinvertebrate fauna at each of the five habitat types in Broke Inlet in the three seasons between November 2007 and May 2008.

4.2 Comparison between benthic invertebrates assemblages at different habitat types and seasons

Two-way ANOSIM demonstrated that the species composition of the benthic macroinvertebrate assemblages differed significantly among habitat types and seasons ($p=0.001$). Moreover, the Global R-statistic was greater for habitat type (0.418) than season (0.304). When the same data was subjected to 3-d MDS ordination samples from habitat type 1 formed a relatively distinct band extending down the right side of the plot shown in figure 6a. Habitat types 3 and 10 also formed a closely associated but relatively distinct group while samples from habitat types 4 and 5 formed an intermingled group in the centre of the plot. The species composition in each habitat type was significantly different from that at other habitat types, except for habitat types 5 vs 4 and 5 vs 3. The R-statistic values for these pairs of habitat types the exhibited significant differences in benthic macroinvertebrate composition ($p=0.001$) ranged from 0.302 to 0.698, with the largest values involving the comparison between habitat types 1 and 10. Greater densities of Nereidid Spp. and *Fluviolatus suborta* typified the benthic macroinvertebrate fauna at habitat type 1 and distinguished its fauna from that at all other habitats. Habitat types 3 and 10, although shown to harbor a significantly different fauna both were characterised by the amphipod *Corophium minor* and the polychaete *Scoloplos normalis*, while Capitellid spp and *Paratanytarus grimmii* distinguished the two habitat types. The anthurid *Mesanthura* sp. and the insect larva stage of *Paratanytarus grimmii* typified the benthic faunal assemblage at both habitat types 5 and 4 and distinguished them from the other habitat types (Table 4).

When the samples in the above 3-d MDS plot were coded for season, the majority of those from spring formed a discrete group in the top right of the plot, whereas those from summer and autumn were relatively dispersed throughout the lower half of the plot (Figure 6b). Pairwise ANOSIM comparisons between seasons showed that the species composition of benthic macroinvertebrates differed significantly between spring and summer and spring and autumn ($p=0.001$) with R-statistics of 0.331 and 0.477 respectively. However, no significant differences were observed between summer and autumn ($p=0.12$). The benthic macroinvertebrate fauna in spring was characterised by the bivalve *Arthritica semen* and polychaetes Capitellid spp. and Nereidid spp., whereas summer and autumn were characterised by the polychaete *Armandia intermedia* and the anthurid *Mesanthura* sp. (Table 5).

