INVESTIGATIONS OF THE REFERENCE CONDITION APPROACH AND INTERTIDAL ECOLOGY OF MINAS BASIN, BAY OF FUNDY, WITH REFERENCE TO THE IMPACTS OF INTERTIDAL HARVESTING

by

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This thesis is accepted in its present form by the Division of Research and Graduate Studies as satisfying the thesis requirements for the degree Master of Science (Biology).

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ABSTRACT

Intertidal harvesting for clams and bloodworms in the Minas Basin results in physical disturbance of the tidal flats, the biological effects of which are largely unknown. This study examined the applicability of the Reference Condition Approach, a freshwater ecological assessment method, for assessing the biological effects of physical disturbance in the intertidal estuarine environment of the Minas Basin tidal flats. Physical disturbance was found to significantly increase grain size, total benthic organism densities, and nematode worms and harpacticoid copepods. Physical disturbance also significantly lowered densities of tube building polychaetes, specifically maldanids and spionids. The BEnthic Assessment of SedimenT (BEAST) approach showed most promise in the Minas Basin than the River InVertebrate Prediction and Classification System (RIVPACS), however the inclusion of more relevant environmental variables is needed to fully assess model performance and the application of predictive models in the study area.

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CHAPTER 1: INTRODUCTION

1.1 Background

The Estuarine Environment

Benthic organisms are a significant component of estuarine systems, and are often found in high densities (de Deckere *et al.*, 2000). They play a major role in the ecosystem, providing an essential link between primary production and higher trophic levels. Consuming a substantial amount of the total primary production (Herman *et al.*, 1999), benthic organisms of the Minas Basin are a primary food source for many crustaceans, fish and birds (Daborn *et al.*, 1993).

The physical and chemical properties of sediments and surrounding waters such as salinity, redox potential, current velocities and inundation time all have significant effects on the distribution of the benthos (de Deckere *et al.*, 2000). In turn, benthic organisms affect the nature of the surrounding sediments, both directly and indirectly. Bioturbation as a result of feeding activity and burrowing oxygenates the sediment (de Deckere *et al.*, 2000). Mass production of fecal pellets on the sediment surface by some deposit feeders results in higher porosity and less cohesion, thereby enhancing erosion (Rhoads and Young, 1970). Tube and burrow construction by many organisms involves mucous secretion for binding sediment particles that can offer either a stabilizing or destabilizing effect (de Deckere *et al.*, 2000). Indirectly, benthos may affect the intertidal micro-relief as a result of tube construction, burrows, and shells, which may increase local erosion processes (Anderson, 1983).

Processes such as current dynamics, circulation, wind waves, sedimentation and flocculation, consolidation, biological stabilization and bioturbation (Grant *et al.*, 1986;

Dyer, 1998) all impact the behaviour of fine sediments. Ultimately, it is the combined effects of the physical, chemical and biological processes in the sediments, and in the water, that influences the distribution of benthic organisms.

Disturbance

All marine communities experience biological and physical forms of disturbance which affect the spatial and temporal heterogeneity of ecosystems and the relative abundances of the resident species (Denslow, 1985). Dayton (1971) was one of the first to recognize disturbance as a key process influencing community structure. The disturbance regime of an area can be quantified in four dimensions: size, intensity, frequency, and duration.

For the purposes of this study, disturbance is defined as any event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability or the physical environment (White and Pickett, 1985). It refers to the damage or mortality itself, meaning the *effect* of some external agent or force, and not the force itself (Sousa, 2001). The definition, intended to be general, encompasses environmental fluctuations (e.g. decreased salinity due to spring freshets) and destructive events (e.g. ice scour and wave energy), which are typically perceived as natural for a particular system. Anthropogenic disturbances, perceived as not natural in such systems, could also be included in this definition. Anthropogenic disturbances include digging for clams and bloodworms, dyking salt marshes, releasing raw sewage into marine waters, and constructing causeways.

Although the term ‘disturbance’ invokes negative connotations in the human context, it is actually beneficial to many species. An enrichment disturbance model (Figure 1.1) was well described by Pearson and Rosenberg (1978). Some degree of disturbance results in increasing food resource availability (i.e. decomposition of mortalities) and the creation of open space for larval recruits and mobile fauna, thereby increasing availability of habitat for more opportunistic species. Those species that do not compete well in stable environments are usually referred to as the 'r-strategists' or opportunists. The freeing of space and food resources leads to the development of a colonization community, characterized by high population density and relatively low taxa richness and biomass. Organisms exhibiting an r-strategy are typically small, short-lived species with high reproductive rates and dispersal abilities (Newell *et al.*, 1998), and rely on disturbed patches for survival. Examples of Minas Basin r-strategists are *Capitella capitata* and *Chaetozone setosa* (Hitchcock, 1999). After a period of time a transitional community will develop, which tends to have lower population densities than the colonizing community. This is a result of the typical r-strategist life history of rapid reproduction, followed by exceeding the carrying capacity of the environment and a prompt decline in numbers (Newell *et al.*, 1998). As the 'boom and bust' of the colonizing community progresses, new species (typically larger) are also colonizing the disturbed area through bedload transport, larval settlement, and migration from the patch edges, resulting in an overall increase in number of taxa. These new, larger species combined with the initial colonizing species contribute to an overall increase in total area biomass (Figure 1.1). Finally, an equilibrium community develops which is mostly dominated by the larger K-strategists who are better adapted to stable environments. These species allocate more resources towards non-reproductive activities and have evolved to become more efficient and specialized competitors with lower reproductive rates. Populations are usually at or near carrying capacity (Pearson and Rosenberg, 1978). Examples of Minas Basin K-strategists are *Nephtys* spp. and *Macoma balthica* (Hitchcock, 1999).

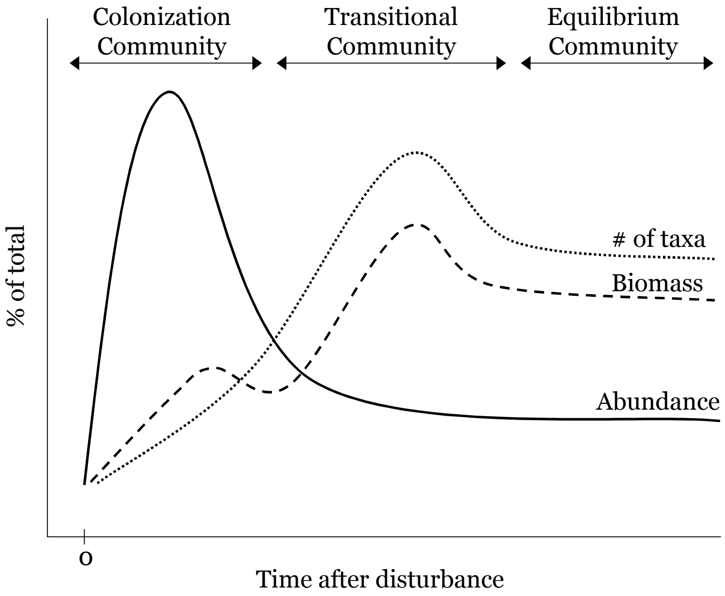

Figure 1.1. Theoretical illustration of an ecological succession gradient in benthic communities after disturbance. The solid line indicates total organism abundance, the dashed line the total biomass, and the dotted line the number of taxa. Originally adapted from Pearson and Rosenberg (1978), modified from Querbach (2002).

During the summer months, the Minas Basin tidal flats can experience frequent physical disturbances through storm events that produce stronger than average waves and/or storm surges. Each winter, the system experiences considerable intertidal ice formation resulting in scouring of the tidal flat surface and associated displacement of organisms; the resutling impact of disturbance is intense enough to keep the ecosystem in an early stage of successional development (Daborn, pers. comm.; (Gordon Jr. and Desplanque, 1983). The theoretical equilibrium community described by Pearson and Rosenberg (1978) may not be found in such naturally disturbed intertidal areas.

Study Designs and Bioassessment

The most reliable approach for experimentally testing the effects of various disturbances is in a laboratory setting, where all variables can be simulated and/or controlled. In field experiments this is not possible, and one is forced to choose control and test sites that are as similar as possible, and manipulate the variable of interest. The most common approach to this type of impact study in the field has been the Before-After, Control-Impact (BACI) study design.

The BACI (Before-After, Control-Impact) design was proposed by Bernstein and Zalinski (1983) and Stewart-Oaten *et al.* (1986) as a way of assessing impacts. Measures of species abundance would be taken at two sites (the site of a putative impact and a similar control site where no impact was expected) on several occasions both before and after the onset of the putative impact. Repeated measurements would thereby allow one to determine if any observed changes at the site of the putative impact are part of a pre-existing cycle of change, or directly attributable to the effects of the impact. The use of a single control led to criticism of this method (Underwood, 1992), who suggested that a natural change at the control site which was coincidentally similar to that caused by the impact at the other site could lead to the impact going undetected. Alternatively, a change at the control site from before to after the onset of the putative impact while the other site remained unchanged, could result in an impact being diagnosed where there was none. Underwood proposed the solution of using several randomly-selected control (or reference) sites.

In the past, the difficulty of field variability, particularly in river systems, has been ameliorated by choosing test and control sites that are in close proximity (Reynoldson *et al.*, 1997), however tidal flats are spatially complex ecosystems. Morrisey *et al.* (1992) and Stark *et al.* (2003) examined spatial variation in the distribution of soft-sediment macrofauna, and found significant faunal variation at scales ranging from 1 m to 3.5 km. The significant small-scale patchiness of the benthic communities in both studies lowers confidence in any assumed similarity between adjacent sites simply because of their proximity. This idea is further supported by Underwood and Chapman (1996), who found more faunal variability at the tens of centimeters scale than at the thousands of meters scale. These studies suggest that a study design with control and impact sites in close proximity may not be an appropriate solution to reducing variability in the field. The Reference Condition Approach, used in this study and described below, may offer a more suitable alternative.

Macrobenthos in soft sediment environments are the most widely used component of the marine biota for studies of environmental effects and assessing ecosystem health (Gray, 1977). Advantages of using macrobenthos in studies include well established taxonomy, relatively low mobility, ease of quantifying density, and some knowledge of responses to pollution (Ellis *et al.*, 2000). Examination of the community level of biological organization is most typical for environmental impact studies, mostly based on structural changes in communities, and there is growing evidence that stressed ecosystems result in biotic impoverishment, reduced productivity, and favourable conditions for opportunistic species (Rapport, 1989).

Ecosystem health

Humans have caused ecosystem-scale changes for hundreds of years in the Bay of Fundy, but does this mean that we have degraded its health? The answer to this question depends upon whom you ask, and is largely perceived by individual perspective and personal relationships to the resources. There is no right answer, and each perception provides an important aspect of the larger picture. When applied to an ecosystem, the terms health and integrity can be used to mean its overall condition or status over the short term (hours to decades), whereas quality and change are meant to represent a view over a longer term (decades to centuries) (Wells, 2005).

Biological integrity is defined by the US Environmental Protection Agency as "...the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (USEPA, 2002). Underlying this definition are three principles:

1. biota spans a variety of spatial and temporal scales,
2. a living system includes elements of biodiversity and the processes that generate and maintain them, and
3. living systems are embedded in dynamic evolutionary and biogeographic contexts (Karr, 1999).

The attractiveness of the ecosystem health approach stems from an analogy to human health sciences. As doctors diagnose patients (i.e., determine the existence and/or cause of a condition), so too may diagnoses be determined for ecosystems. Currently, we lack consensus on the usefulness of this analogy in environmental science (Mark *et al.*, 2003). However, it may not be necessary to define ecosystem health in order to reap scientific and management value from it. The concept, however one sees it, can be very useful in communicating messages.

Applying the concepts of ecosystem health and ecological assessment into a dynamic ecosystem such as the Minas Basin is difficult. Biota and environmental variables vary in healthy ecosystems (Bailey *et al.*, 2003), thus any ecological assessment or conservation decisions must consider the role of disturbance in shaping them. This embodies the ultimate paradox of ecological conservation, "...that we seek to preserve what we must change" (White and Bratton, 1980).

The Reference Condition Approach

The Reference Condition Approach (RCA) is an ecological assessment method. The unavoidable difficulty is that assessments require a norm or standard that a state can be compared to (Mark *et al.*, 2003). Knowledge of if and how a system deviates from what the expected standard, is no easy task given the dynamic nature and complexity of ecosystems. This problem is further confounded by large gaps in our knowledge of complex ecosystems such as in the Minas Basin, making it difficult to determine the range of natural variability. The difficulty of distinguishing variation caused by natural and anthropogenic factors, plus cumulative impacts, add even more complexity to assessments. The RCA acknowledges and accounts for the full range of natural variability by carefully selecting reference sites, and provides a benchmark for the amount of normal variation in the ecosystem.

The Reference Condition, for the purposes of this study, is defined as:

> The condition that is representative of the comprehensive description of data from a collection of similar, minimally impacted, natural sites, grouped by selected physical, chemical, and biological characteristics.[1]

The Reference Condition Approach (RCA) was originally developed for freshwater systems, and is an approach used to determine environmental health of rivers world-wide. The RCA is the basis for river classification in the United Kingdom (Wright *et al.*, 1984), Australia's National River Health Program (Moverley and Hirst, 1999), and Environment Canada's Aquatic Biomonitoring Program (Reynoldson *et al.*, 2001).

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[1] Modified from Reynoldson *et al.* (1997) and US EPA (2002)

The intent of the reference condition approach is not to document and quantify variation in the system, but to detect divergence from the natural state. The defining feature of the approach is that it depends on a set of reference sites that are considered to be of high ecological quality, and representative of the best examples of a particular area (Clarke *et al.*, 2003). Accepting that natural conditions are difficult to find, reference sites that are minimally impacted by human activity may serve as the best available alternative (Reynoldson *et al.*, 2001).

Fundamental to the RCA is that reference sites are used in lieu of traditional replicated control sites (Reynoldson *et al.*, 1997; Norris and Thoms, 1999). Each reference site acts as a replicate, and takes into account spatial variability described by the studies mentioned previously.

Ideally, the set of reference sites should encompass the spectrum of environmental conditions in undisturbed areas that could influence community structure. Reference sites are carefully selected *a priori* and are meant to encompass the broad range of habitats found in the study area – in this case, the Minas Basin intertidal flats. The types of biological conditions that occur in the tidal flats are characterized by grouping all reference sites based on faunal community similarities (Figure 1.2). This provides an objective method of creating groups of reference sites, against which suspected impacted (or 'test') sites having similar environmental characteristics may be compared (Reynoldson *et al.*, 1997).

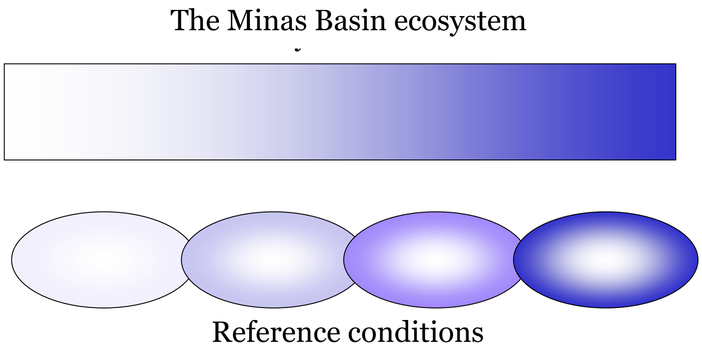

Figure 1.2. The relationship between the ecosystem, a continuum, and the groups of reference conditions found within. Reference conditions should describe all possible natural variation within each ecosystem type. Overlapping reference conditions are recognized to avoid falsely compartmentalizing the continuum.

Two approaches used to assess the degree of disturbance (or degree of impairment) at test sites are the River InVertebrate Prediction And Classification System (RIVPACS) and the BEnthic Assessment of SedimenT (BEAST) (for a detailed comparison see Reynoldson *et al.* (1997). The RIVPACS approach was developed and first applied to freshwater systems by Wright *et al.* (1984). Models such as these, built using multivariate statistics, can be used to predict the expected macroinvertebrate community at a site based on environmental variables. The condition of the test site can be assessed, and expressed in terms of the deviation of the observed community from the expected community (Hose *et al.*, 2004).

The BEAST method was first used by Reynoldson *et al.* (1995) in Ontario's Great Lakes region. Similar to RIVPACS, this approach also uses predictions of group membership of each test site using a discriminant model. The macrofauna data for a test site and the associated group of reference sites are merged into one spreadsheet and plotted in ordination space. Probability ellipses (between 90% and 99.9%) are drawn around the reference sites, and the degree of divergence of the test sites can be quantified (Reynoldson *et al.*, 1997).

In the current study, the RIVPACS and BEAST assessment approaches were applied to the Minas Basin intertidal flats. Both approaches have been shown to be useful in freshwater systems, but this study is the first application in a North American estuarine environment.

1.2 Introduction to the Study Area

The Minas Basin, Nova Scotia (Figure 1.3), is a semi-enclosed body of water that can be classified as an estuary based on the description by Pritchard (1955). Moving east from Parrsboro, the Minas Basin and Cobequid Bay together form a triangular body of water approximately 80 km long and 29 km wide at the western end. Bousfield and Leim (1959) estimated the total surface area of the basin to be 1,127 km^2. Approximately 180,000 people sparsely populate the watershed and coastline of the Basin (Percy, 2001), with Truro being the largest of the urban centres with a population of 16,000 (Nova Scotia Community Counts).

Figure 1.3. Regional map illustrating the Minas Basin study area.

The Minas Basin, in the upper reaches of the Bay of Fundy, is famous for having the highest tidal range in the world. The world's highest recorded tide of 16.27 m was measured at Burntcoat Head, with the average tide being 12 m. Easily eroded Triassic sandstone cliffs combined with high tidal energy results in a large amount of suspended sediment and extensive expanses of exposed tidal flats at low tide. Bousfield and Leim (1959) estimated that low tide in the Minas Basin reveals approximately one-third of the basin area, or approximately 400 km^2 of tidal flats.

The Minas Basin is commonly separated into three geographic regions (Percy, 2001): the central Minas Basin, the Southern Bight, and Cobequid Bay (Figure 1.4).

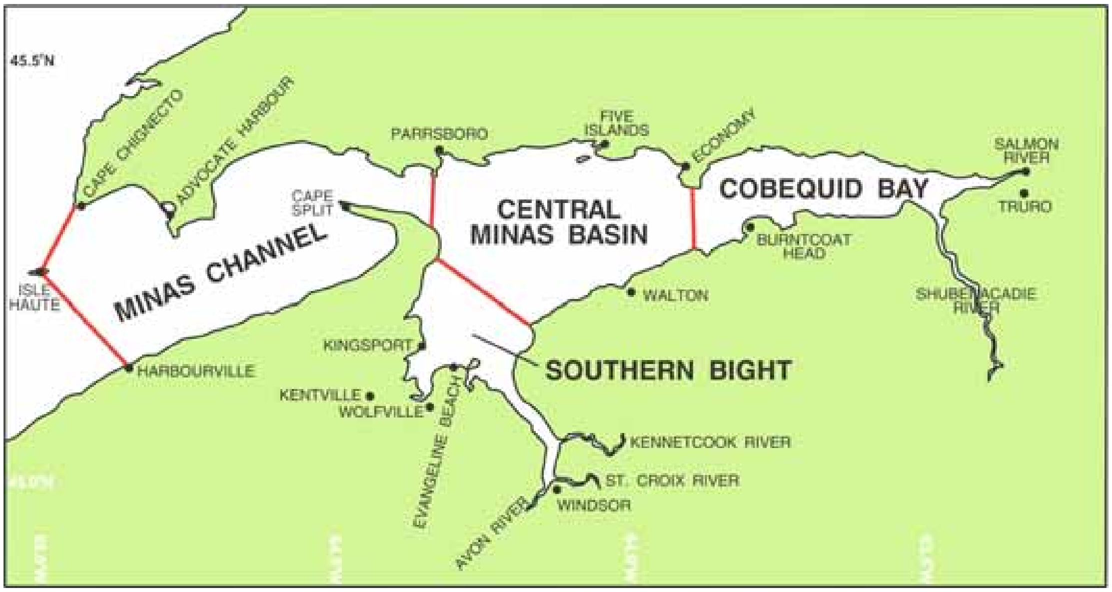

Figure 1.4. Map of the Minas Basin area illustrating the boundaries of the Central Minas Basin, the Southern Bight, and Cobequid Bay. Map by Lesley Carter, modified from Percy (2001).

Central Minas Basin

Bousfield and Leim's 1950 survey found the Central Minas Basin to have summer surface temperatures up to and above 15˚C, shore salinities just under 30‰, "lake-like" wave action with no surf (Bousfield and Leim, 1959), and the average depth is 15 to 20 metres below lowest low water (CHS Chart D7-4010). The north shore of this area, specifically from Five Isalnds to Economy Point, is where the majority of commercial clam digging occurs. The Central Basin extends southward and eastward into two distinct sub regions, the Southern Bight and Cobequid Bay respectively.