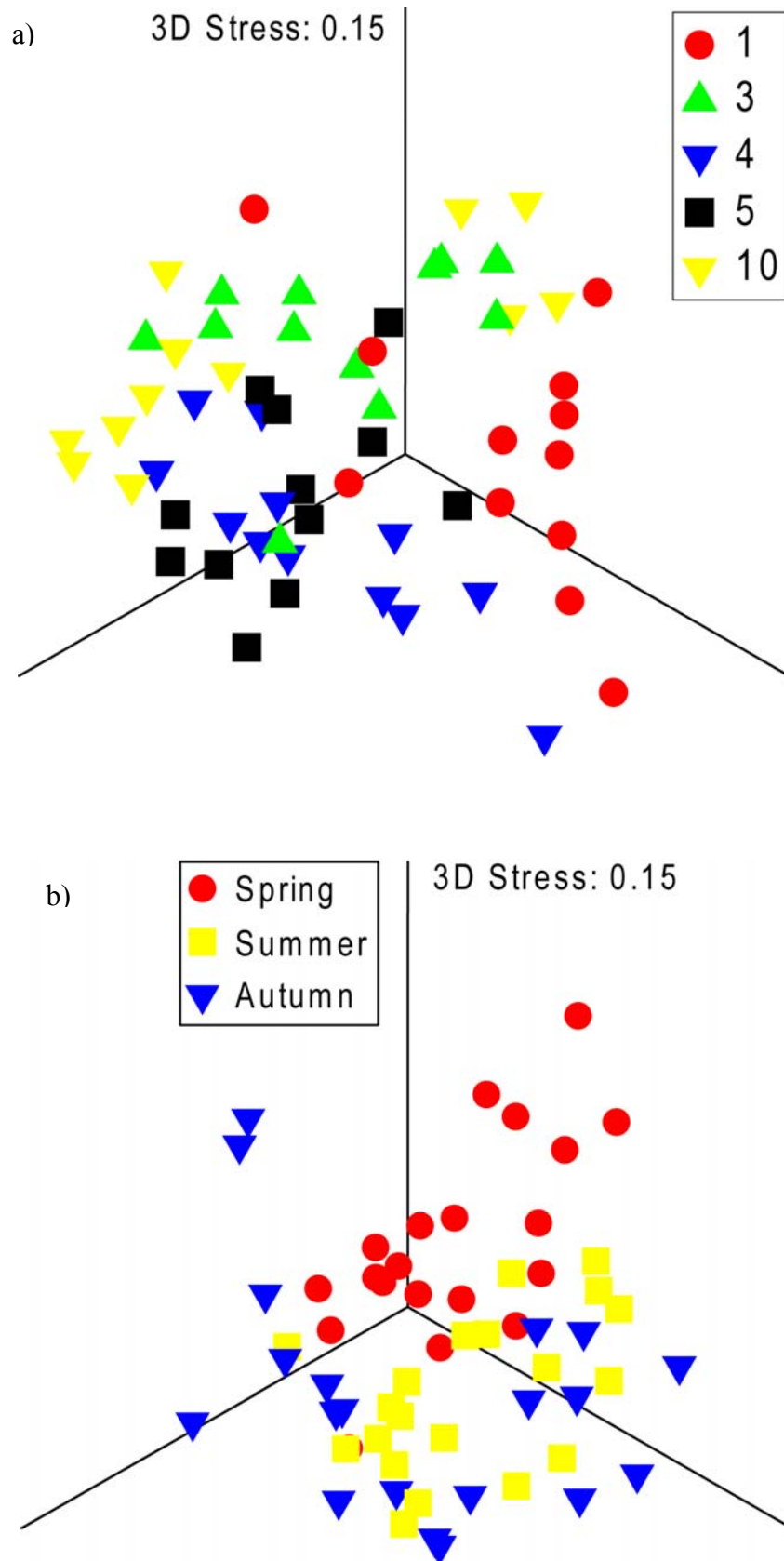


Figure 6: Three-dimensional MDS ordination on the abundance of benthic macroinvertebrate species in samples collected at five habitat types in Broke Inlet and three seasons between November 2007 and May 2008. In a) samples coded for habitat type and b) samples coded for season.

Table 4: Species identified by a Two Way SIMPER as those which typified the benthic macroinvertebrate fauna at each of the five habitats types samples in Broke Inlet, and those that distinguished the faunas at each pair of habitat types. The associated average similarity and dissimilarity percentages, respectively, are also shown. The habitat type at which distinguishing taxa were most abundant is provided in superscript. Grey shading represents those pairs of habitat types that did not contain significantly different faunal compositions.

| | 1 | 3 | 4 | 5 | 10 |
|----|--|--|---|---|---|
| 1 | 32.75% | | | | |
| | <i>Paratanytarus grimmii</i> <i>Fluviolanatus subtorta</i> Nereidid spp. Mesanthura sp. | | | | |
| 3 | 72.25% | 38.81% | | | |
| | Capitellid spp. ⁽³⁾ <i>Scoloplos normalis</i> ⁽³⁾ Nereidid spp. ⁽¹⁾ <i>Fluviolanatus subtorta</i> ⁽¹⁾ | <i>Corophium minor</i> <i>Scoloplos normalis</i> <i>Fluviolanatus subtorta</i> Capitellid spp. | | | |
| 4 | 77.79% | 73.47% | 36.67% | | |
| | Nereidid spp. ⁽¹⁾ Mesanthura sp. ⁽⁴⁾ <i>Fluviolanatus subtorta</i> ⁽¹⁾ <i>Paratanytarus grimmii</i> ⁽⁴⁾ <i>Palaemonetes australis</i> ⁽¹⁾ | Mesanthura sp. ⁽⁴⁾ Nereidid spp. ⁽⁴⁾ Capitellid spp. ⁽³⁾ <i>Scoloplos normalis</i> ⁽⁴⁾ <i>Pontomyia</i> sp. ⁽⁴⁾ | Nereidid spp. Mesanthura sp. <i>Paratanytarus grimmii</i> | | |
| 5 | 78.94% | | | 38.39% | |
| | Nereidid spp. ⁽¹⁾ <i>Scoloplos normalis</i> ⁽⁵⁾ Capitellid spp. ⁽¹⁾ <i>Fluviolanatus subtorta</i> ⁽¹⁾ | | | Nereidid spp. Mesanthura sp. <i>Scoloplos normalis</i> <i>Armandia intermedia</i> | |
| 10 | 82.32% | 62.33% | 76.89% | 69.48% | 53.82% |
| | Capitellid spp. ⁽¹⁾ Nereidid spp. ⁽¹⁾ <i>Palaemonetes australis</i> ⁽¹⁾ <i>Fluviolanatus subtorta</i> ⁽¹⁾ Mesanthura sp. ⁽¹⁾ | Capitellid spp. ⁽³⁾ Cirolanidae sp. ⁽³⁾ <i>Paratanytarus grimmii</i> ⁽³⁾ <i>Armandia intermedia</i> ⁽¹⁰⁾ | Mesanthura sp. ⁽⁴⁾ Nereidid spp. ⁽⁴⁾ <i>Scoloplos normalis</i> ⁽⁴⁾ <i>Armandia intermedia</i> ⁽¹⁰⁾ | <i>Scoloplos normalis</i> ⁽⁵⁾ Nereidid spp. ⁽⁵⁾ Mesanthura sp. ⁽¹⁰⁾ <i>Corophium minor</i> ⁽⁵⁾ <i>Armandia intermedia</i> ⁽¹⁰⁾ | <i>Armandia intermedia</i> <i>Scoloplos normalis</i> <i>Corophium minor</i> |

Table 5: Species identified by a Two Way SIMPER as those which typified the benthic macroinvertebrate fauna at each of the three seasons sampled in Broke Inlet, and those that distinguished the faunas at each pair of habitat types. The associated average similarity and dissimilarity percentages, respectively, are also shown. The season at which distinguishing taxa were most abundant is provided in superscript. Grey shading represents those pairs of seasons that did not contain significantly different faunal compositions.

| | Spring | Summer | Autumn |
|--------|--|---|--|
| Spring | 41.39% <i>Arthritica semen</i> Nereidid spp. Capitellid spp. <i>Paratanytarus grimmii</i> | | |
| Summer | 69.83% Capitellid spp. ^(Sp) Nereidid spp. ^(Su) <i>Armandia intermedia</i> ^(Su) <i>Scoloplos normalis</i> ^(Su) <i>Arthritica semen</i> ^(Sp) | 42.62% <i>Scoloplos normalis</i> <i>Paratanytarus grimmii</i> <i>Armandia intermedia</i> Mesanthura sp. | |
| Autumn | 78.92% <i>Armandia intermedia</i> ^(Au) Capitellid spp. ^(Sp) Nereidid spp. ^(Sp) <i>Paratanytarus grimmii</i> ^(sp) Mesanthura sp. ^(Au) | | 36.25% <i>Armandia intermedia</i> Mesanthura sp. |

4.3 Comparisons between benthic macroinvertebrate assemblages among habitats in each season

Attention was next focused on examining the extent of the differences in benthic macroinvertebrates composition among habitat types, after any confounding influences due to differences among seasons had been removed. One-way ANOSIM tests for habitat types were thus carried out separately for the data recorded in each seasons. These tests demonstrated that the benthic macroinvertebrate composition differed significantly among habitat types in all cases ($p < 0.05$) and that the extent of the differences was greatest in spring ($R = 0.493$) and summer ($R = 0.476$) and substantially lower in autumn ($R = 0.285$).

Pairwise comparisons between habitat types for samples collected in spring were significant in seven of the ten comparisons ($R = 0.583 - 0.906$; $p < 0.05$). No significant differences were found between habitat types 4 vs 5, 4 vs 3 and 5 vs 3 in this season. MDS ordination of this data showed that samples from habitat types 1 and 10 formed discrete groups, while those from 3 and 5 were intermingled and those from habitat type 4 were more dispersed (Figure 7a). A similar trend in differences between the faunal composition of habitat types was observed in summer, with six of the ten pairwise comparisons exhibiting significant differences ($p < 0.05$), and the most pronounced differences occurring between habitat types 1 and 10 ($R = 0.938$). These results were reflected by the 3-d MDS plot constructed from data collected in spring, in which habitat type 1 formed a distinct group on the left of the plot, and was located the greatest distance from habitat 10. While samples from habitat type 5 also formed a relatively tight group, those from habitat types 3 and 4 were relatively dispersed. In autumn, only four out of the ten pairwise comparisons produced significant results, namely habitat types 3 vs 4, 3 vs 5, 10 vs 4 and 10 vs 5. MDS ordination analysis supports these results with habitat type 3 forming a distinct group at the bottom of the plot, with those from and habitat types 4 and 5 occupying the upper regions (Figure 7c).