Southern Bight

The southern bight is formed by the convergence of several rivers - the Kennetcook, St. Croix, and Avon at the southern end, and the Gaspereau, Cornwallis, Canard, Habitant, and Pereau Rivers at the western end. Extensive mudflats with large but variable *Corophium* populations are found here, which attract migrating Semipalmated Sandpipers and many other species of migratory shorebirds every summer (Shepherd and Boates, 1999). This area is also where some commercial groundfish and baitworm harvesting takes place.

Cobequid Bay

Cobequid Bay is 30 km long, with a relatively constant width of 8 km (Dalrymple, 1977). Cobequid Bay drains many rivers and streams, of which the Salmon River and Shubenacadie River are the largest. Warm, fresh water from the rivers and solar radiation on the tidal flats result in warmer temperatures during the summer and lower salinity in Cobequid Bay waters compared to other areas of the Minas Basin. In Cobequid Bay, some endemic populations of zooplankton can be found whose normal range is south of the Bay of Fundy or in the warmer waters of the Southern Gulf of St. Lawrence; these are separated from their nearest outside populations by distances exceeding that of the normal larval transport range (Bousfield and Leim, 1959). A specific example of this is the extremely abundant *Canuella canadensis* (Jermolajev, 1958) which is restricted to the Shubenacadie River.

The Minas Basin is relatively pristine compared to heavily built-up or industrial areas in the region such as Saint John Harbour. However, human pressures such as harvesting of certain fish and invertebrate species, lack of sewage treatment, and some land uses are recognized by local citizens and government agencies alike as posing a threat to the overall health of the ecosystem (BoFEP, 2001).

History of research in the Minas Basin

Many zoologists in the late 1800's and early 1900's did not include the upper reaches of the Bay of Fundy in their studies. Whiteaves (1901) published a still widely used marine species identification key, and mentioned only six species from the Minas Basin (2 sponges and 4 molluscs). Kindle (1917) reported on some intertidal and shallow water collections made at Kingsport and Cheverie Point and added 12 species to the Whiteaves list. Shortly after, Leim spent the summers of 1919 to 1921 studying the life history of the shad in the Basin while at the same time doing as much general collecting as possible (Leim, 1924). After a 20-year gap, Baillie and Klawe in 1946 (in Berkeley and Berkeley, 1956) expanded upon knowledge of the polychaete species. Bousfield and Leim (1959) were the first to compile a substantial invertebrate faunal list for the Minas Basin and Channel with 195 invertebrate species catalogued (Yeo, 1977).

In the early 1970's our knowledge of the Bay of Fundy greatly improved when feasibility studies on tidal power were conducted (Moyse, 1978), over an 8 year period (Daborn, pers. comm.). In the late 1970's the National Research Council funded several large-scale studies to document the fauna of the intertidal flats at Scots Bay and the Southern

Bight area (Gratto, 1978; 1979). Since then, studies have tended to be more ecological in nature, examining relationships between species and their surrounding environs. For example, Wilson (1988; 1988; 1989; 1991) investigated competition and predation in soft-sediment communities of the Minas Basin, and the effect of winter ice has been studied in detail by Gordon and Dadswell (1984), Gordon and Desplanque (1983), and Partridge (2000). Many studies have endeavoured to quantify human impacts with emphasis on baitworm harvesting (Shepherd, 1993), flounder trawling impacts (Brylinsky *et al.*, 1994), and tidal barriers (Coon, 1999).

Investigations of specific ecosystem components are still also conducted alongside the more ecosystem-based studies. Contaminants (Chou *et al.*, 2000; Jones *et al.*, 2001), the endangered Atlantic Salmon (Amiro, 2003), foraging site selection of migrating birds (Hicklin and Smith, 1984), and estimates of salt marsh productivity (Hargrave *et al.*, 1983) are just a few examples.

1.3 Study Objective

The purpose of this study was to apply the Reference Condition Approach, an ecological assessment method, to a temperate intertidal estuarine environment. The impact assessed was human physical disturbance resulting from baitworm harvesting and clam digging.

The study area included the intertidal flats of the Minas Basin, from Parrsboro Harbour clockwise around the Basin to Blomidon. The reference condition approach was applied to both the Minas Basin intertidal meiofauna and macrofauna.

CHAPTER 2: METHODS

This chapter provides an overview of the methods used in this study. It begins with a discussion of the rationale used for reference and test site selection, followed by specific field and laboratory methods, and concludes with the statistical methods used to apply the BEAST and RIVPACS assessments.

2.1 Linkages between Biota and Environment

The BEAST and RIVPACS approaches both use environmental variables to make biota-related predictions for each test site, thus it is important that the habitat variables measured at each site are relevant and appropriate to the biota studied. Variables were chosen based on a primary literature review of the major physical variables that influence soft bottom communities (summarized in Table 2.1).

Table 2.1. List of environmental variables measured at each site and a brief rationale for the inclusion of each variable in this study.

Variable Measured	Rationale for inclusion based on literature review
Grain size	Many environmental variables are associated with grain size. Intertidal areas with coarser grain size tend to have well-flushed, highly oxygenated deposits low in organic matter as a result of higher wave energy or tidal currents. Conversely, areas with finer grain size tend to have slow flushing resulting in the build up of organic matter and microbes leading to abrupt vertical gradients of oxygen, nutrients and geochemical factors such as redox potential, sulphides and pH (Alongi, 1998). The most important factors determining grain size are wave action and current velocity (Gray, 1981).
Distance from shore	Aerial exposure time has been shown to be a major environmental factor in shaping community structure (Craig, 1976; Delgado *et al.*, 2003), and increasing aerial exposure was found to suppress species diversity by Bursey (2003). Distance from shore was the best available surrogate for exposure time.
Organic Carbon	Percent organic carbon was measured, as organic matter in sediments is an important food source for benthic fauna. An overabundance of organic matter, however, can result in reduced species richness, abundance and biomass due to oxygen depletion and build-up of ammonia and sulphides linked with the breakdown of the excess material (Shine, 2004).
Inorganic Carbon	Inorganic carbon in sediments is a measure of available carbon for production to occur by primary producers.
Chlorophyll *a*	Benthic diatoms increase sediment stability through the production of extracellular polymeric substances (carbohydrates) that act to bind sediment grains (Yallop *et al.*, 1994). Underwood and Smith (1998) found benthic diatom chlorophyll *a* to be closely correlated with carbohydrate concentrations and sediment stability. Chlorophyll *a* was measured both as a function of resource availability and possible measure of sediment stability.
Presence/absence of anoxic layer	Reduced sediments are indicative of a low oxygen environment, and most fauna will avoid burrowing beneath the anoxic layer (Gray, 1981). Redox potential is the best measure of the degree of oxygen within the sediments, however visual presence/absence of an anoxic layer was the best possible surrogate.

A review of the literature revealed that grain size has a profound influence on benthic community structure (Gray, 1981; Maurer and Leathem, 1981; Lee and Huh, 1988; Bursey and Wooldridge, 2003; Rodriguez-Villanueva *et al.*, 2003). Grain size distribution itself is largely determined by relative wave energy. In areas of high tidal ranges, such as the Minas Basin, grain size is also indicative of the extent of tidal currents in the area. The resulting grain size distribution is closely associated with organic material, pollutants, oxygen, anoxic layer depth, etc. (Figure 2.1, also Table 2.1).

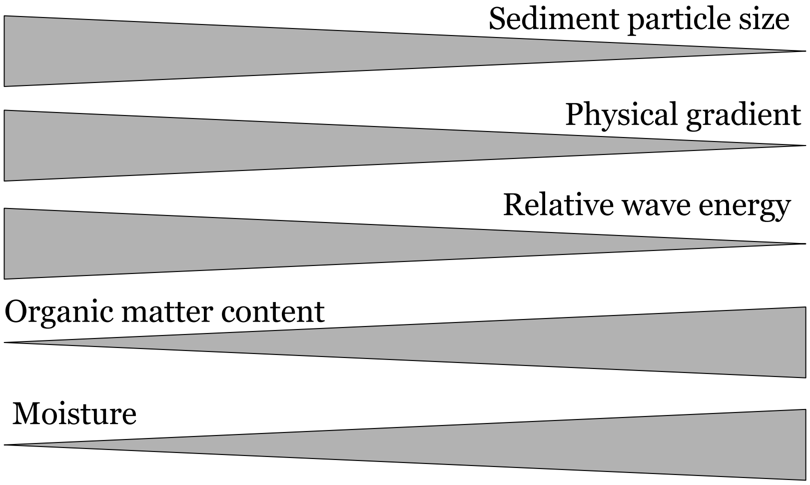

Figure 2.1. Gradations in the sedimentary, physical and chemical structure of tidal flats. Adapted from Alongi (1998).

As grain size is associated with a large number of environmental variables, it was used as the primary factor by which the reference sites were stratified (described below in Section 2.2). The second most important factor was distance from shore, which served as a proxy for aerial exposure time, also shown to have a major influence on intertidal faunal distribution (Peletier, 1996; Bursey and Wooldridge, 2003; Delgado *et al.*, 2003).

Many other environmental variables are relevant to the structure of intertidal communities but were not included given the time and resource constraints of this study. At the micro-habitat scale, bacterial concentrations could be measured as a potential food source for infauna, water content (sediment porosity) as a function of sediment stability and drainage, redox potential, and aerial exposure time (Table 2.1). At the macro-habitat, or regional scale, water column properties such as temperature, salinity and turbidity, as well as tides and currents, are also factors influencing faunal distribution.

2.2 Site Selection

Reference Sites

Bousfield and Leim (1959) estimated that low tide in the Minas Basin reveals one-third of the Basin area, or approximately 400 km^2 of tidal flats (Figure 2.2). The multivariate statistical methods used in the RCA require a sufficient number of sites to characterize the variability of reference conditions for the study area. Althouth no definite guidelines exist for the number of sites required (Reynoldson *et al.*, 2001), Reynoldson and Wright (2001) have suggested a minimum of 50 sites to construct RCA-type models. Given the size of the study area, 50 reference sites were thought to be appropriate (Reynoldson, pers. comm.). Reference sites were allocated by stratifying using sediment grain size and distance from shore (previously discussed) with the intent of capturing the full range of tidal flat variation.

While several intertidal sediment characterization maps of the Southern Bight area along the south shore of the Minas Basin are reported in the literature (Dalrymple, 1977; Shepherd, 1993; Partridge, 2000), similar maps for the north shore are lacking (Craig, 1976). Given the patchy map coverage for the north shore, the inconsistency of detail among maps, and the age of the sediment maps, new surficial sediment maps were created for this study based on visual observations.

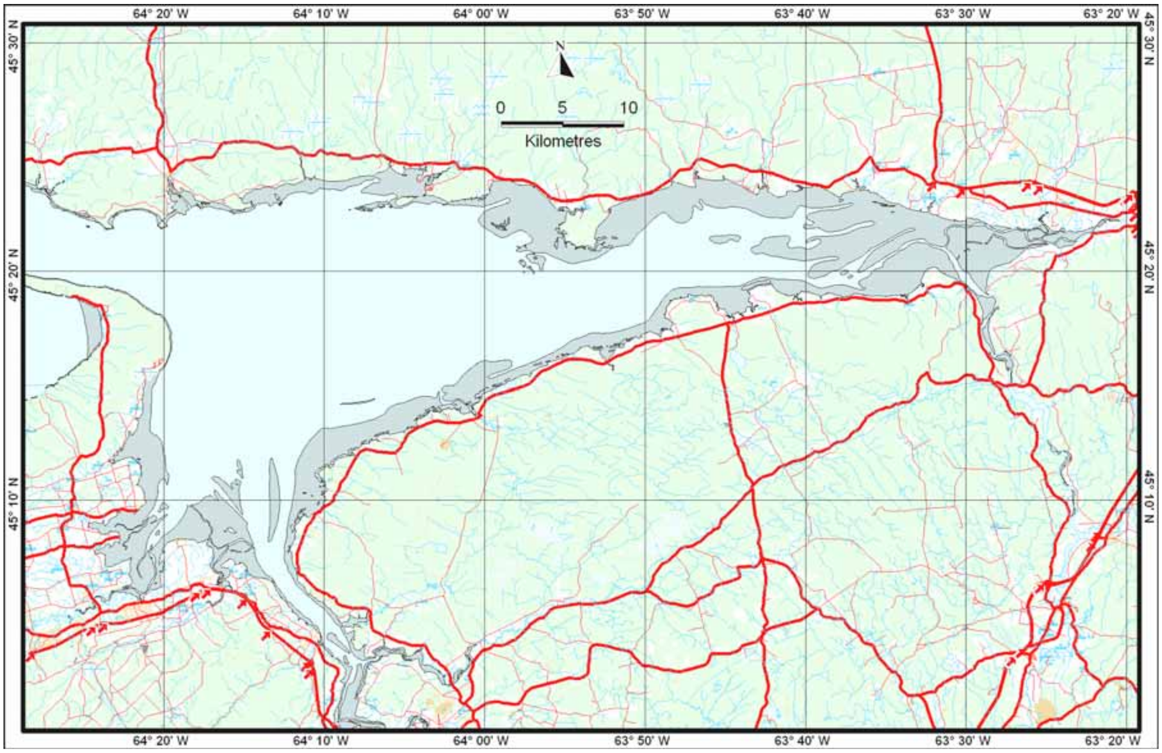

Figure 2.2. Map of the Minas Basin study area showing the extent of the tidal flats exposed at low tide (hatched). Map by Stanley Johnson, Fisheries and Oceans Canada (from the Natural Resources Canada NTDB 1:50,000 digital map series).

The shoreline of the Minas Basin was examined at low tide over a period of 2 weeks in late June of 2002. High and open roadside vantage points were used for viewing exposed tidal flats with Pentax 8x24 binoculars. Tidal flats were classified into one of five categories based on visual characteristics as seen through the binoculars. Tidal flat appearance was categorized as 1) sand with ripples or waves, 2) flat sand, 3) firm mud, 4) soft mud, or 5) very soft mud. The extent of each tidal flat category was roughly sketched onto a 1:50,000 map of the Minas Basin. Areas that had clam or bloodworm harvesting activity at the time of viewing were noted in detail (Figure 2.3) as potential test sites, and not included in the classification exercise; only undisturbed areas were of interest for reference site selection. Areas of exposed bedrock were also noted, as they were not appropriate for sampling.

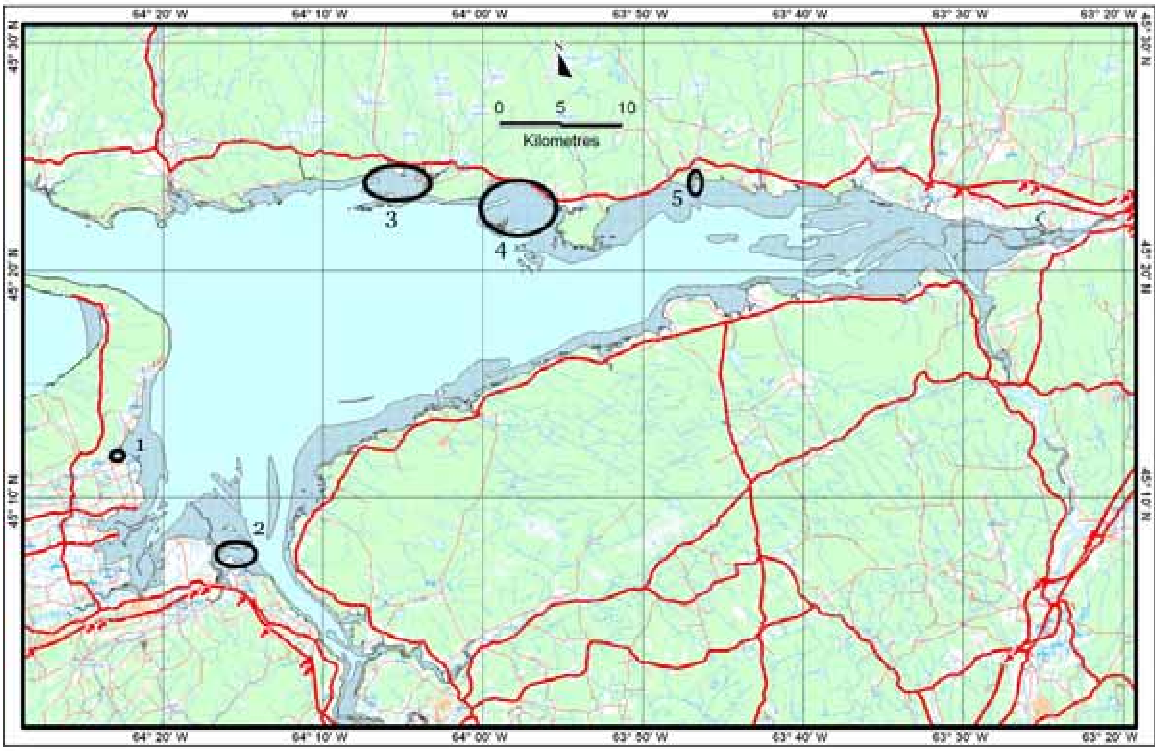

Figure 2.3. Tidal flats of the Minas Basin highlighting the extent of areas where the author found clam or baitworm harvesting activities in 2002. (Detailed observations: 1. Three harvesters in cove near shore. 2. About 15 harvesters in area, disturbance extensive. 3. Several harvesters, two weirs and ATVs on flats. 4. A few harvesters, four weirs. 5. One harvester, small patch of disturbance.)

With the shore of the Minas Basin roughly classified based on visual characteristics, as a proxy for grain size, the intertidal tidal flats were further divided into high, mid, and low intertidal areas based on distance from shore. This tripartite division was used to distribute the reference sites between high, mid, and low tide areas as a proxy for aerial exposure time. In summary, this tidal flat classification exercise resulted in five different categories of sediment appearance (from sand with waves to very soft mud) plus three divisions based on distance from shore (high, mid, and low intertidal). This resulted in 15 separate tidal flat categories among which the reference sites were to be distributed (Table 2.2).

Table 2.2. Minas Basin tidal flat classification scheme designed for reference site selection, with final site numbers included.

	Sand with ripples (1)	Flat sand (2)	Firm mud (3)	Soft mud (4)	Very soft mud (5)
High intertidal	7, 8	40	11, 12, 13, 16, 17, 25, 27	1, 2, 15, 24	9, 18, 26
Mid intertidal	29	4, 5, 6, 41	14, 22, 23, 36	19, 28, 33, 35	34, 38, 39
Low intertidal		10, 31	3	30, 37	20, 21, 32

Even distribution of reference sites between each tidal flat category was attempted, (however sampling difficulties did not always allow for this). As 40-50 reference sites encompassing the range of intertidal variation were required, distribution of potential reference sites between each of the 15 tidal flat types required approximately three sites per cell in Table 2.2. Three potential sampling sites for each tidal flat type were located on the detailed 1:50,000 tidal flat classification map. Every effort was made to ensure these three sites were distributed broadly over the entire study area.

Site coordinates (Universal Trans Mercator) were taken from the 1:50,000 tidal flat classification map and programmed into a hand held Garmin GPS V unit, used to locate sites in the field. Final reference site locations are shown in Figure 2.4.

Test Sites

Test site selection was determined based on observations of digging activity during the intertidal classification exercise (previously noted) and visual evidence of physical disturbance such as 1) visible rows or furrows as a result of bloodworm harvesting, 2) unusually potholed tidal flat surface as a result of clam harvesting (Appendix A), or 3) active clam or bloodworm harvesters in the area. Based on observations during the shoreline classification exercise, it was noted that there was no harvesting activity in the Parrsboro area, or along the Noel shore (the south shore of Cobequid Bay from Maitland to Tennycape). As reference sites were relatively evenly distributed in the tidal flats, it was also desirable to have test sites evenly distributed around the Basin. Therefore, two to three weeks prior to sampling, the areas with no visual evidence of regular clam or bloodworm digging (Parrsboro – T1, Noel Shore – T7, and Moose Cove – T8) were experimentally disturbed. The action of bloodworm harvesting was reproduced with a hand-held rake, in order to replicate the physical disturbance caused by commercial and recreational harvesting. Time constraints and inexperience resulted in no organism removal from these areas, and small disturbance areas (approximately 1 m^2). All final test site locations are shown in Figure 2.4 and photos of each test site are provided in Appendix B.