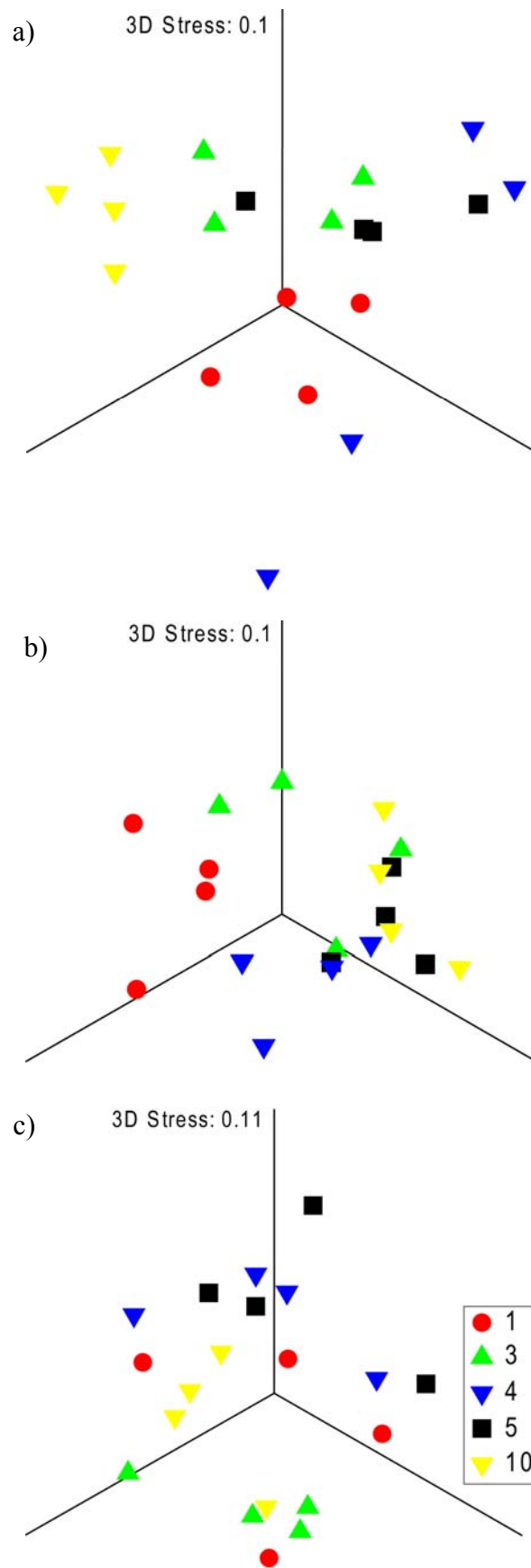


Figure 7: Three-dimensional MDS ordination on the abundance of benthic macroinvertebrate taxa recorded at the five habitat types in Broke Inlet in a) spring, b) summer and c) autumn.

4.4 Relationships between faunal composition and habitat classification

The RELATE procedure showed that the arrangement of the rank orders between sites representing each of the five habitat types in the resemblance matrix constructed from the faunal composition data in both spring and summer (Figure 8b; 8c) was significantly correlated with that derived from enduring environmental data (Figure 8a) used to classify these habitat types $p=0.001$, $Rho=0.441$ in spring and $p=0.001$, $Rho=0.367$ in summer respectively. Such results indicate that the pattern of spatial differences exhibited by the benthic macroinvertebrate fauna in spring and summer was well reflected by that of the environmental characteristics used to distinguish habitat types. However, the resemblance matrices constructed from the benthic macroinvertebrate data recorded in autumn (Figure 8d) were clearly not correlated with the enduring environmental matrix, $p>0.05$, $Rho=0.104$.