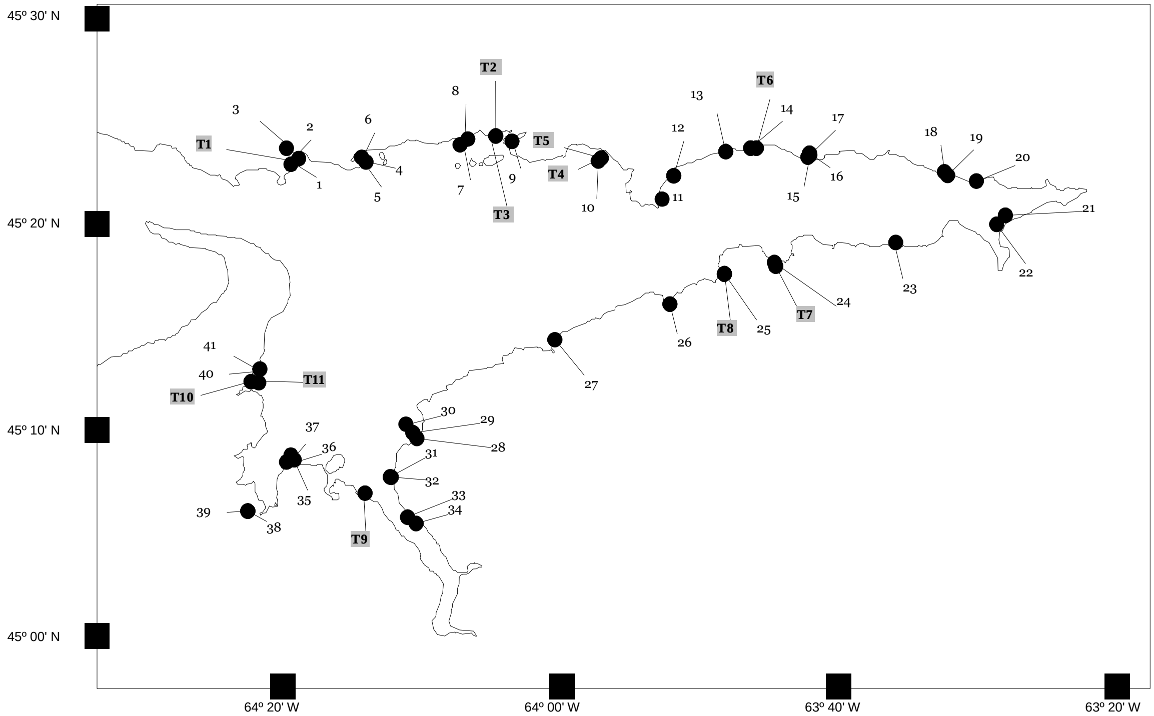

Figure 2.4 Sampling site locations within the Minas Basin tidal flat study area. Sites 1-41 represent all reference sites and Sites T1-T11 represent all test sites (bold and shaded).

2.3 Sampling Methods

Field sampling was conducted between July 5-23, 2002, at low or ebb tide. All sampling was carried out on foot or with the assistance of a hovercraft, weather permitting. The hovercraft was rented in the Parrsboro area to expedite sampling along the north shore and Cobequid Bay (resources did not allow for the hovercraft to be used for the entire sampling period). Figure 2.5 illustrates the sampling protocol carried out at each site, described below. During one tidal cycle, as many sites were sampled as possible, after which the samples were immediately processed in the laboratory for preservation and storage.

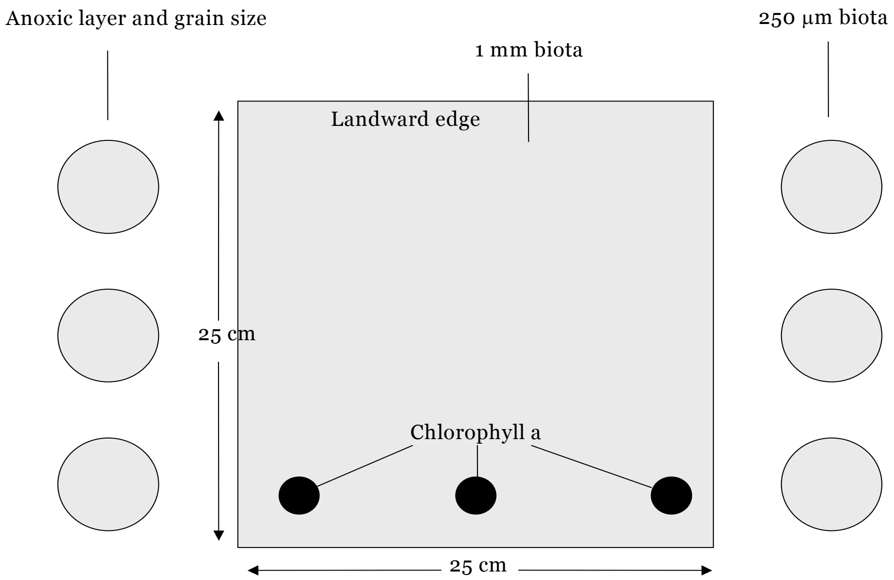

Figure 2.5 Schematic for all reference and test site field sampling procedures.

Selecting Sites

The hand held GPS unit (Garmin IV) was used to locate sites in the field, however actual sampling sites were occasionally modified from the original coordinates entered into the GPS (e.g., due to exposed bedrock). Upon reaching the approximate site area, a 25 cm^2 quadrat was thrown randomly to the west onto the tidal flat. With a specific sampling site selected, the GPS unit was used to record the final coordinates of the site (based on an average of 50 measurements to ensure accuracy). A glass scintillation vial was then marked with the site number, placed in the sediment below the quadrat and a photo was taken for site documentation and future reference.

Chlorophyll **a**

Field Methods

A plastic syringe with the tip cut off was used to gather three replicate plugs of sediment from the inside lower (seaward) edge of the quadrat for chlorophyll *a* analysis. The plunger was drawn up as the syringe was gently pushed into the upper 2 cm of the sediment. Upon retrieval, the plunger was pushed down to release the sample into a glass scintillation vial, and the rubber tip was wiped along the vial lip to capture as much of the sample as possible. All three plugs were pooled in the field. To minimize degradation, chlorophyll samples were immediately sheltered from light and placed in an ice-filled cooler within one hour of collection. Once in the lab, chlorophyll *a* samples were taken from the portable cooler and stored in a freezer until analysed.

Laboratory Methods

Amount of chlorophyll a was measured following the methods of Brylinsky (Greenberg *et al.*, 1998; undated, modified from Greenberg et al., 1998). However, as this analysis was carried out over a two-day period, the spectrophotometer was left on overnight with the acetone reference cuvette inside. Unfortunately, the reference cuvette dried up, which was not discovered until all remaining samples were analyzed on the second day. Although chlorophyll *a* analysis was carried out for all samples, it is not known which samples were compared to full or empty reference cuvettes, rendering all results erroneous. Chlorophyll *a* values are therefore not considered further in this study.

Anoxic Layer and Grain Size

Field Methods

A sediment corer was made specifically for this study (Figure 2.6). The sediment corer consisted of a piece of 4 mm thick black PVC plumbing pipe with an internal diameter of 3.4 cm (area = 9.07 cm^2). This was cut to a length of 30 cm and cut in half lengthwise. One seam was secured with duct tape to hinge the pipe, allowing it to be opened along the other side for visual inspection of the core.

The corer was pushed into the sediment until it either encountered bedrock or reached a maximum depth of 15 cm prior to extraction. Upon opening a core for visual inspection, anoxic layer depth was measured with a ruler to the nearest millimeter if visible. Three cores were spaced evenly along the outside edge of the left edge of the quadrat.

In core samples where the anoxic layer was visible, the sediment above the anoxic layer was horizontally divided into equal halves. The top half was placed in a 120 ml container and labelled with "Site #, Top", while the bottom half was placed in another 120 ml container and labelled with "Site #, Bottom" (all containers used were Fisherbrand™ inert polypropylene). The three cores were pooled in the field and analyzed as one sample. In core samples where no anoxic layer could be seen, the core was evenly divided horizontally into top and bottom halves as above.

The Fisherbrand™ jars containing the sediment samples were stored in a labelled box at room temperature (approximately 20°C) until laboratory analysis.

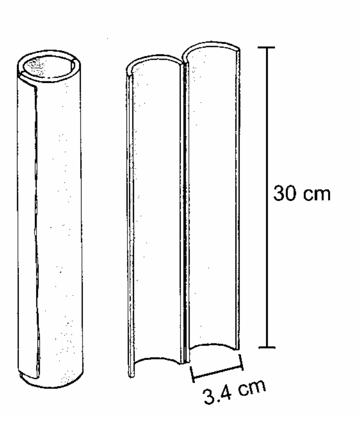

Figure 2.6. The sediment corer constructed specifically for this study, as described in the text. Illustration by Tim Fedak.

Laboratory Methods

A Coulter Laser LS230 was used to analyze all samples for particle size. This instrument uses laser-based technology (based on the Fraunhofer and Mie theories of light scattering) to analyze particles in suspension between 0.04 and 2 mm; for coarser sediments (>2 mm), additional steps were taken to determine percentage of gravel.

Fine sediment samples with no gravel:

A small 2 mm mesh sieve was placed on top of the input opening to remove gravel from the sample. If any sediment was retained in this sieve, the sample was deemed "Coarse", labelled and stored for coarse sediment analysis described below. Enough sediment was placed into the Coulter Laser LS230 to reach an obscurity level of 8-12% (usually between 0.5 – 3.0 g) and the analysis sequence was run. Output was a histogram printout and Excel™ file for each sample, from which percentages of clay, silt and sand were calculated.

Coarse sediment samples with gravel:

Coulter Laser analysis was run as described above. Each sample was then dried and weighed to determine total dry weight. All samples were wet sieved through two screens – a 2 mm sieve on top of a 64 µm sieve. Sediment retained in the 2 mm sieve (gravel) was transferred into a crucible and labelled "Site #, >2mm". Sediment retained in the 64 µm sieve (sand) was transferred to a separate crucible and labelled "Site #, <2mm". Anything less than 64 µm (silt and clay) was discarded (this had been analyzed previously). The wet fractions were then dried in an oven at 60°C for 24 hours (until completely dry), and dry sample weight was recorded. Percentage (by dry weight) of gravel, sand, silt and clay was then calculated.

Carbon Analysis

Field Methods

One 4 oz. plastic container was used to collect and store samples for carbon analysis. Samples were collected by scraping the surface of the sediment with the plastic lid of the jar (each scrape approximately 5 cm long and 1 cm deep). Three surface scrapes were collected, pooled in the field, and analyzed as one sample.

Laboratory Methods

Total Carbon:

Approximately 250 mg of freeze-dried sediment was weighed into combustion crucibles, and moistened with a small amount of deionized water to keep the sample from blowing out during analysis. The samples were left overnight to allow the water to evaporate, leaving a crust on the upper surface. The following day, one scoop (equal to one gram) of both copper and iron crystals was added to each crucible. Using a high temperature combustion WR-112 Leco Corporation Carbon Determinator, single samples were analyzed with an industry standard being analyzed every 7 trials to ensure that there were no equipment malfunctions.

The LECO analyzer determines the total carbon content of a sediment sample by measuring the thermal conductivity of the released gas upon combustion. Output was recorded as % total carbon per sample. Precision and accuracy were estimated to be ± 0.03 wt.% based on replicate analyses of calibration standards.

Organic Carbon:

Approximately 250 mg of freeze-dried sediment was weighed into combustion crucibles, to which approximately 1.5 ml of 1M HCl was added in order to remove any inorganic carbon. The sample was allowed to dry overnight, and the addition of 1.5 ml of 1M HCl followed by drying was repeated twice. After the third night, analysis was carried out as described for total carbon.

The LECO analyzer determines the organic carbon content of an acidified sediment sample by measuring the thermal conductivity of the released gas upon combustion. Output was recorded as % organic carbon per sample. Precision and accuracy were estimated to be ± 0.03 wt.% based on replicate analyses of calibration standards.

Inorganic Carbon:

Inorganic carbon (%) was calculated using the equation:

$$\text{Inorganic carbon (\%)} = \text{Total carbon (\%)} - \text{Organic carbon (\%)}$$

Distance from Shore

The final coordinates for each site sampled were taken from the hand held GPS unit and mapped on the 1:50,000 base maps used in the tidal flat classification exercise. The distance from each sampling site to the nearest point of coastline was measured on the base map and converted to meters.

Biota – 250 μm and Greater

Field Methods

The sediment corer (previously described) was used to collect benthic samples for fauna retained with a 250 μm mesh. Three cores were collected, evenly spaced along the outside edge of the right boundary of the quadrat. All three cores were placed in one large Ziploc bag until sieved in the lab (i.e. samples were pooled in the field). Due to the speed of the flood tide, no cores were obtained for site 12.

Upon returning to the laboratory, the Ziploc bag was emptied into a 250 µm geological sieve and rinsed several times. A flexible waterspout was used to gently rinse the sample in order to remove as much sediment as possible. The sample was then transferred from the geological sieve to a 500 ml Mason jar and preserved with 10% buffered formalin until the organisms were sorted and identified.

Laboratory Methods

Samples were retained on a 250 μm geological sieve, and gently rinsed to remove excess formalin and any remaining sediment. Each sample was then placed in a large glass dish (25x25x5 cm) and examined with a dissecting microscope one spoonful at a time (approximately 25 ml) in a glass Petri dish.

Reynoldson *et al.* (2001) compared three levels of taxonomic identification (family, genus, and species) and their performance with the reference condition approach, and found predictive model performance at the family level of identification had the greatest ability to detect disturbance. Family level of identification was therefore used as the target when identifying organisms in the laboratory. Organisms were sorted and identified to family level using the 'Keys to the Fauna and Flora of Minas Basin' by Bromley and Bleakney (1984). All but three taxa were identified to the family level; the Order Harpacticoida and Phyla Nematoda and Nemertea were left at the higher level, as they were too difficult and time consuming to identify further. After identifying and enumerating all organisms, samples were placed in glass vials with 70% ethanol (EtOH) with glycerine for long-term storage.

Biota – 1 mm and Greater

Field Methods

To sample benthic macrofauna at each site, the entire 25 cm^2 quadrat was excavated to a maximum of 12" and placed in the apparatus constructed for field sieving (Figure 2.7). A field sieve was made specifically for this study, consisting of window screen (1 mm mesh) cut into sections approximately 55 x 90 cm. For each sampling site, one piece of window screen was placed between two 52 x 32 x 8 cm wire baskets, stacked one on top of the other.

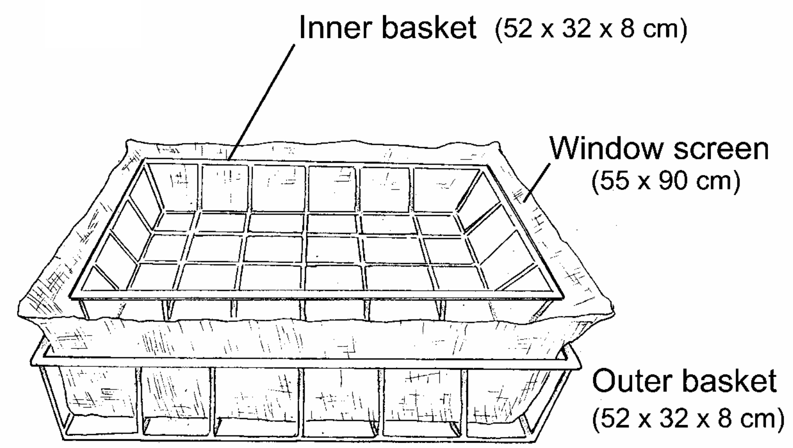

Figure 2.7. Field sieve constructed specifically for this study, as described in the text. Illustration by Tim Fedak.

For macrofauna collection, the entire 25 x 25 cm quadrat was excavated and placed in the field sieve. Sieving was conducted on site at the nearest water source. Once the bulk of the sediment was washed from the sample, the top wire basket was removed. The window screen containing the macrofauna was then gently folded and placed in a large Ziploc bag for more thorough sieving and preservation in the laboratory.

Upon arrival at the laboratory after sampling, the Ziploc bag containing the window screen and macrofauna was placed into a 15 L plastic bucket. The bucket was subsequently placed into a large sink with a flexible waterspout. The Ziploc bag was rinsed several times, with contents going into the bucket each time. After that the window screen contents were backwashed into the bucket to remove organisms from the screen. Once the screen was free of all macrofauna, the bucket contents were slowly poured through a 1 mm geological sieve. The sample was gently rinsed to remove any remaining sediment, transferred to a 500 ml Mason jar, and preserved with 10% buffered formalin until the organisms were sorted and identified.

Laboratory Methods

Samples were retained on a 1 mm geological sieve, and gently rinsed to remove excess formalin and any remaining sediment. The identification, sorting, and weighing procedure was carried as described above for the 250 µm samples.

2.4 Statistical Analysis

Statistical analysis was carried out using SYSTAT V. 8.0 and PRIMER (Plymouth Routines in Multivariate Ecological Research) V. 5.2.9. PRIMER includes a wide range of univariate, graphical and multivariate routines for analysing biological monitoring and associated physico-chemical data.

Description of Intertidal Communities

Overall differences in the intertidal communities and environmental variables were examined and described in Chapter 3. Differences between reference and test sites were examined using the SYSTAT Kruskal-Wallis test (assumes non-normal distribution of data). The BIO-ENV routine in PRIMER was used to find the best fit between environmental variables and biota (Clarke and Warwick, 2001). Principal Components Analysis (PCA) and multidimensional scaling (MDS) plots were used to visually portray similarities and differences between sites.

The Reference Condition Approach

Multivariate methods were used to classify reference sites, predict membership of test sites and determine the level of impairment using two techniques – the Benthic Assessment of Sediment (BEAST) and the River Invertebrate Prediction and Classification System (RIVPACS) (Reynoldson *et al.*, 1997; Reece *et al.*, 2001; Reynoldson *et al.*, 2001). The initial steps for the RIVPACS and BEAST procedures are the same, and are shown in Figure 2.8. The rest of this chapter provides a detailed summary of the statistical procedures for both assessment methods.

Classification of Reference Sites into Biological Groups

Classifying reference sites into similar groups is based on both the taxa present and their respective densities at each site. Data are often transformed (log, square root, or fourth root) to minimize extreme variance in abundances (Clarke and Warwick, 2001). However, given the homogenous nature of the taxa found throughout the study area, faunal abundances were left untransformed to retain the effect of numerical dominance. The cluster analysis routine of PRIMER was used to classify reference sites into similar groups based on similarities among sites. The similarity measure used was the Bray-Curtis, shown to be useful at discriminating sites using benthic fauna. The dendrogram clusters were formed using group averaging, which uses the average similarity of individual nodes.

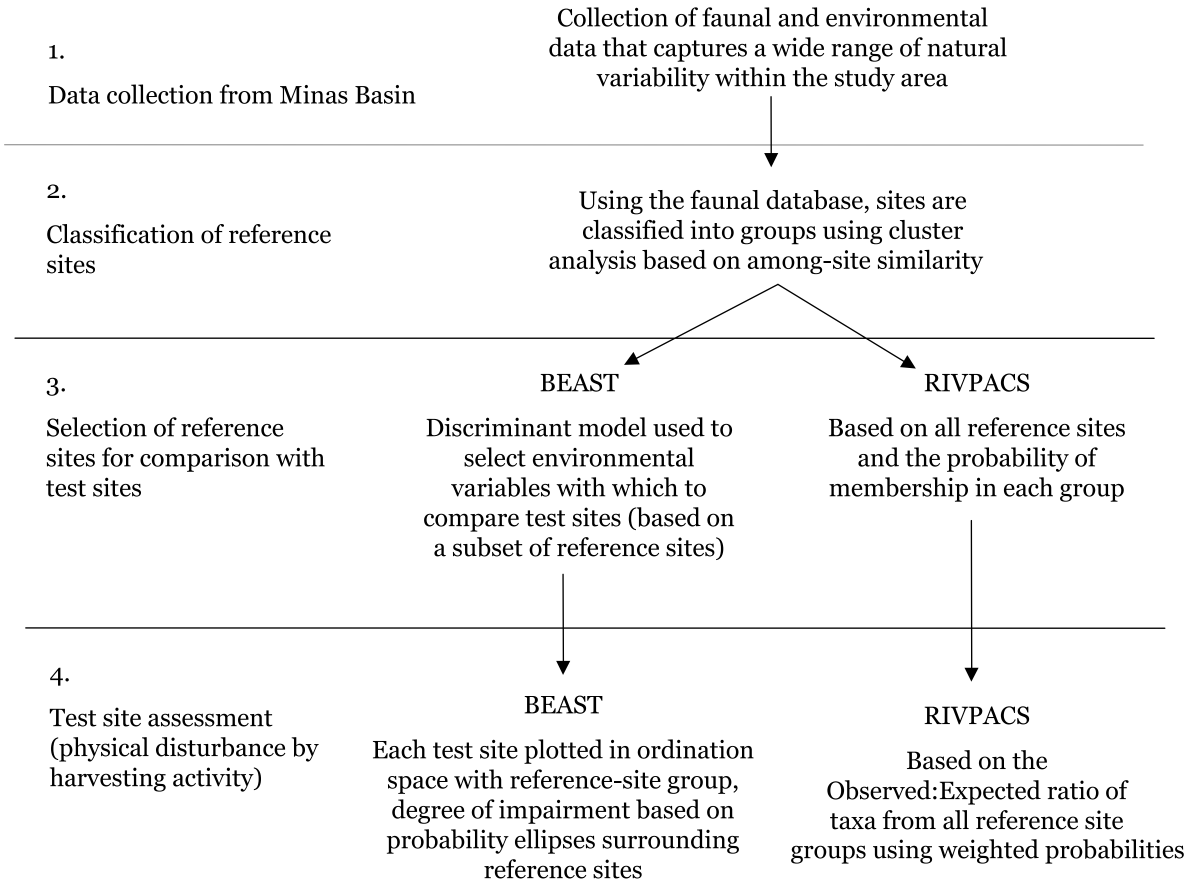

Figure 2.8. Flowchart of the two assessment methods, BEAST and RIVPACS, used in this study to determine the impact of physical disturbance on the intertidal fauna of the Minas Basin (modified from Reynoldson *et al.*, 1997).

After the groups were identified by cluster analysis, differences between them were verified in two ways (both using PRIMER):

1. The Analysis of Similarities (ANOSIM) routine tests for differences between groups (defined *a priori* using cluster analysis) using permutation/randomization methods on a similarity matrix. The output is a 'Global R statistic' and the significance level of the statistic. The higher the Global R value, the more confidence one has in the group differentiation.

2. The Similarity Percentages (SIMPER) routine examines the contribution of each species to the average Bray-Curtis dissimilarity between groups of samples, as well as species-specific contributions to similarity within a group. The output is the average similarity of sites within groups and the average dissimilarity between groups as a percentage. It also includes each taxon's contribution to within group similarity and between group dissimilarity (also in percent).

Ultimately, the number of groups used for analysis is selected by looking at the structure of the dendrogram, the performance of the discriminant models, and the distribution of sites in ordination space (MDS routine in PRIMER) (Reynoldson *et al.*, 1995).

Correlation of Biological Data with Environmental Variables

The presence, density and abundance of macrofauna depends on large-scale geographic and ecological processes, life histories, as well as more local variables such as flow regimes and food availability. Detailed information at the microhabitat level is difficult and time consuming to attain. In a statistical modelling approach such as this, the aim is to select a small but appropriate suite of environmental predictor variables that can be measured consistently at all sites (Clarke *et al.*, 2003).

Variables that are directly influenced by the disturbance under consideration are not included in RCA analysis (Clarke *et al.*, 2003). In the present study, the impact of human disturbance in the form of clam and baitworm harvesting is being assessed, thus it is important that harvesting activity does not influence any of the environmental variables used in model construction. Thus, significant differences between all reference sites and test sites were tested for each variable using a Mann-Whitney test (the nonparametric analog of the two-sample t-test). Any environmental variables that differed significantly between reference and test sites were removed from further analysis.

Before conducting any analyses, habitat data were first checked for any necessary transformations, as discriminant analysis assumes equal within-group variances for all variables (Joy and Death, 2003). Transforming data can minimize variance and remedy lack of symmetry, therefore approximating normality. Transformation of a variable typically reduces the number of outliers, and is more likely to produce normality.

Using SYSTAT, the degree of skewness (lack of symmetry of distribution) and kurtosis (measure of the peakedness of a distribution) were computed. If kurtosis divided by the standard error of kurtosis, or skewness divided by the standard error of skewness, were greater than two, then that variable was transformed. A positive value was square root transformed and a negative value was $\log_{10}$ transformed (summary in Table 2.3).

Table 2.3. Listing of environmental descriptors measured for all reference sites sampled in the Minas Basin mudflats and their required transformations.

Variable	Type of transformation
Latitude (decimal degrees)	None
Longitude (decimal degrees)	None
Gravel Top (%)	Square-root
Sand Top (%)	None
Silt Top (%)	None
Clay Top (%)	None
Gravel Bottom (%)	Square-root
Sand Bottom (%)	None
Silt Bottom (%)	None
Clay Bottom (%)	None
Total Inorganic Carbon (%)	Square-root
Total Organic Carbon (%)	Square-root
Anoxic layer (presence/absence)	Log
Distance from shore (m)	Square-root
Classification (scale of 1-5, firm-soft)	None

Stepwise discriminant function analysis was used as a guide in selecting the appropriate subset of environmental variables for use in the predictive model. Discriminant function analysis produces ordination axes (discriminant axes) that are weighted linear combinations of the environmental predictor variables (Clarke *et al.*, 2003). These discriminant axes maximise discriminatory ability by selecting the aspects of variation in the environmental variables that differ most between the biological groups. The final selection of the environmental variables that provides the best fit (i.e. lowest error rate) for the model is attained by iteration (Reynoldson *et al.*, 2001; Joy and Death, 2003). Combinations of variables used are based on both stepwise discriminant function analysis (DFA) in SYSTAT and the BIOENV procedure in PRIMER. The cross-validation option was used to check if sites were allocated to the correct groups. This process, also known as jackknifing, predicts group membership of each site separately and avoids biased assessment (Joy and Death, 2003).

Stepwise DFA uses the spreadsheet containing all reference site environmental data (transformed) and a column indicating to which group each reference site belongs (based on cluster analysis dendrogram). Test site data are not included at this point. The forward stepwise selection routine is run (Reynoldson *et al.*, 2001), which takes the variable with the highest F-ratio (largest difference between groups) and includes it as a predictor variable. Any correlation of that variable and all others is then removed, the F-ratios re-calculated, and the variable with the next highest F-ratio is chosen. The F-ratio calculations are automatically repeated until all variables have been considered.

The accuracy of predictions can be verified by determining how well the reference sites were predicted to belong to the correct group. Predicted and actual group membership is compared to give a group and total error rate in percent.

Predicting Group Membership of Test Sites

A key aspect of the reference condition approach is determining the appropriate group of reference sites against which to compare the test sites. Predictions are calculated by linking the biotic-based groups formed by cluster analysis to the environmental variables selected by stepwise DFA at each test site.

After a subset of predictor environmental variables have been selected, complete DFA is run on the spreadsheet of appropriately transformed environmental variables that includes all reference and test sites. As in stepwise DFA, the input spreadsheet also includes a ‘Group’ column that indicates to which group each reference site belongs (based on the cluster analysis dendrogram). The ‘Group’ variable for each test site is left blank, the complete DFA routine is run, and the output spreadsheet includes prediction values for all sites belonging to each group.

Assessment of Test Sites

Once test sites have been predicted to the appropriate biological community based on environmental descriptors, they may undergo assessment. Detailed test site assessment using the BEAST and RIVPACS approaches are described below.

The BEnthic Assessment of SedimenT (BEAST) Approach

The BEAST method (Reynoldson *et al.*, 1995; Bailey *et al.*, 2003) compares reference and test sites by plotting both in ordination space. The method constructs probability ellipses around the cluster of reference sites. Variation between reference sites plotted in ordination space in any group is assumed to represent the normal range of variation Therefore, if a test site falls within a cluster of reference sites it can be considered unimpaired, and if it falls beyond a reference site cluster it is considered different and impaired (Reynoldson *et al.*, 2001). The further away from reference a test site is, the more impaired it is deemed. This degree of impairment is quantified by creating probability ellipses around the reference sites using SYSTAT.

Four bands, or probability ellipses are used for assessment (Wright, 1995). Band 1 is the area within the 90% probability ellipse and considered “the same as reference” (i.e., any test site within Band 1 is unimpaired). Band 2 is the area between the 90% and 99% probability ellipses and is “possibly different than reference”. Band 3 is between the 99% and 99.9% probability ellipses and is “different from reference”. Band 4 is outside the 99.9% ellipse and is considered “very different from reference” (Figure 2.9).

The discriminant model was run for the 11 test sites disturbed by clam harvesting or baitworm digging. Each site was compared to the reference site group to which it had the highest probability of belonging. Each test site was plotted in ordination space and assessed as being the same as, possibly different, different, or very different from reference (Reynoldson *et al.*, 2001).

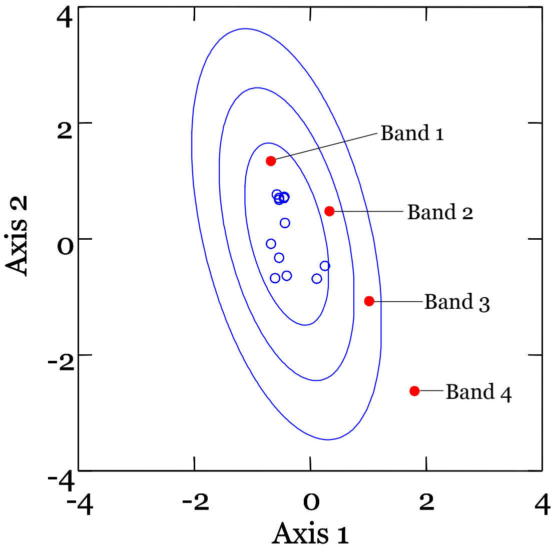

Figure 2.9. Example assessment of four test sites using the BEnthic Assessment of SedimenT (BEAST) approach. Reference sites (open circles) are ordinated with test sites (filled circles) and the degree of impairment corresponds to the Band within which the test site falls (described in text).

Misclassification

Classification of test sites to the wrong reference group is a concern in BEAST assessment. As a precaution, any test site having a >25% probability of belonging to a reference group, was compared to each of those reference site groups.

The River InVertebrate Prediction And Classification Scheme (RIVPACS) Approach

The RIVPACS approach provides a taxon-by-taxon prediction of what is expected at a site if it is in reference condition, based on its environment. The following RIVPACS model construction outline is based on the publications of Barmuta *et al.* (2002), Moverley and Hirst (1999), and Reynoldson *et al.* (1997).

Model Construction

1. As in BEAST assessment, reference sites are placed into groups based on the faunal data using cluster analysis, and predictive environmental variables are chosen using forward stepwise discriminant function analysis. Discriminant function analysis output is a spreadsheet of the probability of each reference site and each test site belonging to each group.
2. The probability of occurrence of a taxon is calculated by summing the products of the probability outcomes of step 2 and the observed percentage frequency that each taxon was found in each group. The sum is the overall (or weighted average) probability of occurrence.
3. The number of predicted taxa are calculated by summing the probabilities of all taxa with >50% overall probabilities of occurrence (also called expected taxa, E).
4. Observed taxa (O) are the number of taxa found at a site that were *also* predicted taxa.
5. To calculate an O/E ratio, the number of observed taxa is divided by the number of expected taxa. The closer this ratio is to 1.0 the closer a site is to the model's expectation.

Using the probability outputs of the discriminant function analysis for each test site, site-specific probabilities of occurrence are calculated for each taxon at each test site. For example (Table 2.4), a test site has an 8% probability of belonging to Group 1 and a 92% probability of belonging to Group 2, and the amphipod *Corophium* was present in 43% of Group 1 reference sites and 100% of Group 2 reference sites. The probability of group membership (0.08 and 0.92) is multiplied by the frequency of occurrence (43 and 100%) for each group. This is done for each taxon, and the sum of the products for all groups is the expected probability of finding each taxon in a test site *if* it is comparable to the reference condition.

Table 2.4. Example calculation of the probability of taxon occurrence at a test site, based on probabilities calculated by discriminant function analysis. Reference sites are grouped using the faunal community and test sites are predicted to belong to each group based on environmental descriptors (modified from Bailey, 2003).

Reference Group	**Frequency of taxon in reference group (%)**	**Probability that test site belongs to group**	**Contribution to probability that taxon will occur at test site**
Group 1	43	.08	3
Group 2	100	.92	92
Probability that taxon will occur at test site if it is in reference condition			95%

The calculated probability of each taxon at each site is only taken into account as contributing to the expected number of taxa (E) if it has a >50% probability of occurring. Moverley and Hirst (1999) suggested that using only taxa with >50% probability of occurrence was an arbitrary cut-off. Hawkins *et al.* (2000), however, have since tested various models and concluded that using only taxa with >50% probability of occurrence leads to a more robust model compared to those using all taxa. Additionally, taxa that have low probabilities of occurrence are difficult to predict with any certainty.

The expected number of taxa (E) for a site is calculated by summing all taxon probabilities >50% (or 0.50). The number of observed taxa (O) is the number of taxa that are found at a site *and are also* predicted taxa (i.e. taxa with >50% probability of occurrence). The observed and expected taxa are examined together as a ratio (O/E), and a perfect model would result in the observed and expected taxa at each site to be equal.

As a perfect model is unlikely in practice, there is variation in the O/E value for each reference site that indicates an imperfect relationship between the faunal community and the environment and diversity of within-group community composition (Bailey *et al.*, 2003). This variation of O/E captures the distribution of the errors in predicting taxa and is used as a basis against which to compare individual test site O/E values.

The more variation among reference sites that is explained by the measured environmental descriptors, the more robust the model will be, and the more sensitive the assessment of test sites will be (Bailey *et al.*, 1998). The objective of a test site assessment is to determine whether or not the test site could be part of the reference site distribution (Bailey *et al.*, 2003).

Residual variation is variation that is not described by the model (i.e. the environmental descriptors measured in the study). Residual variation should be as small as possible. Falsely concluding that a test site is not encompassed by reference variation is a Type I error, or false positive – falsely concluding that an impacted site is encompassed by reference variation is a Type II error, or false negative. Decreasing residual error increases model robustness, and reduces the likelihood of making either type of error (Bailey *et al.*, 2003).

Misclassification

The actual value of the misclassification error rate is less important in this procedure than in the BEAST, as RIVPACS uses all probabilities of a site belonging to each group rather than just the group with the highest probability (Joy and Death, 2003). Therefore, sites with an affinity for two or more groups can be misclassified but still provide adequate predictions for a model.

Evaluation

Determination of site impairment depends on the range of the distribution of the O/E ratios for the reference sites, which theoretically provides the model error. A test site is considered equivalent to a reference site if the O/E ratio is within two standard deviations of the mean of the reference site O/E ratios (Wright *et al.*, 1984). The greater the deviation from the range of reference site O/E values, the greater the suspected impairment (Table 2.5).

Table 2.5. Relationship between O/E ratios for test sites and assessing the level of impairment with the RIVPACS approach (modified from Reynoldson, 1997).

O/E Ratio standard deviations	Band #	Assessment
<2	1	Same as reference
2-4	2	Possibly different from reference
4-6	3	Different from reference
>6	4	Very different from reference

Comparison of Approaches

The BEAST and RIVPACS approaches both use multivariate methods for defining reference groups which is attractive because it requires no *a priori* assumptions to create faunal groups from the reference sites, or when comparing test sites to reference groups (Reynoldson *et al.*, 1997). Some disadvantages of the approaches are that the initial model construction is statistically complex which can discourage prospective users, the costly nature of establishing the reference database, and the possibility of not measuring relevant environmental variables. Advantages include the standard procedure which makes the approach transferable to most areas, the large amount of data produced that can be used for other analyses or studies, and the creation of a baseline survey for a geographic area. The BEAST and RIVPACS approaches are complementary in nature, and using both to assess sites can compensate for the individual weakness of each approach (Reynoldson *et al.*, 1997). Strengths and weaknesses of both approaches are outlined in Table 2.6.

Table 2.6. Strengths and weaknesses of the BEAST and RIVPACS approaches.

	Strengths	**Weaknesses**
BEAST	- Incorporates quantitative changes in community assemblages - Results expressed visually - Assessment statistically less complex than RIVPACS	- Misclassification of test sites of concern, particularly if the predictive model is not robust - No biological predictions are made for any site
RIVPACS	- Uses weighted probabilities - Produces site-specific biological predictions	- Uses only presence/absence of taxa, model does not respond until a taxon is absent - Assessment is statistically more complex than the BEAST - Results expressed as numerical tolerance limits

CHAPTER 3: RESULTS AND DISCUSSION

INTERTIDAL COMMUNITIES OF THE MINAS BASIN

3.1 Introduction

The chapter begins with an outline of field sampling and collected data, followed by a general overview of the major taxa and trends found in the fine (250 µm) and coarse (1 mm) mesh data sets. Statistics were performed with the Kruskal-Wallis test for significance to account for non-normal distribution. Linkages between environmental variables and biota are reviewed, as well as brief comparisons between reference and test sites. The chapter ends with a summary and discussion of the findings.

3.2 Results

Field sampling occurred over an 18-day period in July 2002 with only minor problems encountered. Although the hovercraft was able to reach sites that otherwise would have been inaccessible, high winds and rough water prevented it from being used to its full potential. The hovercraft was used for 2.5 days out of the 4 that were originally planned. This resulted in several sites being sampled closer to shore than originally intended.

Every effort was made to distribute the reference sites broadly over the entire Minas Basin study area and sample a variety of tidal flat types. Of the 45-50 desired reference sites, sampling limitations resulted in 40 reference sites with fine mesh samples (site 12 was not sampled) and 41 reference sites with coarse mesh samples.

3.2.1 Environmental Data

Description of Environmental Variables at Reference Sites

Fifteen environmental variables were measured in this study (Table 3.1). A complete list of environmental descriptor values for reference and test sites is provided in Appendix C.

Table 3.1. Range of the 15 environmental variables measured at all reference sites in the Minas Basin study area.

Variable (units)	Minimum	Maximum
Latitude (decimal degrees)	45.092	45.403
Longitude (decimal degrees)	-63.474	-64.375
Gravel top (%)	0	21
Gravel bottom (%)	0	32
Sand top (%)	7	92
Sand bottom (%)	6	93
Silt top (%)	4	58
Silt bottom (%)	4	55
Clay top (%)	3	43
Clay bottom (%)	3	41
Total organic carbon (%)	0.083	0.985
Total inorganic carbon (%)	0.033	0.548
Anoxic layer (0=absence, 1=presence)	0	1
Distance from shore (m)	50	1200
Classification (scale of 1-5, firm-soft)	1	5

There are many regional differences in the study area that were not measured in this study such as turbidity, salinity and temperature (previously described in Chapter 1). Continuing with a regional breakdown of north shore, Cobequid Bay, and Southern Bight, environmental data were examined for variations, and some grain size differences were found to be statistically significant. Figure 3.1 portrays the sediment composition by percentage dry weight at each reference site. Application of a Kruskal-Wallis test found Cobequid Bay to have significantly higher percentages of clay top, clay bottom, and silt bottom. Conversely, Cobequid Bay had the lowest percentages of gravel bottom ($p<0.05$ for all). Tidal flat classification scores ranged from one (sand with waves) to five (very soft mud), and were generally higher in Cobequid Bay indicating finer sediment, a thicker sediment layer, higher interstitial water content, or a combination of these variables.

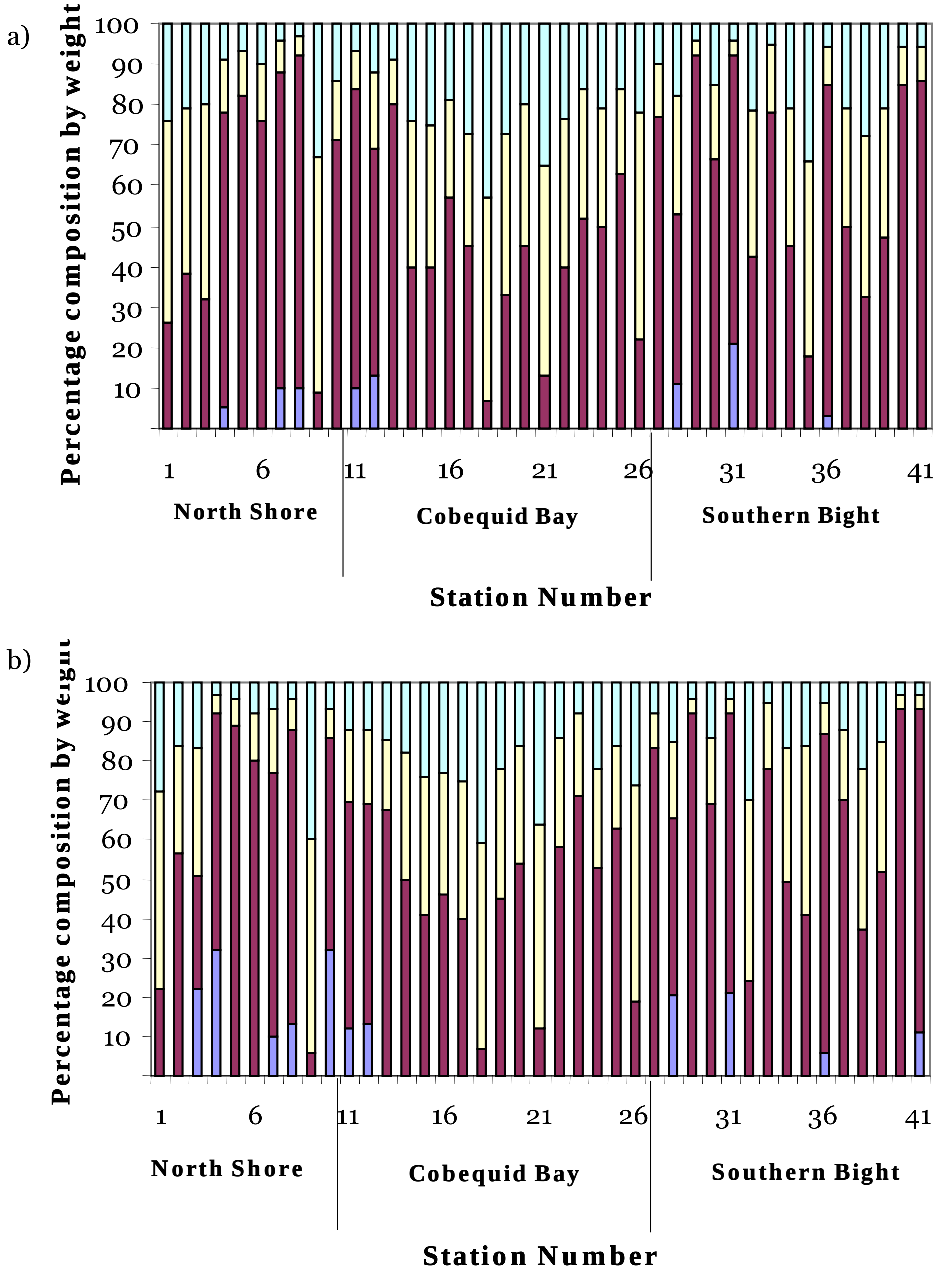

Figure 3.1. Stacked histograms showing the composition of (a) the top and (b) bottom of the grain size cores. From top to bottom, column sections represent percentages of clay, silt, sand and gravel by dry weight.