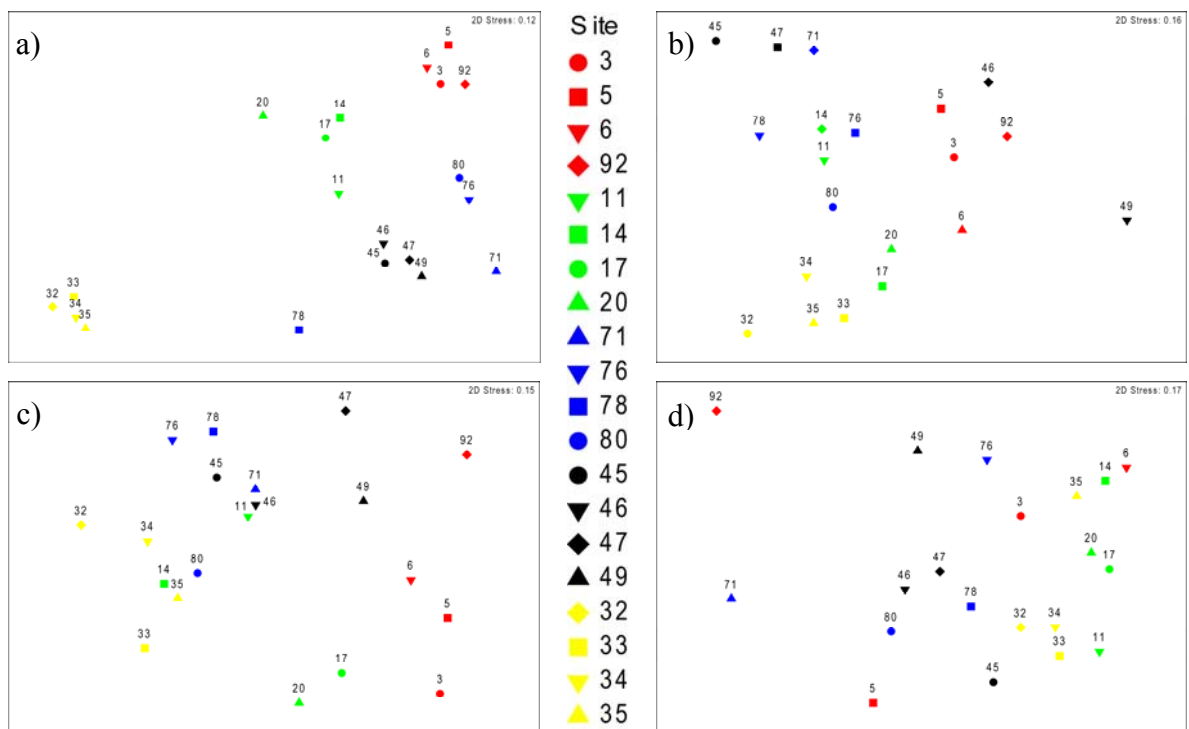


Figure 8: Two-dimensional MDS ordinations on a) the average enduring environmental variables recorded in each of the habitat types and the average benthic macroinvertebrate faunal assemblage present in each habitat type in b) spring, c) summer and d) autumn.

5.0 Discussion

This study has demonstrated that the composition of the benthic macroinvertebrate assemblages within the nearshore waters of Broke Inlet is significantly influenced by habitat type in spring, summer and autumn 2007/2008. However, these habitat differences were far more pronounced in the first two of these seasons. Furthermore, in spring and summer, the pattern of spatial differences in the faunal composition among habitat types was well reflected by that of the enduring physical characteristics employed to identify those habitat types ($p=0.01$, $Rho=0.441$ in spring and $p=0.01\%$, $Rho=0.367$ in summer). However, no significant match was detected between the faunal and enduring environmental variable matrices in autumn ($p=0.164$, $Rho=0.110$), which reflected the fact that faunal differences between habitat types were reduced.

The observed seasonal changes in the extent to which i) the various habitat types were characterised by significantly different faunal assemblages and ii) the spatial pattern among habitat types in the faunal matrix was correlated with that in the environmental matrix, is most likely influenced by whether the bar of the estuary is open or closed. Dye and Barros (2005a; b) and Dye (2006) found that changes in the density of both macrobenthos and meiobenthos in estuaries were related to the bar state (*i.e.* open or closed). Various other studies have also linked changes in the density and distribution of macrobenthos in coastal lagoons to the period of isolation from the sea (Castel, 1992; Guelorget & Perthuist, 1992; Koutsoubas *et al.*, 2000; Teske & Woodridge, 2001), during which concurrent changes in physicochemical variables such as salinity, dissolved oxygen content and temperature occur, and which are thought to lead to reductions in faunal diversity and abundance (Holland *et al.*, 1987; Guelorget & Perthuist, 1992; Warwick & Clarke, 1993; Koutsoubas *et al.*, 2000). Furthermore, as some estuarine benthic macroinvertebrate species are also known to occur in marine waters (Kennish, 1990), bar closure prevents both the migration of adults and also the recruitment of their larvae into estuaries as many species spawn during summer when the mouths of many seasonally open estuaries on the south coast are typically closed (Kalejita & Hockey, 1991; Quijón & Jaramillo, 1993; Sardá *et al.*, 1995).