A principal components analysis (PCA) biplot is one way of representing original variables on a plot of data on the first two principal components. The first principal component (PC 1) has the largest variance between data points, and subsequent PCs are defined as the projected variable which is uncorrelated with the earlier PCs and has maximal variance. Variables are represented as arrows, whose lengths are proportional to the standard deviations of those variables, and the orientation of the arrows relates to the correlations between the variables and the principal components. The biplot for the environmental variables of all reference sites (Figure 3.2) clearly shows that PC 1 is driven by grain size, thereby explaining most of the between-site variance (51%). Total organic carbon, also along this axis, tends to be a function of grain size as smaller particles (particularly clay) are more adsorbent than the larger particles such as sand and gravel. The second principal component (PC 2) has a strong geographical component, and also includes the presence/absence of anoxic layer and distance from shore, accounting for 14 % of the variation.

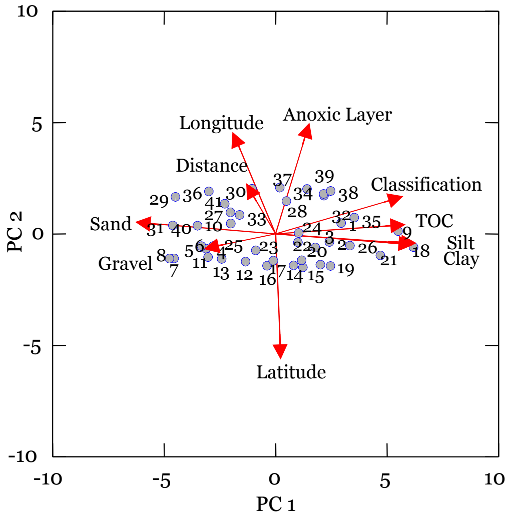

Figure 3.2. Principal components analysis biplot of all reference sites (hatched circles, sites numbered) based on 15 environmental variables measured. Variables are represented as arrows (‘TOC’ = total organic carbon; ‘classification’ as described in Chapter 2). Length of an arrow represents the magnitude of influence within the principal component.

The closer sites are in Figure 3.2, the more similar their measured environmental variables are. Referring back to Table 2.2, where all reference sites were classified into different habitat types based on distance from shore and sediment appearance, it was noted that most of the sites within each habitat type are found close together in Figure 3.2. For example, sites 9, 18, and 26 were classified as “high intertidal, very soft mud”, and are found clustered together, as are sites 34, 38, and 39 which were classified as “mid intertidal, very soft mud”. The largest classified group was “high intertidal, firm mud” containing seven sites, and all but one of these was found well clustered in Figure 3.2. Thus, the principal components analysis of the environmental data lends support for the *a priori* tidal flat classification method based on distance from shore and visual appearance.

Description of Environmental Variables at Test Sites

The only two environmental variables at test sites showing significant variation from the average of the reference sites was the presence/absence of anoxic layer and gravel content in the top layer of the sediment core samples ($p \leq 0.05$ for both). Thirty-nine percent of all reference sites had a visible anoxic layer, whereas the anoxic layer was only visible at 9% of test sites. The average gravel content of the 41 reference sites was 0.5%, with a maximum of 4.6% gravel at site 31. Eighty-one percent of all reference sites had zero gravel, whereas 36% of the test sites had zero gravel content. Average test site gravel content was 2.2% with the maximum of 6.8% at site T5. The other grain size measures (sand, clay and silt content) also showed lower values in test sites when compared to reference sites, however, they were not significantly different.

3.2.2 Biota

All fine mesh samples collected a total of 10,015 specimens representing 27 taxa in 8 phyla. The coarse mesh samples collected 26,204 specimens representing 33 taxa in 6 phyla. A grand total of 37 taxa were identified and of these, 22 were found in both mesh sizes, 5 taxa were found only in the fine mesh samples and 10 taxa were found only in the coarse mesh samples.

Although Warwick *et al.* (1990) suggest the genus level may be the optimum taxonomic level for most efficient discrimination for bioassessments, several studies (Gray *et al.*, 1990; Warwick, 1993; Somerfield and Clarke, 1995; Worm *et al.*, 2002) have found minimal loss of information when identifying to the family level compared to the species level for the purposes of bioassessments, saving both time and money. Identification of specimens to the family level was the intent of this study.

Of the taxa identified to family level, two have only one species present in the study area and were frequently encountered when processing samples. These are *Mya arenaria* (Family Myidae), also known as the Soft-Shelled Clam, and *Macoma balthica* (Family Tellinidae). Within the family Corophiidae there is one genus (*Corophium*) and five species found in the Minas Basin (Bromley and Bleakney, 1984). The most common and abundant of the species is *Corophium volutator*, commonly referred to as the mud shrimp. For simplicity in the text of this thesis the families Myidae, Tellinidae, and Corophiidae are referred to by the genera within – *Mya, Macoma,* and *Corophium* respectively. Table 3.2 provides a summary of all taxa identified and enumerated in both the fine and coarse mesh samples.

Table 3.2. List of all taxa encountered (family level identification or higher), frequency of occurrence and average densities within the 250 µm and 1 mm collections. Families with only one genus present in the study area are listed in brackets, "-" denotes taxon not found. Taxonomy based on Bromney and Bleakney (1984).

Taxonomic listing	**250 µm mesh**		**1 mm mesh**	
	Frequency (% of reference sites)	Average density (/m^2)	Frequency (% of reference sites)	Average density (/m^2)
Phylum Coelenterata				
Class Anthozoa				
Family Edwardsiidae	7	0	-	-
Phylum Mollusca				
Class Gastropoda				
Family Pyramidellidae	5	0	32	48
Hydrobiidae	2	0	2	0
Naticidae	-	-	7	0
Nassariidae	7	0	15	16
Buccinidae	-	-	2	0
Columbellidae	-	-	2	0
Class Bivalvia				
Family Mytilidae	-	-	2	0
Anomiidae	-	-	2	0
Pholadidae	-	-	2	0
Myidae (*Mya*)	15	0	22	16
Tellinidae (*Macoma*)	27	368	59	240
Veneridae	2	0	-	-
Phylum Annelida				
Class Polychaeta				
Family Sabellidae	12	1,103	7	16
Cirratulidae	5	0	7	16
Maldanidae	20	0	29	64
Orbiniidae	10	0	10	64
Capitellidae	83	8,453	54	288
Glyceridae	2	0	5	0
Spionidae	61	4,778	59	1,920
Nephtyidae	17	1,103	34	16
Phyllodocidae	20	0	32	32
Nereidae	46	368	54	64
Syllidae	20	368	7	16
Phylum Arthropoda				
Order Thoracica				
Family Balanidae	2	0	-	-
Order Cumacea				
Family Diastylidae	5	0	17	16
Order Isopoda				
Family Janiridae	2	0	5	0
Order Amphipoda				
Family Gammaridae	-	-	15	0
Corophiidae (*Corophium*)	78	31,607	78	4,880
Caprellidae	-	-	2	0
Order Mysidacea				
Family Mysidae	-	-	12	0
Order Decapoda				
Family Hippolytidae	-	-	32	32
Order Diptera				
Family Chironomidae	2	0	2	0
Order Harpacticoida	51	1,470	-	-
Order Calanoida	2	0	2	0
Phylum Rhyncocoela	22	0	37	32
Phylum Nematoda	88	9,556	-	-
	n=27		**n=33**	

Description of Biota at Fine Mesh Reference Sites

Twenty-seven taxa were identified in 40 reference site samples (Table 3.2, Appendix D). All but three groups of organisms (harpacticoid copepods, nematodes and nemertean worms) were identified to the family level. The number of individuals collected in the reference site core samples ranged from 7 to 499 (equivalent to 2,572 to 183,321 individuals/m^2), and the number of taxa per site ranged from three to 11. The mean and median number of individuals collected per station was 163 and 116 respectively.

The reference sites with the richest community composition were sites 2 and 37 (11 taxa), followed by sites 1, 6, and 36 (10 taxa). The sites with the highest total organism densities were sites 15, 3, 34, and 2 each having 499, 454, 387, and 385 individuals respectively. Conversely, the reference sites with the lowest diversity were sites 8, 20, and 39, with only three taxa. The lowest organism densities were found at sites 20, 8, 22, and 7 each having seven, 11, 17, and 21 individuals respectively.

Nematodes, capitellids and the amphipod *Corophium* were the most abundant and widespread taxa, found at 80% or more of the reference sites (Figure 3.3). Spionids, harpacticoid copepods, and nereids were the next most frequent and abundant, occurring at 45 to 65% of all reference sites. Sabellids and nephtyids were locally abundant, as they occurred at few sites, but were in relatively high numbers. Taxa that were local and rare are located in the bottom left-hand corner of Figure 3.3 (not labelled). Distribution maps of the common taxa in the fine mesh samples are provided in Appendix E.

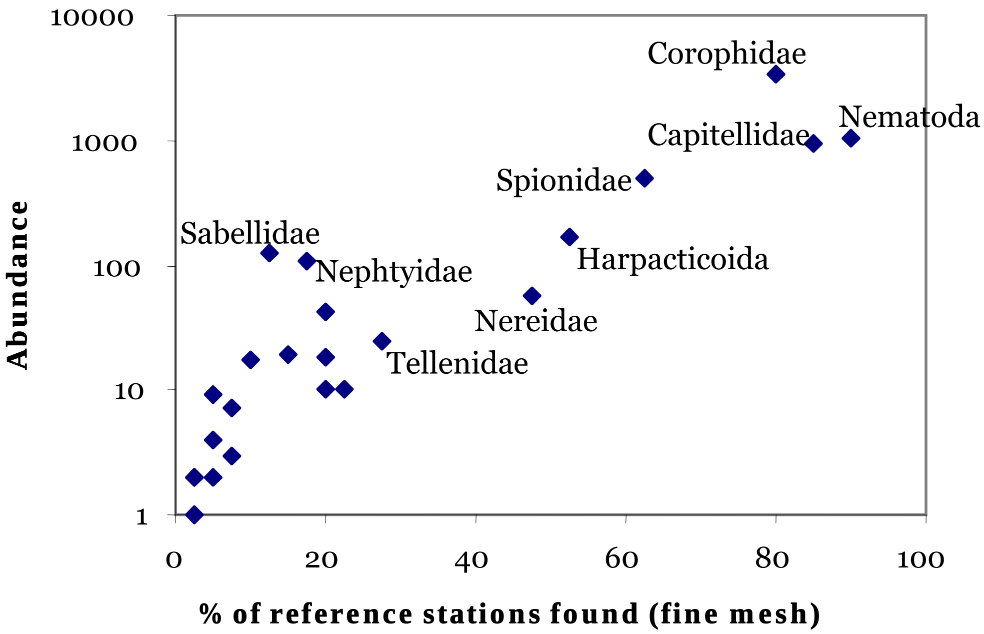

Figure 3.3. Frequency-abundance diagram for reference sites showing all taxa in the 250 µm mesh samples. Frequency equals taxa occurrence as a percentage of all reference sites; abundance equals the total sum of individuals for all reference sites ($\log_{10}$ scale).

Regional Variation

In general, fewer taxa and fewer individuals per site were encountered in Cobequid Bay than the north shore or the Southern Bight, however these differences were not significant. *Corophium* and capitellid densities were highest along the north shore and the Southern Bight. Harpacticoid copepods were slightly higher in the Bight area than elsewhere and they occurred less frequently in Cobequid Bay. Spionid densities were significantly higher along the north shore compared to Cobequid Bay and the Southern Bight (p=0.002).

Multivariate Analysis

PRIMER was used to map the biological community as an MDS plot (Figure 3.4), which graphically portrays the degree of similarity between sites. Sites that have similar biological communities will be closer together, and sites that are different will be farther apart. Some sites that are geographically a few meters apart, such as sites 1 and 2, 7 and 8, and 38 and 39 are plotted close together indicating they have similar biological communities, however this is not always the case (e.g., site 4 is well removed from 5 and 6) . Generally, there is no clear biological distinction between the geographic regions previously discussed, however some sites from Parrsboro and the north shore area (sites 1-9) are plotted closely with several sites in the Southern Bight (sites 35-41). More detailed analysis of community composition is provided in Chapter 4.

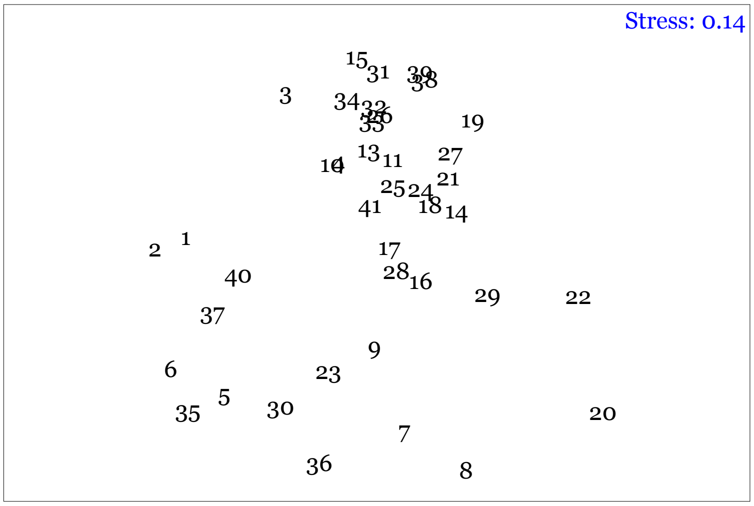

Fig 3.4. MDS plot of the fine mesh biological community for all reference sites. Numbers represent sampling sites (1-10 = North Shore, 11-26 = Cobequid Bay, 27-41 = Southern Bight).

Using all measured environmental variables, the BIOENV procedure in PRIMER was run to select the environmental variables which best fit the biological patterns seen in the fine mesh reference sites. An extremely weak relationship was found (18%) with only two variables, total organic carbon and mudflat classification, contributing to this relationship.

Description of Biota at Fine Mesh Test Sites

Of the 11 test sites sampled there were 18 taxa encountered, all of which were observed at in the reference sites. The total number of individuals collected in the test site samples ranged from 158 to 546 (equivalent to 57,986 to 200,382 individuals/m^2), and the mean and median number of individuals collected per site were 317 and 242 respectively. The average number of individuals per site was significantly higher than the average at reference sites (p=0.002). The number of taxa per site ranged from 5 – 12, with the lowest at site T5 at Economy and the highest at site T1 in Parrsboro harbour.

Significantly higher densities of capitellids (p=0.005) and nematodes (p=0.002) were present at test sites than reference sites. In general, some test sites also showed some notable variation when compared to reference sites. For instance, the highest total organism densities were 546 and 528 individuals at sites T9 and T1 respectively. Site T9 contained primarily *Corophium*, however this may have included dead individuals, and site T1 contained primarily nematodes and sabellids. Sites T4, T5 and T8 all had very high densities of capitellids, and sites T10 and T11 had the highest densities of nematodes at 156 and 268 respectively (57,252 and 98,356/m^2). In comparison to reference sites the test sites showed higher numbers of organisms, as well as higher densities of particular opportunistic families, such as capitellids and nematodes; however these variations were not seen at all test sites and no generalizations can be inferred.

Description of Biota at Coarse Mesh Reference Sites

Fourty-one reference sites were sampled, revealing the presence of 33 taxa (Table 3.2, Appendix F). All but two groups of organisms (calanoid copepods, and nemertean worms) were identified to the family level. The number of organisms collected in the reference quadrat samples ranged from 8 – 1,929 (equivalent to 128 – 30,864 individuals/m^2), and number of taxa per site ranged from 1 – 13. The mean and median number of organisms collected per 625 cm^2 quadrat is 489 and 314 respectively.

The reference sites with the richest community composition were sites 6 and 36 with 13 taxa each, and sites 2, 12, and 37, all with 12 taxa. The sites with the highest total organism densities were sites 10, 41, and 31 having 1,929, 1,699, and 1,643 individuals respectively. Conversely, the reference sites with the lowest diversity were sites 40 with only one taxon, site 7 with two taxa, and sites 3, 13, 18, and 22, each with three taxa. The lowest organism densities were found at sites 40, 8, and 7 each having 8, 15, and 20 individuals respectively.

The amphipod *Corophium* was the most common and abundant of all taxa, occurring at approximately 80% of all sites (Figure 3.5). Occurring in between 55 and 60% of all reference sites were the spionid, capitellid, and nereid worms, along with the bivalve *Macoma*. Orbiniids were locally abundant, and occurred in high numbers in the few sites where they were collected. Taxa that were local and rare are in the bottom left-hand corner of Figure 3.5 (not labelled). Distribution maps of the most common taxa in the coarse mesh samples are provided in Appendix G.

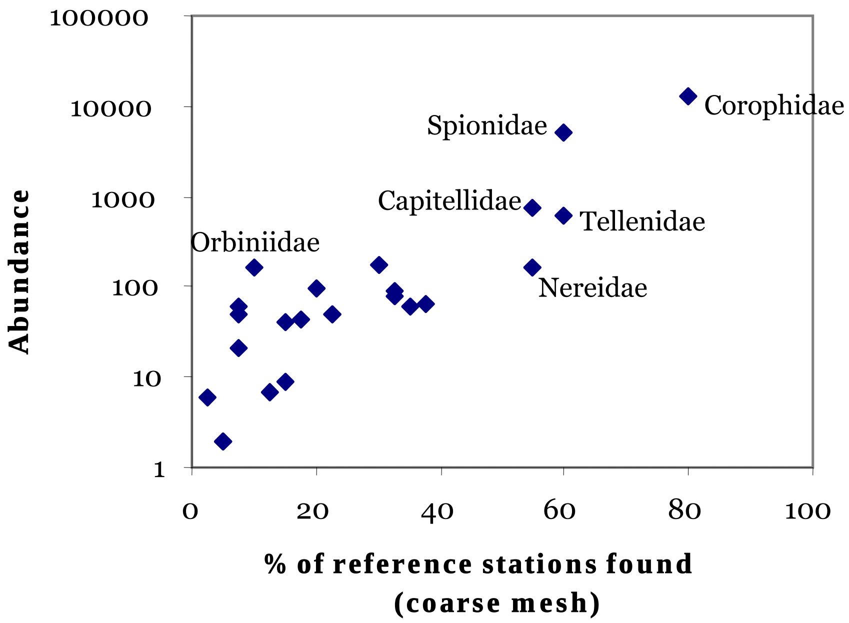

Figure 3.5. Frequency-abundance diagram for reference sites showing all taxa in the coarse mesh data set. Frequency equals taxa occurrence as a percentage of all reference sites; abundance equals the total sum of individuals for all reference sites ($\log_{10}$ scale).

In examining relationships between biological elements of the reference community, some relationships with the amphipod *Corophium* were considered important to present. A strong negative relationship was found between polychaetes and amphipods when expressed as percent dominance at each reference site (Figure 3.6a). The same relationship was found in the fine mesh samples, however it was weaker (R^2=0.5736). When the total numbers of organisms at each reference site were plotted against the number of *Corophium* at each site, a strong positive relationship was found (Figure 3.6b). This suggests that *Corophium* was the primary contributer to high faunal densities. The outlier six sites with little or no *Corophium* but high total organism density all had high densities of spionids. This relationship was not seen with the fine mesh sample data.

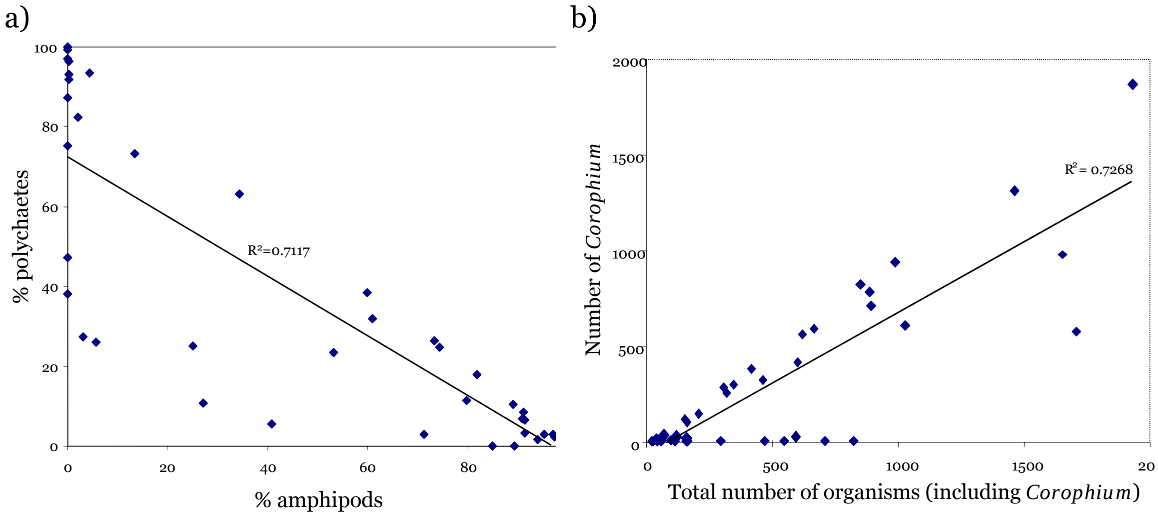

Figure 3.6. Scatter plots presenting the relationship between (a) percent dominance of amphipods and polychaetes, and (b) the total number of organisms compared to total number of *Corophium* in each of the coarse mesh (1 mm) samples.

Regional Variation

Keeping with the regional breakdown previously discussed, the biological variation was much stronger with the coarse mesh fauna. There were significantly more taxa per site and more individuals per site in the Southern Bight than in Cobequid Bay and the north shore. The Southern Bight had an average of eight taxa per site, while Cobequid Bay and the north shore had averages of five and six respectively. Capitellid densities were also significantly higher in the Southern Bight. In Cobequid Bay, *Corophium*, maldanid, nephtyid and spionid densities were lowest. Conversely, *Mya*, nereid, and *Macoma* densities were highest in Cobequid Bay ($p \leq 0.003$ for all).

Multivariate Analysis

PRIMER was used to map the biological community as an MDS plot (Figure 3.7), and the resulting plot was remarkably similar to the fine mesh results (Figure 3.4).

Interestingly, the sites that are geographically close and showed a high degree of similarity in the fine mesh MDS plot generally show the same degree of similarity in the coarse mesh MDS plot, below. As in the fine mesh MDS plot there is no clear biological distinction between the geographic regions previously discussed, however the geographic congruity of the sites is slightly more evident. The eight sites in the lower right-hand corner are all located in Cobequid Bay, and the same north shore/Southern Bight cluster is seen on the left-hand side of the plot. More detailed analysis of community composition is provided in Chapter 4.

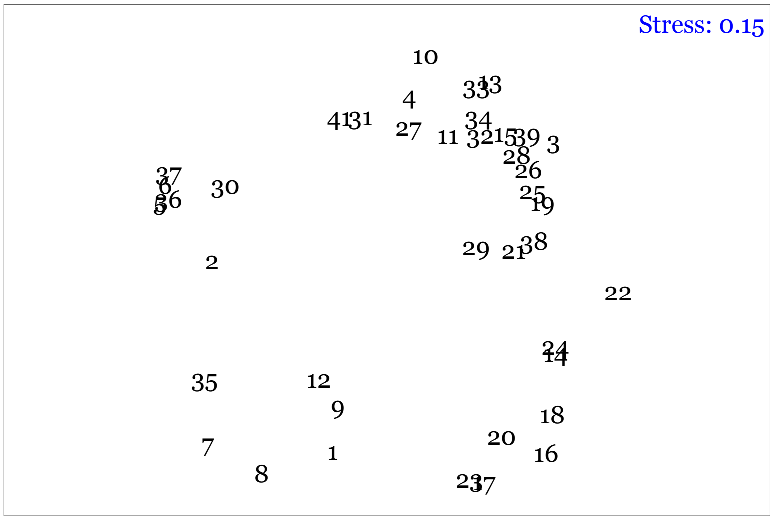

Fig 3.7. MDS plot of the coarse mesh biological community for all reference sites. Numbers represent sampling sites (1-10 = North Shore, 11-26 = Cobequid Bay, 27-41 = Southern Bight).

Using all measured environmental variables, the BIOENV procedure in PRIMER revealed a stronger relationship than the fine mesh samples, with 29% of the variation in the biological community accounted for using longitude, total inorganic carbon, and total organic carbon.

Description of Biota at Coarse Mesh Test Sites

Of the 11 test sites sampled there were 20 taxa encountered, all of which were observed at the reference sites. The total number of individuals collected ranged from 113 at site T1 to 1,617 at site T3 (equivalent to 1,808 to 25,872 individuals/m^2), and the mean and median number of individuals collected per site was 562 and 284 respectively. The number of taxa per site ranged from four to 11, with the lowest at sites T5 (Economy) and T7 (Noel Bay) and the highest at site T10 at Houston Beach.

There were more consistent differences seen between reference and test sites in the coarse mesh data. On average, there were higher average densities of capitellids and *Corophium*, and lower densities of maldanids and spionids at test sites. Test sites also displayed an increase in community dominance by amphipods (primarily *Corophium*) with reference sites having an average of 48% amphipods and test sites having 60%. Conversely, a slight reduction in community dominance by polychaetes was noted, with reference sites having an average of 36% and test sites having 30%. There were no increases in total organism density as seen in the fine mesh samples.

3.3 Discussion

The frequency diagrams representing the fine and coarse mesh faunal samples (Figures 3.3 and 3.5 respectively) are very similar. *Corophium*, capitellids and spionids were found frequently and abundantly in both the fine and coarse mesh data sets, with *Corophium* being the most abundant and widespread taxon encountered. The coarse mesh was not able to retain the smaller taxa such as nematodes and harpacticoid copepods, and the larger surface area of the coarse mesh quadrat may have been more effective in sampling nereids and *Macoma* as they were present at a greater percentage of coarse mesh reference sites.

Nematodes were the most frequent taxon encountered in the 250 µm data set, appearing at 90% of all reference sites. Mean and median reference site densities were 13,462/m² and 6,976/m² respectively. The maximum density found was 98,400/m² at site T11, and densities were significantly higher at test sites. Nematodes vary widely with respect to life history, and range in feeding preference from general consumption of detritus and bacteria to being predatory. Little is known about their dispersal mechanisms, however it has recently been suggested that they actively choose settling spots in response to the food quality of the sediment (Ullberg, 2004), suggesting that the disturbed test sites in this study may actually be of higher food quality and/or availability for some fauna. These results are consistent with Schratzberger and Warwick (1998) who found physical disturbance at intermediate levels in sand and mud flats to increase nematode densities.

Maximum *Corophium* density using the fine mesh data was found at site T9 (Avonport, approximately 189,800/m²), an area heavily and frequently dug for bloodworms. When the site was sampled it was observed to have many dead *Corophium* (and/or molts) lying on the surface of the flat, however these were not distinguishable in the lab. It is suspected that the preservative masked the visual differences between live and dead *Corophium* making the density at that site invalid. The next highest density was considerably lower, approximately 123,360/m² at site 31. This value is still much higher than those cited in the literature for the Minas Basin, which range from >46,000/m² reported by Wilson (1989) to >60,000/m² reported by Gratto *et al.* (1983) and Wilson (1988), likely a result of the efficiency of the fine mesh in capturing small individuals. The highest *Corophium* density found with the coarse mesh was at site 10 which captured the equivalent of approximately 29,900 individuals/m².

Evangeline Beach (sites 35-37) is a popular feeding ground for semipalmated sandpipers, and recognized internationally as part of the Western Hemispheric Shorebird Reserve Network. It is unusual that no *Corophium* were encountered in 2002 at any of the Evangeline Beach sites. In the past, *Corophium* have been found far from shore, close to low tide (P. Hicklin, pers. comm.). As the distance from shore at the three Evangeline Beach sites ranged from 350 m to 900 m, it is believed that 900 m might not have been far enough from shore, however, further study to confirm this would be required.

3.3.1 Regional Variation

Cobequid Bay was found to be distinct from other areas of the Minas Basin, with significantly finer sediments and a different faunal community. These differences are most evident in the coarse mesh biological community, with Cobequid Bay being characterized by lower densities of tube builders (maldanids and spionids) that prefer larger grain sizes for tube construction and feeding, and higher densities of *Mya* and *Macoma* clams, which can tolerate the finer sediments and may actually prefer them (Azouzi *et al.*, 2002).

Gray (1981) found wave action and current velocity to be the most important factors influencing grain size, and although these were not measured directly for this study these variables have been studied by others. Amos and Joice (1977) showed that lower wave energy levels are found in the Minas Basin than in the outer Bay of Fundy, even though prevailing winds align with the long axis of the basin. This is likely due to the barrier effect of the Cape Blomidon-Cape Split peninsula (Dalrymple, 1977). Studies by the Atlantic Tidal Power Engineering and Management Committee (1969) indicate that wave energy is concentrated at the tip of Economy Point and Cape Tenny (directly across the basin on the south shore), leaving the waters of Cobequid Bay relatively calm, with little wave energy. Higher summer surface water temperature (Bousfield and Leim, 1959), higher turbidity (Greenberg and Amos, 1983) and lower salinity (Bousfield and Leim, 1959) are also typical of the Cobequid Bay waters.

The biological communities of the north shore and Southern Bight appear to be quite similar, in that they share common taxa. Also, the relative densities of major taxa are similar, such as the densities of sabellids and syllids in the fine mesh data (Appendix E), and densities of maldanids and spionids in the coarse mesh data (Appendix G). This is also supported by the similarity of Southern Bight and north shore reference sites found in the MDS plots of the fine and coarse mesh communities (Figures 3.4 and 3.7 respectively).

3.3.2 Relationship between Biota and Habitat

The BIO-ENV analysis was inconclusive in linking biota and habitat. The poor linkages may be a result of two factors: the suite of environmental variables measured for this study was insufficient and perhaps did not include the most appropriate variables to measure, and biological interactions likely play a large role in structuring the intertidal community (not considered in the BIO-ENV analysis) . Both of these suppositions are explored further in Chapter 4.

3.3.3 Effects of Physical Disturbance

The fine mesh samples revealed significantly higher meiofaunal densities in the test sites than in the references sites. There were also significantly higher densities of capitellids and nematodes. Higher densities of *Corophium* were also observed at test sites, although these were not statistically significant. The coarse mesh samples also saw higher densities of capitellids and *Corophium* at test sites compared to reference sites, as well as a decrease in maldanid and spionid densities, but again these differences were not significant. There was also a slight increase in the dominance of *Corophium* and a slight decrease in polychaete dominance (mainly spionids) in test sites, however this study provided only a snapshot of population levels. Other studies have shown longer-term declines in *Corophium* population levels (Shepherd and Boates, 1999) and infaunal communities as a whole (McLusky *et al.*, 1983; Gee, 1993) following intertidal harvesting.

Digging for bloodworms removes sediment from its original position, completely overturning large sections and exposing the underside to air and wave or current action. Fine sediment is transported and stones and shells previously buried are exposed as a result (Anderson and McLusky, 1981; Underwood and Paterson, 1993; Farrell, 1996). This study supports those conclusions to some extent, as test sites had significantly higher gravel content than reference sites. Site T5 (Economy) had 47% gravel by weight, the highest of all sites, with the second highest content at site T8 (Moose Cove) with 23%.

The primary biological factors affecting sediment stability are microbial and microalgal populations associated with particle surfaces that can cause increased adhesion between particles and effectively alter the granulometry (Probert, 1984). Extracellular products of microorganisms living on grains and within interstices foster stability by means of accumulation of mucilaginous materials, particularly by epipelic diatoms. Some mudflats can harbour diatom densities of up to 1-5 x 10^5/cm^2 (Coles, 1979). The action of bloodworm harvesting results in the burial of fauna and microorganisms that contribute significantly to the tidal flat stability and subsequent resistance to erosion (Grant *et al.*, 1986; de Deckere *et al.*, 2000; van de Koppel *et al.*, 2001; Lelieveld *et al.*, 2003).

Intertidal harvesting may also cause incidental mortality of organisms as a result of physical damage, burial and smothering, or exposure to desiccation and predation (Ambrose *et al.*, 1998; Dernie *et al.*, 2003). This was apparent at station T9, Avonport, where large numbers of dead *Corophium* (or molts) lay on the sediment surface. Any mortality will have immediate effects on the soft sediment community; however, effects on population levels of a species in the longer term will also be dependent upon the degree of habitat damage and the rate of subsequent recovery, discussed further in Chapter 4.

3.3.4. Post-Disturbance Recovery

Overall post-disturbance recovery rates will primarily depend on the energy (i.e., tidal and/or waves) of the site. Evidence of digging activity will disappear more quickly in sandier, high energy environments with more wave action and faster tidal currents than sheltered areas with finer sediments (McLusky *et al.*, 1983). This was noticeable at site T1, which was in the cove on the lee side of the Parrsboro lighthouse. The sediments in that cove are indicative of a low energy area, having high percentages of silt and clay. After 10 days, evidence of experimental disturbance was still quite obvious, with a highly irregular surface with pools of standing water. Stations T7 and T8, experimentally disturbed 19 days prior to sampling, were in higher energy areas. These sites had noticeably higher gravel content (several rocks exposed) but had flat surfaces similar to the surrounding area (Appendix B).

The intensity and scale of any disturbance event influences the recovery dynamics of an ecosystem. Ecological theory suggests that small-scale disturbances at intermediate frequencies may have beneficial effects (Connell, 1978; Caswell and Cohen, 1991), previously discussed in Chapter 1 (Figure 1.1). In support of this theory, the author-disturbed test sites (T1, T7 and T8) could all be considered small in scale (approximately 1 m^2 each), and mostly resulted in increased numbers of taxa and total organism density. At the test sites where harvesting was observed, larger and more frequent disturbance had likely occurred; this was correlated with higher densities of the more mobile and opportunistic taxa (capitellids and *Corophium*), and lower densities of the sessile, tube building taxa (maldanids and spionids). Larger disturbances would require large-scale transport of organisms or significant reproductive output from outside source patches for recovery to occur.

Redfield and Deevy (1952) noted that after a disturbance, bacteria could develop in a matter of hours, which has been shown to facilitate nematode settlement (Ullberg, 2004). As a result of initial bacterial recolonization, organic films composed of adsorbed organic substances, microbial extracellular metabolites and microorganisms such as bacteria and fungi will develop in a relatively short period of time. Probert (1984) reported chlorophyll *a* and gross primary production returned to control levels within 10 days post-disturbance. Similarly, Underwood and Paterson (1993) reported that chlorophyll *a* concentrations recovered within six days.

3.3.5. Mesh Size and Area

The fine and coarse mesh sampling strategies were successful at characterizing the faunal community in different ways. The fine mesh was successful in retaining the larger meiofauna, namely harpacticoid copepods and nematodes. Although the small surface area of the core may have been adequate for sampling the smaller organisms, it was likely insufficient for characterizing the larger fauna such as maldanids, bivalves, or the larger polychaetes. The large size of the quadrat used for the coarse mesh samples resulted in more thorough characterization of the macrofauna and a higher number of taxa enumerated in that data set. Both samples were very time consuming to sort in the laboratory. The fine mesh samples were more difficult to handle under a dissecting scope, particularly the nematodes. Processing the coarse mesh samples was also a slow process, but mostly as a result of the sheer volume of material to sort through (from gravel and cobble to plant litter and leaves). The volume of the coarse mesh samples ranged from less than 250 ml to more than 1.5 L.

CHAPTER 4: RESULTS AND DISCUSSION

THE REFERENCE CONDITION APPROACH

This chapter details the results of the BEAST and RIVPACS assessments. The fine mesh and coarse mesh fauna were analyzed separately, with the fine mesh results outlined first and the coarse mesh results following that. The chapter concludes with an overall comparison and discussion of the approaches.

4.1 Results: Fine Mesh (250 um)

4.1.1 Model Building

Classification of Reference Sites into Biological Groups

Cluster analysis of all reference site taxa (Figure 4.1a) revealed three distinct community groups. Clusters may be identified on a dendrogram by drawing a horizontal line at any similarity level, in this case 20%. Closer inspection of Group 3 biota revealed that these three sites had very low numbers of capitellids, *Corophium*, nematodes, and low total organism abundance. Although the ANOSIM routine in PRIMER found Group 3 to be biologically distinct, total organism abundances for sites 20, 22, and 29 were very low (seven, 17, and 32 respectively), whereas total abundances for most other reference sites ranged between 100 and 200 or more. Unfortunately, based on the limited data collected for this study, the cause(s) for biological impoverishment cannot be determined. The three reference sites in Group 3 were insufficient for the BEAST analysis, for which a minimum of 10 sites to adequately characterize the variability of a community group is recommended (Reynoldson and Wright, 2000). Therefore, reference sites 20, 22, and 29 were removed from further analysis.

The dendrogram was re-created with the remaining 37 reference sites (Figure 4.1b) and two biological groups (dashed line) were formed which were used for RCA analysis. The geographic distinctness of the two communities is strong (Figure 4.2). Group 1 contains 13 reference sites, and covers mainly the Parrsboro to Five Islands shore and the Southern Bight. Group 2 contains 24 reference sites, and encompasses the entire Cobequid Bay area (Economy to Walton), several sites in the Southern Bight, and two sites on the north shore. Of the 15 environmental variables measured, only longitude was significantly different between the two groups (p=0.006).

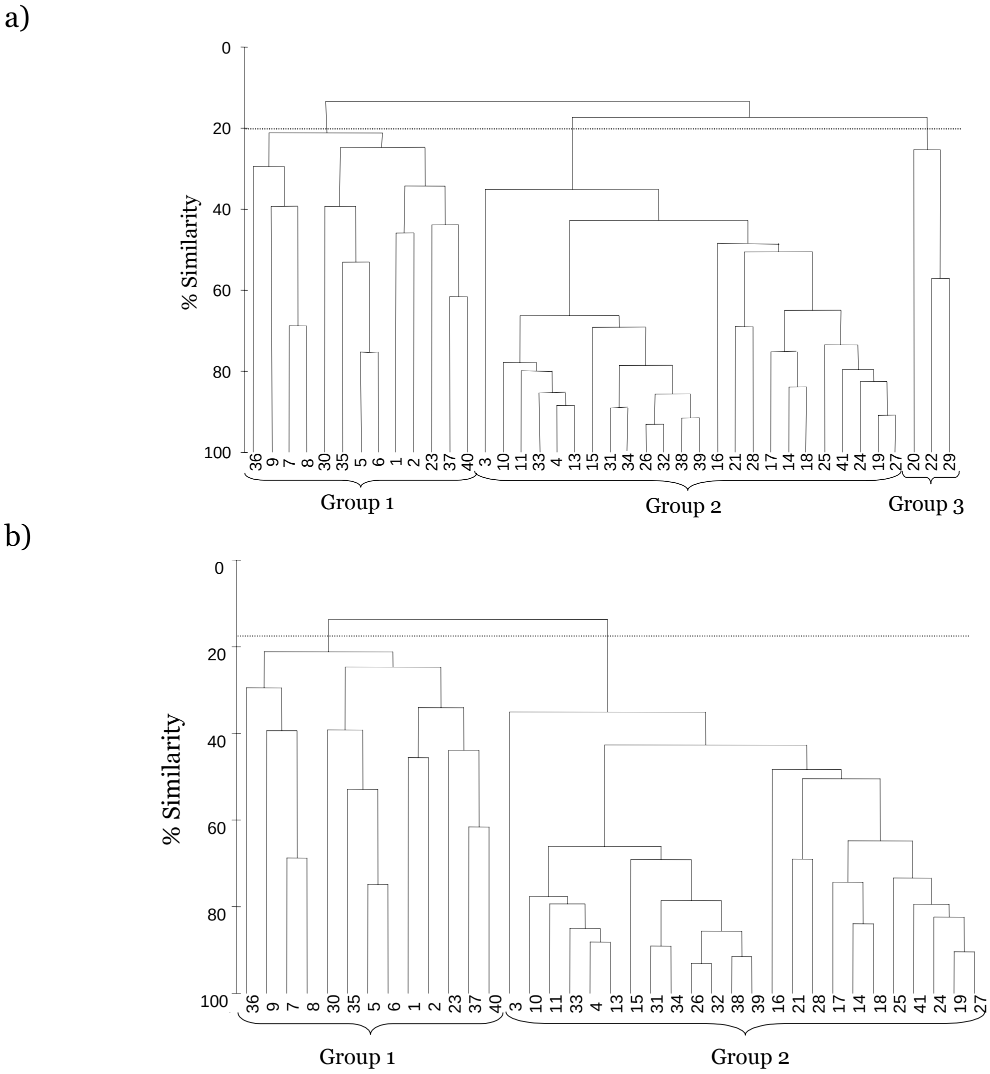

Figure 4.1. Dendrogram plots of cluster analysis based on all biota from 250 µm samples in the Minas Basin mudflats based on (a) all 40 reference sites and (b) the final dendrogram excluding reference sites 20, 22, and 29.