The characteristics of the salinity regime of Broke Inlet exhibited pronounced seasonal differences throughout the study period. Thus, during spring 2007, the estuary was open to the Southern Ocean, and had been as such since early September. Together with freshwater

input via the Shannon, Forth and Inlet rivers from winter rains, these conditions produced a pronounced salinity gradient throughout the system, with salinities around 10-12 occurring at the river mouths, ~17 in Shannon basin, ~22 in the Middle basin and 35 in the entrance channel (Tweedley, unpublished data). Conversely, during autumn 2008, approximately four and a half months after the bar of the estuary had closed and rainfall had reduced markedly over the summer months, salinities were remarkably consistent across the estuary, *i.e.* ~30 throughout the basins and entrance channel. As a component of the current habitat classification scheme is dependent on spatial differences in water quality (*i.e.* location throughout the system), the spatial homogeneity in salinity that was recorded in autumn would have contributed to a reduction in the environmental distinction among habitat types. Changes in salinity have been shown to be a major influence on the spatial and temporal distribution of macrobenthos within estuarine environments (Jones *et al.*, 1986; Rakocinski *et al.*, 1997; Kanandjembo *et al.*, 2001; Hirst, 2004). Given the above, it is thus not surprising that the composition of the benthic macroinvertebrate fauna differed least among habitat types in autumn, and that the pattern of spatial differences in these faunas did not match that exhibited by the enduring environmental data. Furthermore, Platell & Potter (1996) hypothesised that the lack of a pronounced salinity gradient in the nearby Wilson Inlet, another seasonally open system, was partly responsible for the depauperate macrobenthic faunal assemblages present within those waters. Such conclusions have also been made by several other workers (e.g. de Decker & Bally, 1985; Stoner & Acevedo, 1990).

The seagrass *Ruppia megacarpa*, the dominant aquatic vegetation type within Broke Inlet, has been shown to undergo large seasonal differences in percentage cover, shoot density, above and below ground biomass and maximum shoot length due to fluctuations in a range of hydrological parameters that occur in seasonally open estuaries (Carruthers *et al.*, 1999). Although the spatial distribution of *R. megacarpa* throughout Broke Inlet remained relatively unchanged throughout the study macrophyte abundance and biomass in Broke Inlet was greatest in summer and underwent considerable declines in autumn (Tweedley, unpublished data). This coincided with marked reductions in the number of species, density and diversity of benthic macroinvertebrate fauna at almost all habitat types, which is also likely to have contributed to the reduced faunal distinction among habitat types in this season. Seagrass beds have been shown to harbour a significantly greater number of species and individuals than that nearby unvegetated sediment (Orth *et al.*, 1984;

Hutchings *et al.*, 1991; Edgar & Shaw, 1993; Mattila *et al.*, 1999), which has been related to a range of factors including increased food abundance, sediment stability, protection from predators and habitat complexity (Heck & Wetstone, 1977; Heck & Orth, 1980; Connolly, 1995). Furthermore, Platell & Potter (1996) reported that the abundance of certain benthic macroinvertebrate species within Wilson Inlet was correlated positively with the presence of *R. megacarpa*.