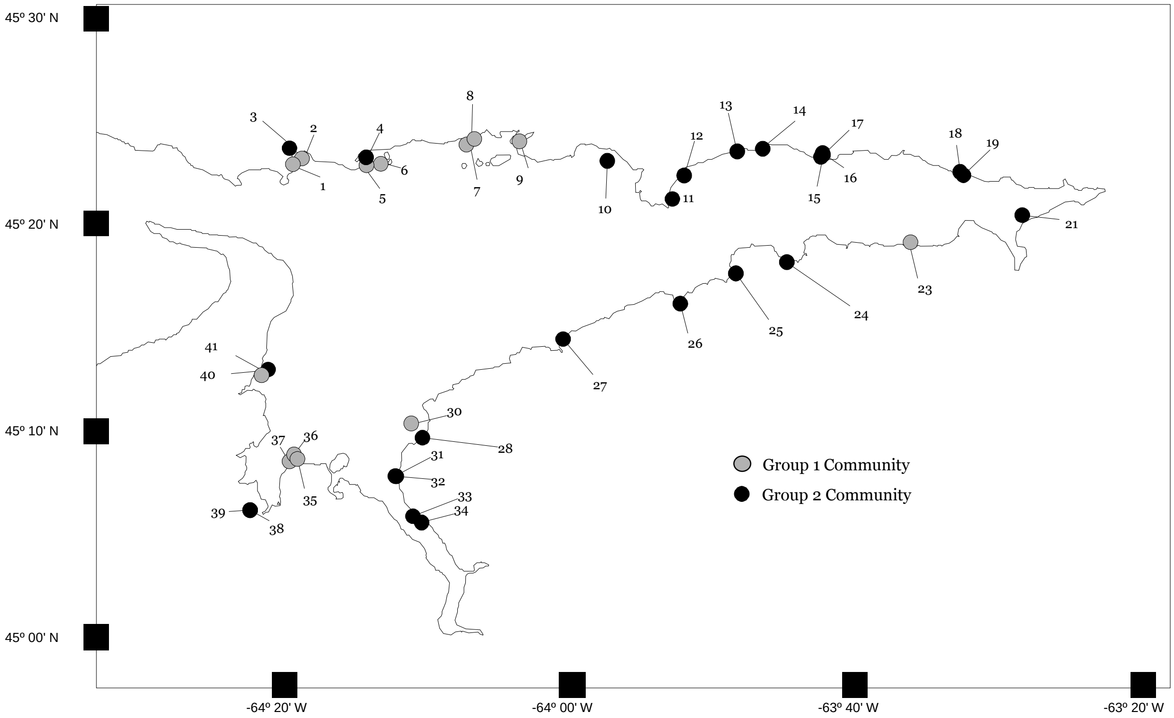

Figure 4.2. Geographic distribution of the invertebrate community groups formed by cluster analysis of the fine mesh (250µm) faunal data from 37 reference sites in the Minas Basin tidal flats (sites 20, 22, and 29 removed). Group 1 biological community represented by grey circles, Group 2 biological community represented by black circles.

Group 1 had higher average taxa richness (number of taxa/site), and lower average densities of organisms than Group 2 (Table 4.1). Capitellids and nematodes are both common to the entire study area, and were both found at 85% of all Group 1 reference sites. In Group 2, capitellids and nematodes were found at 81 and 96% of the reference sites respectively. The major differences between the two groups were the 100% dominance of spionids in Group 1 and *Corophium* in Group 2. There were also group differences between average densities of the nematodes and capitellids. Most striking was the difference in *Corophium* abundances, with Group 1 having only an average of seven individuals per site (not shown) and Group 2 having an average of 127 individuals per site.

Table 4.1. Descriptions of two biological reference groups formed by cluster analysis of fine mesh (250 µm) biota based on 37 reference sites in the Minas Basin tidal flats (based on Figure 4.1b).

Group (# sites)	Average Taxa Richness (±SD)	Average Total Abundance (±SD)	Common Taxa, Frequency of Occurrence in Each Group (Average abundance per site)
1 (13)	8 (3)	114 (109)	Spionids, 100% (31) Nematodes, 85% (41) Capitellids, 85%(8)
2 (24)	6 (2)	209 (125)	*Corophium*, 100% (142) Nematodes, 96%(21) Capitellids, 81%(34)

The SIMPER routine in PRIMER revealed that sites in Group 1 had an average similarity of 28% and the densities of spionids, nematodes, capitellids and harpacticoid copepods together contributed 91% of that similarity. Sites in Group 2 had a higher average similarity of 53% with densities of *Corophium*, capitellids and nematodes contributing 98% of that similarity. Group 1 and Group 2 had a high average dissimilarity of 86%, suggesting they are quite different in composition.

All Group 1 and Group 2 reference sites were plotted in ordination space using the multidimensional scaling (MDS) routine in PRIMER (Figure 4.3a). Characteristics of Group 1 and Group 2 were also overlaid on the MDS plots to show the relevance of each (Figures 4.3b-f). While there was a trend in Group 1 to be defined by lower organism densities and higher taxa richness, the primary difference was the dominance of polychaetes. Conversely, Group 2 was partially characterized by higher densities of organisms and lower taxa richness, and the primary difference was the dominance of amphipods, primarily *Corophium*. Amphipod and polychaete dominance was defined as the percentage of total organism density.

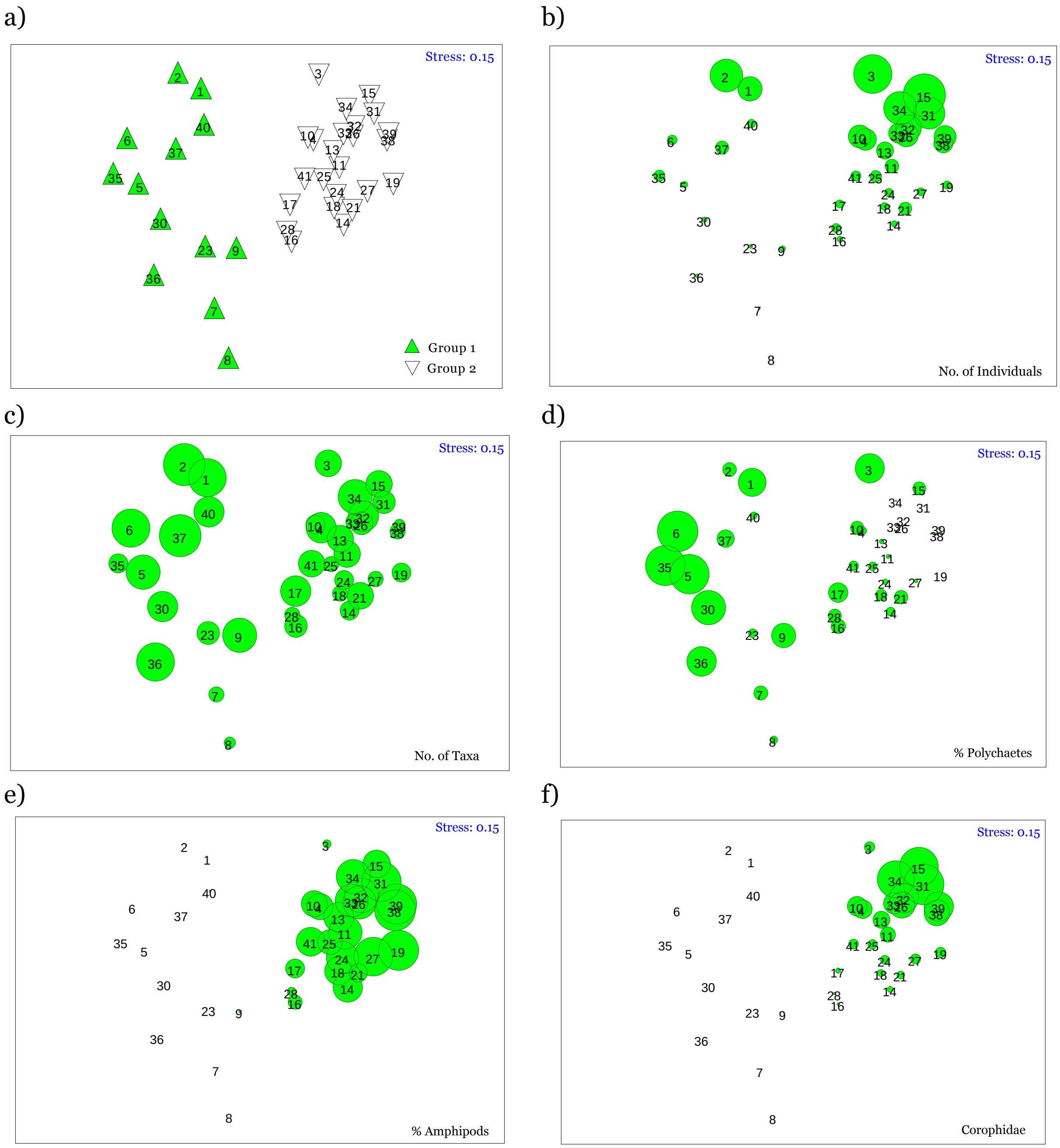

Figure 4.3. Multidimensional scaling ordinations of (a) the two reference site groups used in the 250 µm mesh analysis and relative values of (b) numbers of individuals, (c) number of taxa, (d) percentage of polychaetes, (e) percentage of amphipods, and (f) abundance of *Corophium*. Symbol size represents the importance of each factor in discriminating between groups, and numbers represent reference sites.

Correlation of Biological Data with Environmental Variables

A Mann-Whitney test for significance between reference and test sites was carried out for each of the environmental variables to determine if any were influenced by the effects of intertidal harvesting (Chapter 3). 'Gravel top' and anoxic layer were both found to be significantly different between test sites and reference sites and were both removed from discriminant function analysis. The remaining 13 environmental variables (previously described in Table 3.1) were included as possible predictor variables when building the predictive model by discriminant function analysis (DFA).

Discriminant Function Analysis produces a subset of environmental variables (Table 4.2) that best discriminated the community groupings, and can be used to probabilistically classify sites into the biological groups. This is known as the predictive model, and the accuracy of the model can be described by the proportion of sites it correctly classifies (Table 4.3).

Table 4.2. F-values and group means ± standard deviation for the five environmental variables used as predictor variables for the community groupings based on discriminant function analysis for the 250 µm mesh size data.

Variable	**F-Value**	**Group 1 mean ± SD**	**Group 2 mean ± SD**
Longitude (deg.min)	23.00	-64.18 ± 0.20	-63.93 ± 0.30
Latitude (deg.min)	6.95	45.30 ± 0.11	45.28 ± 0.12
Gravel bottom (% by weight)	14.21	2.4 ± 4.6	5.6 ± 10.4
Distance from shore (m)	5.05	396 ± 333	335 ± 325
Classification (scale of 1-5)	3.33	3.0 ± 1.29	3.7 ± 1.01

Sixty-nine percent of Group 1 reference sites and 75% of Group 2 reference sites were correctly classified using longitude, latitude, gravel bottom, distance from shore, and classification. Overall cross-validated (jackknifed) model accuracy was 73%.

Table 4.3. The number and percentage of 37 reference sites predicted to each community group using discriminant function analysis with selected environmental variables (% correct in parentheses).

Reference site community group	Number of sites predicted to Group 1	Number of sites predicted to Group 2
1	9 (69%)	4
2	6	18 (75%)
Total	**15**	**22**

Latitude and longitude are spatial descriptors that likely act as proxies for regional differences in environmental variables that were not directly measured. For example, turbidity increases as one moves from the Minas Channel to Cobequid Bay. In the central Minas Basin, turbidity also increases as one moves from the Parrsboro area down to the Southern Bight. Tidal current velocities, which are typically strongest along the north shore also, play a major role in shaping the benthic community (Canadian Hydrographic Service, 1997).

Predicting Group Membership of Test Sites

The output of complete discriminant function analysis in SYSTAT is a spreadsheet detailing the probability that each site will belong to each group (test site predictions shown in Table 4.4). Based on environmental variables, seven sites were predicted to belong to Group 1 and four sites were predicted to belong to Group 2. Test site predictions were very strong except for sites T2, T8, and T11 (all <75% for the highest prediction), and the habitat data at these sites were examined in more detail to determine if they were misclassified.

Table 4.4. Probabilities of each test site belonging to one of two reference community groups based on discriminant function analysis using longitude, latitude, gravel bottom, distance from shore, and classification as predictor variables for the 250 μm mesh biota. Shading indicates the highest probability value.

Site	Predicted Group (based on habitat)	Probability Group 1	Probability Group 2
T1	1	0.959	0.041
T2	1	0.668	0.332
T3	1	0.789	0.211
T4	1	0.904	0.096
T5	1	0.994	0.006
T6	1	0.998	0.002
T7	2	0.044	0.956
T8	1	0.549	0.451
T9	2	0.009	0.991
T10	2	0.000	1.000
T11	2	0.425	0.575

4.1.2 Assessment of Test Sites

The construction of predictive models is common to the RCA. These models are derived by classification of fauna and linking that classification to habitat attributes with DFA. There are a number of different ways of assessing the extent to which test sites match the reference sites to which they have been predicted. The most typically used are the BEAST and RIVPACS assessment methods. BEAST evaluates test sites in ordination space by the variation of the biota around the group centroid, whereas RIVPACS assesses test sites using all reference sites and weighted probabilities of taxon occurrence (described in Chapter 2). Test sites were assessed for the fine mesh size data set using both methods, outlined in detail below.

The BEnthic Assessment of SedimenT (BEAST)

BEAST assessment was carried out using the predictive model described above (Table 4.2). Individual test sites were plotted in ordination space against the reference sites of the Group to which it was predicted to belong (from Table 4.4) using the MDS routine in PRIMER. These plots were re-drawn in SYSTAT and a simple customized program (created by Trefor Reynoldson, Acadia University) was run to build the probability ellipses.

The overall model accuracy rate of 73% is well within the range of other RCA studies, which have achieved accuracy rates of 62% in the Fraser River (Reynoldson *et al.*, 2001), 76% in Great Britain (Wright *et al.*, 1984), 52% in the Great Lakes region and 75% in the Australian Capital Territory (see case studies in Bailey *et al.*, 2003)).

Each test site was compared to the predicted reference group and assessed accordingly (Table 4.5). The habitat descriptors at the test sites with prediction values lower than 75% were examined in greater detail, and two test sites were thought to be misclassified (T8 and T11). The westerly location and low gravel content of test site T2, combined with the surrounding reference sites being classified in Group 1, suggest that site T2 was correctly classified into Group 1. Test site T8, biologically similar to Group 2, has an easterly location, high gravel content, and high classification, all characteristics of Group 2. The nearest reference site (25) several meters away was also classified as Group 2, further suggesting that this test site was misclassified by the predictive model. Test site T11 had habitat characteristics of both groups, but the westerly location of the site suggests it was likely misclassified by the predictive model and belongs to Group 1. Thus, six test sites fell within reference (Band 1), four test sites were found possibly different from reference (Band 2), and one test site was found very different from reference (Band 4).

Table 4.5. BEAST assessments for all test sites using the 250 μm mesh size community data. Shading indicates sites with weak predictions that were compared to both reference groups.

Site	Predicted Group (Probability)	Habitat Similarities of Test Site Compared to Predicted Reference Group	Biological Comparison of Test Site to Predicted Reference Group	Band
T1	1 P = 0.959	Westerly location, no gravel	Biologically similar to reference but higher number of total individuals	1
T2	1 (correct) P = 0.668	Westerly location, no gravel	Biologically similar to Group 1 but higher *Corophium*	1
T3	1 P = 0.789	Westerly location, far from shore, low classification[1]	Biologically similar to reference but higher *Corophium* and total individuals	2
T4	1 P = 0.904	Westerly location, low gravel, far from shore, low classification	Not similar to reference - higher capitellids and *Corophium*, lower spionids, higher total individuals	2
T5	1 P = 0.994	Westerly location, far from shore, low classification	Not similar to reference - higher capitellids and *Corophium*, lower spionids	2
T6	1 P = 0.998	No gravel, far from shore, low classification	Similar to reference but very high *Corophium* and higher total individuals	2
T7	2 P = 0.956	Easterly location, close to shore	Similar to reference but higher nematodes	1
T8	1 (possibly misclassified) P = 0.549	Shows characteristics of both groups (easterly location, high gravel, high classification)	Biologically similar to Group 2	1
T9	2 P = 0.991	High classification	Similar to reference but lower capitellids, and higher total individuals	1
T10	2 P = 1.000	High gravel, close to shore, high classification	Not similar to reference - low *Corophium*, high nematodes	4
T11	2 (possibly misclassified) P = 0.575	Shows characteristics of both groups (westerly location, high gravel)	Biologically similar to Group 1	1

It is interesting to note that the four test sites in Band 2 (T3 – T6) all fell within Band 1 when plotted with reference Group 2. For example, test site T6 had a 99.8% probability of belonging to Group 1 and fell in Band 2 when plotted with Group 1 reference sites (Figure 4.4a). When plotted within Group 2 reference sites it fell within Band 1 (Figure 4.4b), which

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[1] Classification refers to the intertidal classification exercise described in Chapter 2.

was also the case for test sites T3, T4, and T5. Recall that the Group 2 community is comprised of high organism densities, higher capitellid densities, and a dominance of the amphipod *Corophium* (Table 4.1). Although likely still representing a reference state, this group may consist of sites that are inherently more disturbed – either by waves, tidal currents, or other environmental variables – however, further study is required to test this hypothesis.

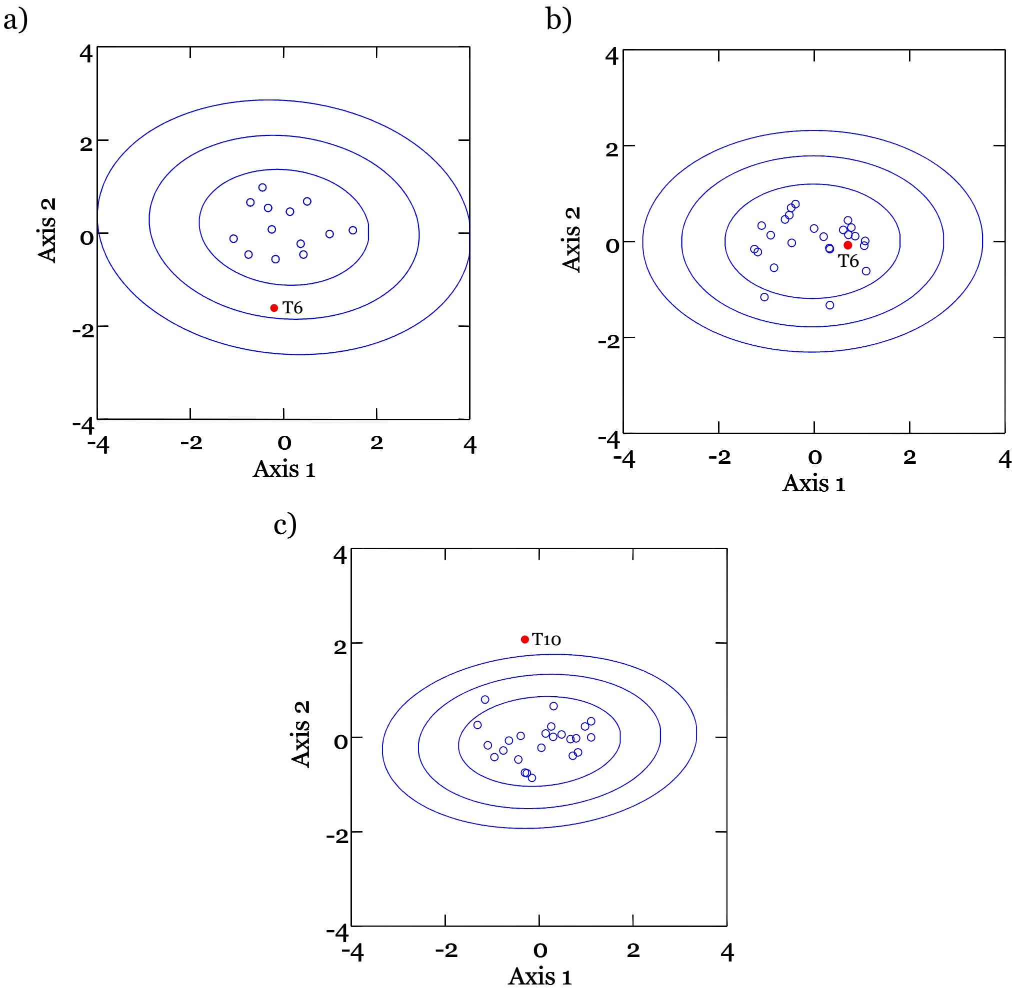

Figure 4.4. Ordination of reference sites (open circles) and selected test sites (shaded and labelled) based on cluster analysis of the 250 μm mesh size faunal community: (a) test site T6 falls within Band 2 when plotted with Group 1 reference sites (correct assessment), (b) test site T6 plotted with Group 2 reference sites, and (c) test site T10 falls within Band 4 when ordinated with Group 2. Probability ellipses around the reference sites represent 90, 99, and 99.9% (moving away from centre).

Three test sites were disturbed by the author before sampling (T1, T7 and T8) due to lack of intertidal harvesting activity in these areas, and are examined in more detail. Site T1 had 10 days of recovery before sampling. It was in close proximity to reference sites 1 and 2 (both Group 1), was predicted to belong to Group 1, and fell within Band 1 when compared to reference. Site T1 had a comparable number of taxa and similar community, but a higher number of total organisms (528), primarily driven by the presence of 285 sabellids.