Several approaches for classifying and/or predicting habitat types in coastal environments have been adopted throughout the world. While there are numerous ways of distinguishing among such schemes, one major difference depends on whether they have been based on (i) the distribution of particular species (Paine, 1966; Estes & Palmisano, 1974) or taxa e.g. benthic macroinvertebrates (Ellis *et al.*, 2006) or fish (Monaco *et al.*, 1992; Araújo & Costa de Azevedo, 2001), (ii) abiotic criteria (e.g. Dethier 1992; Digby *et al.* 1998; Zacharias *et al.* 1998; Roff & Taylor 2000) or (iii) a mixture of (i) and (ii) above (e.g. Mumby & Harborne 1999; Zacharias *et al.* 1999; Allee *et al.* 2000; Connor *et al.* 2004; Madden *et al.*, 2005). One advantage of using biology as the basis of such classification schemes is that there is clearly no need to correlate biological distributions with physical parameters. However, as many types of fauna are unable to be mapped remotely, and direct sampling over the necessary spatiotemporal scales is often time-consuming and expensive (Roff & Taylor, 2000). In contrast, classifications based on abiotic variables are typically applicable to a range of biota, and the type of data employed in such schemes can often be measured from maps that are readily available. For example, remotely sensed imagery and Geographical Information Systems (GIS) can provide data on coastal geomorphology, bathymetry, chlorophyll levels, water temperature and benthic substratum type, each of which either directly influences faunal distribution or provides surrogates for influential variables.

Several abiotic classification schemes have been developed which require, their finer levels, data for various *in-situ* environmental variables, such as salinity, dissolved oxygen, sediment grain size, macrovegetation biomass and turbidity (e.g. Connor *et al.*, 2004; Madden *et al.*, 2005). However, as with classifications based mainly on biota, these schemes also require an extensive prior field sampling in order to produce meaningful results. Furthermore, in estuaries that become seasonally isolated from marine waters, such as many of those in south-western Australia, obtaining sufficient *in-situ* environmental data to account for the dramatic seasonal and inter-annual changes that are associated with bar openings maybe particularly time-consuming (Potter & Hyndes, 1999). Therefore, like the current scheme, many approaches to

habitat classification employ temporally enduring environmental variables which act as surrogates for *in-situ* variables (e.g. Roff & Taylor, 2000; Valesini *et al.*, 2003; Valesini *et al.*, in prep).

The classification procedure used during this study parallels that used by Wildsmith *et al* (2005) and Wildsmith (2008) to successfully distinguish spatial differences in the distribution of the benthic macroinvertebrate faunas of both nearshore marine and estuarine systems in south-western Australia. Despite several studies reporting that the use of abiotic variables are largely ineffective at distinguishing between fauna in coastal waters (Robinson & Levings, 1995; Stevens & Connolly, 2004), Wildsmith (2008) found that the characteristics of benthic macroinvertebrate faunas in the permanently open Swan-Canning Estuary not only differed significantly among the habitat types generated by the Valesini *et al* (in prep) classification scheme but that the faunas were more related to differences in habitat type than those among a suite of *in-situ* environmental variables including salinity, water temperature, dissolved oxygen, sediment grain size and organic matter and redox depth. Moreover, the underlying pattern in the spatial distribution of benthic macroinvertebrates among habitat types was well matched with that defined by the enduring environmental variables of those habitat types, thus indicating that the habitat type classification scheme provides a sound basis for predicting the occurrence of benthic macroinvertebrate taxa throughout the estuary. While results from the current study demonstrate that the extent to which differences in habitat type “explain” the extent to which differences in benthic macroinvertebrate assemblages were not as great as those detected by Wildsmith (2008), they were still reasonable, particularly when the estuary remains closed for more extended periods, water quality conditions within Broke Inlet becomes more homogenous which is reflected by reduced differences in benthic macroinvertebrate assemblages among habitat types.

In summary, a habitat classification scheme developed by Valesini *et al* (in prep) which produced an accurate and distinct group of benthic macroinvertebrate habitat types in the permanently open Swan Canning Estuary was applied to the seasonally open Broke Inlet. Although a significantly different benthic macroinvertebrate assemblage was detected among habitat types in each season, matching between the faunal assemblage and the enduring environmental variables only occurred during spring and summer *i.e.* periods of an open or recently closed connection to the ocean. It is hypothesised that the non significant matching in autumn reflected the more homogenous water quality conditions

present during that season caused by the closing of the bar four months earlier and the seasonal changes in biomass of the dominant macrophyte *Ruppia megacarpa*. Therefore, this study has demonstrated that the use of habitat classifications schemes which employ enduring environmental variables can distinguish between benthic macroinvertebrate faunas in different habitat types albeit currently on when the bar is open or had recently closed. These habitat classification schemes based on enduring environmental variables warrant further investigation and work is currently underway investigating fish and habitat type relationships in Broke Inlet and other south-western Australian estuaries.

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