Sites T7 and T8 both had 19 days of recovery before sampling and were a few meters apart from reference sites 24 and 25, respectively. Both test sites had the same taxa present as their closest reference site plus an extra one (T7) and three (T8). Total organism density was approximately double at both test sites, driven by densities of capitellids, *Corophium* and nematodes. Site T7 was predicted to belong to Group 2 and was in Band 1 when ordinated with the Group 2 reference sites. Site T8 was likely misclassified to Group 1 with a low prediction value of 55%. Site T8 shows biological characteristics of both groups with high taxa richness and nematode densities comparable to Group 1, and the high total organism, *Corophium*, and capitellid densities that are typical of Group 2.

The River InVertebrate Prediction And Classification Scheme (RIVPACS)

As described in Chapter 2, RIVPACS uses the weighted probability of taxon occurrence for all reference sites, regardless of grouping, so misclassification is less of a concern. Observed number of taxa (O) and the expected number of taxa (E) for each reference site were plotted (Figure 4.5), allowing one to immediately compare the range of values for all references sites. A perfect model would result in the observed taxa and expected taxa at each site to be equal (Figure 4.5, dashed line, $R^2=1$). The model for the 250 µm mesh size data set finds a high degree of variability in the distribution of observed and expected values (R^2=0.06), very poor results compared to other studies. The case studies outlined in Bailey (2003) reported R^2 values of 0.44 for the Fraser River, and 0.30 and 0.49 for the Australian Capital Territory for the autumn and spring samples, respectively.

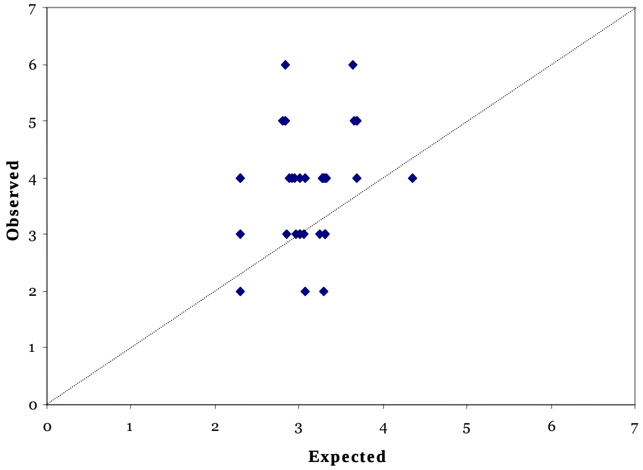

Figure 4.5. Observed number of taxa plotted against expected number of taxa for reference sites from the Minas Basin tidal flats using the 250 µm mesh size data set (R^2=0.06). Dashed line represents the ideal model O/E distribution.

The average O/E ratio and standard deviation of the reference sites was 1.24 ± 0.34; test site O/E values are provided in Table 4.6. These O/E values found no impaired test sites, as the range of Band 1 (encompassing 2 standard deviations) was quite large. For example, a test site would have to have an O/E value of less than 0.56 or greater than 1.92 to fall within Band 2, possibly impaired. A standard deviation of 0.34 is quite high (see case studies in Bailey *et al.*, 2003). This is most likely a result of the low number of taxa present in the study site with averages of 8 and 6 taxa per site in reference Groups 1 and 2, respectively, with a maximum of only 12 taxa. Maximum taxa richness recorded from previous freshwater studies are 20 in the Great Lakes, 25 in the Fraser River, and 30 taxa in the Australian study (detailed in Bailey, 2003).

Table 4.6. Number of observed taxa, expected taxa, O/E values, and final assessment for each of the 11 test sites for the 250 µm mesh size data.

Site	Location	Observed Taxa	Expected Taxa	O/E Ratio	Assessment Band
T1	Parrsboro harbour	4	2.82	1.42	1
T2	Five Islands	4	2.88	1.39	1
T3	Five Islands	4	2.81	1.42	1
T4	Economy	4	2.30	1.74	1
T5	Economy	3	2.84	1.06	1
T6	Saints Rest	3	2.84	1.05	1
T7	Noel Bay	4	3.29	1.22	1
T8	Moose Cove	4	2.95	1.36	1
T9	Avonport	3	3.32	0.90	1
T10	Houston Beach	3	3.33	0.90	1
T11	Houston Beach	5	3.02	1.66	1

The RIVPACS approach did not provide meaningful results for assessment of physical disturbance, as the spread of reference site O/E values was extreme, with values ranging from a low of 0.61 at site 19 (Debert Beach) to a high of 2.12 at site 2 (Parrsboro). To better understand where the weakness of the model lay, O/E averages and standard deviations were calculated for each of the two reference groups (Table 4.7). Group 1 had a much higher O/E standard deviation (0.414 compared to 0.255).

Table 4.7. Comparison of RIVPACS model average O/E values and standard deviations based on two reference groups identified by cluster analysis of the 250 µm fauna.

	Group 1	Group 2
Mean O/E value	1.421	1.139
Standard deviation	0.414	0.255

Recalling the outcome of the SIMPER analysis of the reference groups, Group 1 was found to have only 28% similarity between sites. With this in mind and inspecting the reference site cluster analysis and MDS ordinations (Figures 4.1 and 4.3a), it is possible that the Group 1 reference condition would be better characterized with two separate groups: one group containing four sites 7-9 and 36, and the other group containing the nine remaining sites. The difference within Group 1 is particularly clear with respect to the number of individuals at each site (Figure 4.3b), spionid and nematode densities (not shown), and percentage composition of polychaetes (Figure 4.3d) and harpacticoids (not shown).

4.1.3 Comparison of BEAST and RIVPACS (250 um mesh size)

The BEAST assessment found four sites in Band 2 and one site in Band 4. The RIVPACS assessment found no difference between reference and test sites. Possible explanations for questionable RIVPACS model performance are the small number of taxa identified in the study area, a lack of taxonomic distinction between reference groups, a lack of sensitivity of the benthic meiofauna to the environmental variables considered in this study, and the macrofaunal density changes that result from physical disturbance. The small size of meiofauna such as nematodes and harpacticoid copepods may render them more sensitive than macrofauna to environmental variables not considered in this study, such as geological make-up of the sediments, chemical contaminants, interstitial water content, micro-relief, waves and currents, and concentrations of benthic microalgae, to suggest a few. If more relevant environmental variables had been measured for this study, latitude and longitude may not have emerged as the top two predictor variables in discriminant function analysis.

RIVPACS was not a very powerful assessment mechanism, as the reference site O/E values were highly variable. There was very little taxonomic distinctness between Group 1 and Group 2 (summarized in Table 4.8). For example, the major taxonomic groups were present in both Groups 1 and 2, in particular the dominant and defining taxa such as the amphipod *Corophium*, harpacticoid copepods, and nematodes. Most taxa present in one group but not in another (Hydrobiidae, Janiridae, etc.) were present in such low frequency that they were not considered with the RIVPACS approach as they did not have a >50% probability of occurring at any site. The other major factor influencing the high variation in reference site O/E values is the overall low number of taxa present in the study area, further discussed in Section 4.3.

Table 4.8. Frequency of occurrence of the dominant taxonomic groups found in Group 1 and Group 2 reference sites. Groups based on cluster analysis of faunal community of the 250 µm mesh size data set.

Taxonomic listing	**Percentage of reference sites with taxon present (%)**	
	Group 1	**Group 2**
Phylum Coelenterata		
Class Anthozoa		
Family Edwardsiidae	14	4
Phylum Mollusca		
Class Gastropoda		
Family Pyramidellidae	14	0
Hydrobiidae	7	0
Nassariidae	14	4
Class Bivalvia		
Family Myidae	7	19
Tellinidae	21	31
Phylum Annelida		
Class Polychaeta		
Family Sabellidae	29	4
Maldanidae	50	4
Orbiniidae	21	4
Capitellidae	79	88
Glyceridae	0	4
Spionidae	100	42
Nephtyidae	43	4
Phyllodocidae	36	12
Nereidae	36	54
Syllidae	57	0
Phylum Arthropoda		
Class Crustacea		
Order Cumacea		
Family Diastylidae	14	0
Order Isopoda		
Family Janiridae	7	0
Order Amphipoda		
Family Corophiidae	43	100
Order Harpacticoida	57	50
Phylum Rhyncocoela	36	15
Phylum Nematoda	86	92

4.2 Results: Coarse Mesh (1 mm)

4.2.1 Model Building

Classification of Reference Sites into Biological Groups

Cluster analysis of reference site taxa revealed three distinct community groups (Figure 4.6). Clusters may be identified on a dendrogram by drawing a horizontal line at any similarity level, in this case approximately 12%. Groups 1 and 3 could both be subdivided further into two groups, however RCA analysis should have a minimum of 10 reference sites per group (Bailey *et al.*, 2003). Although Group 2 had only eight reference sites, Groups 2 and 3 were found to be quite different biologically (discussed below) and were kept separate. Reference site 40 was a distinct outlier in cluster analysis (not shown) and was removed from analysis.

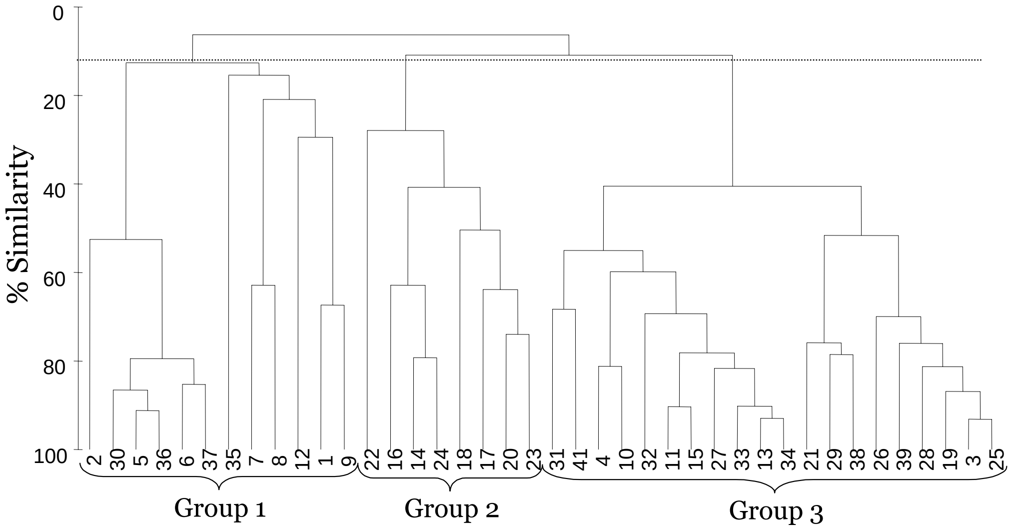

Figure 4.6. Cluster analysis dendrogram based on 1 mm biota for 40 of the original 41 reference sites in the Minas Basin mudflats (after removal of site 40, an outlier).

The geographic separation of the three communities is strong (Figure 4.7). Group 1 contained 12 reference sites, and covers mainly the Southern Bight and Parrsboro to Five Islands area. Group 2 contained eight reference sites, all of which are in Cobequid Bay

(Economy to Walton). Group 3 is the largest (20 reference sites) and the most geographically dispersed, with sites spread throughout the Minas Basin.

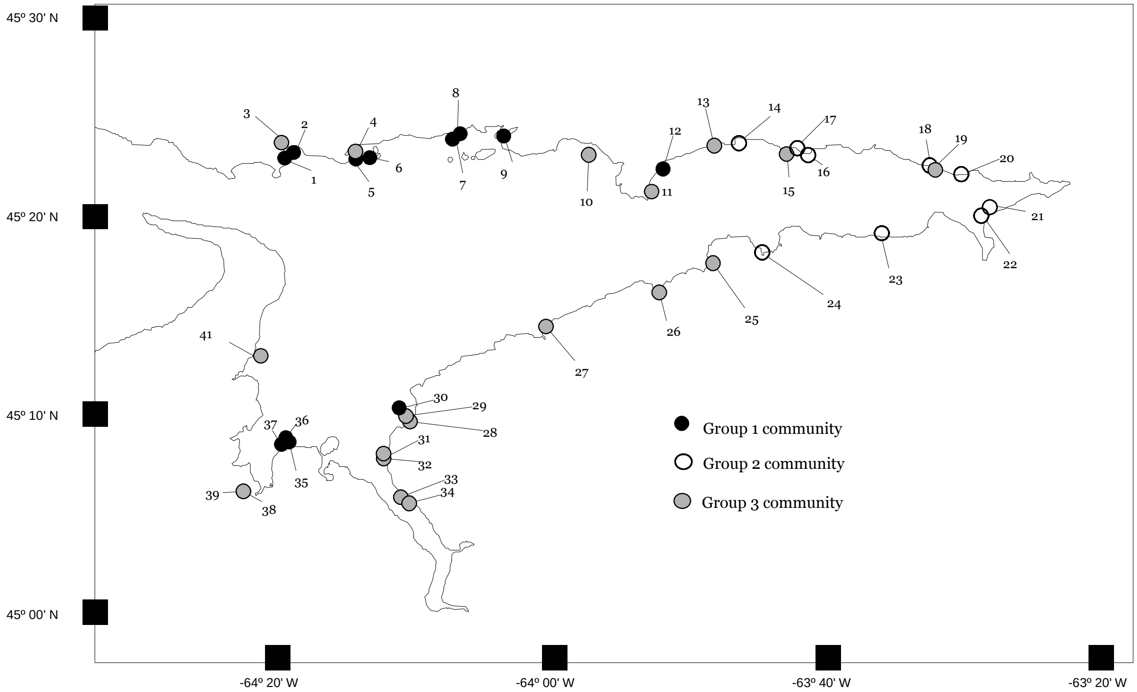

Figure 4.7. Geographic distribution of the invertebrate community groups formed by cluster analysis of the coarse mesh (1 mm) faunal data from 40 reference sites in the Minas Basin tidal flats (site 40 removed). Group 1 biological community represented by black circles, Group 2 by open circles, and Group 3 by grey circles.

Group 1, confined primarily to the north shore and the Southern Bight, had the highest average taxa richness (number of taxa/site) and is dominated by high densities of Spionid worms (Table 4.9). Group 2, containing 8 reference sites found only in Cobequid Bay (Bass River to Noel Bay) is dominated by *Macoma* clams, followed by *Corophium* and nereid worms. The lowest taxa richness and total organism densities of organisms were found in Group 2. The 20 reference sites in Group 3 show no geographical pattern, and are distributed throughout the Basin. *Corophium* is the dominant organism at these reference sites, followed by capitellid and nereid worms. The highest densities of organisms were found in Group 3, with an average of 775 in each 625 cm^2 quadrat (equivalent to 10,000 organisms/m^2).

Table 4.9. Descriptions of three biological reference groups formed by cluster analysis of coarse mesh (1 mm) biota based on 40 reference sites in the Minas Basin tidal flats (based on Figure 4.6).

Group (# sites)	Average Taxa Richness (±SD)	Average Total Abundance (±SD)	Common Taxa, Frequency of Occurrence in Each Group (average abundance per site)	Group Similarity
1 n=12	9 (4)	331 (276)	spionids, 100% (258) capitellids, 67% (11) maldanids, 67% (12) nephtyids, 67% (4)	28%
2 n=8	4 (1)	69 (42)	*Macoma*, 100%(31) *Corophium*, 88% (21) nereids, 88%(10)	44%
3 n=20	6 (2)	775 (541)	*Corophium*, 100% (616) capitellids, 70% (31) nereids, 60% (3)	52%

The SIMPER routine found that Group 1 sites had an average similarity of 28%, with densities of spionids, capitellids and maldanids together contributing 91% of that similarity. Group 2 had a higher average similarity of 44% with densities of *Macoma*, *Corophium* and nereids contributing 95% of that similarity. Finally, Group 3 had the highest average similarity of 52% with high densities of *Corophium* contributing to 96% of that similarity. The average dissimilarity between Group 1 and Group 2 was 94%, Group 1 and Group 3 was also 94%, and Group 2 and Group 3 was 89%. The high dissimilarity between groups suggests they are very different with respect to their biological community composition.

All reference sites were plotted in ordination space using the multidimensional scaling (MDS) routine in PRIMER (Figure 4.8a). Characteristics of the three groups were also overlaid on the MDS plots to show the relevance of each (Figures 4.8b-f). Group 1 was defined by low organism density, high taxa richness, and the dominance of polychaetes (predominantly *Nephtys*, not shown). Group 2 was defined by low organism density, low taxa richness, and the dominance of bivalves. Group 3 displayed a range of total organism density and taxa richness, and was heavily dominated by amphipods.

Correlation of Biological Data with Environmental Variables

The same environmental data used for the fine mesh RCA analysis was used for the coarse mesh analysis. Therefore, gravel top and anoxic layer were not included when building the predictive model, as they varied significantly between reference and test sites.

The output of the DFA routine was the subset of environmental variables that generates the best predictive ability for the community groupings referred to as the predictive model (Table 4.10), and the model accuracy in percent (Table 4.11).

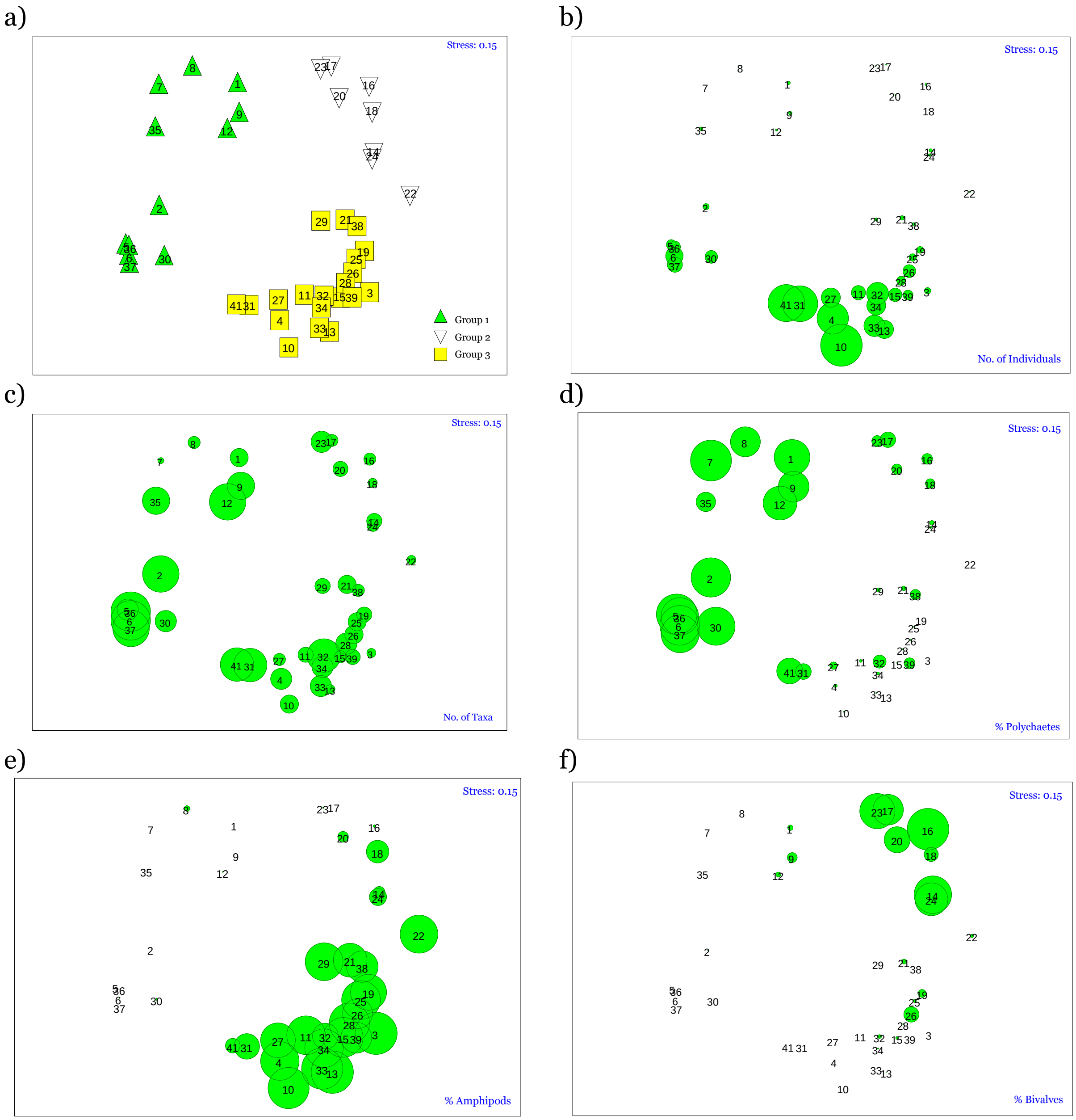

Figure 4.8. MDS ordination of (a) the three reference site groups used in the 1 mm analysis and relative values of (b) numbers of individuals, (c) number of taxa, (d) percentage of polychaetes, (e) percentage of amphipods, (f) and percentage bivalves indicating their importance in discriminating between groups.

Table 4.10. F-values and group means ± standard deviation for the four environmental variables used as predictor variables for the community groupings based on discriminant function analysis for the 1 mm data set.

Variable	**F-Value**	**Group 1 mean ± SD**	**Group 2 mean ± SD**	**Group 3 mean ± SD**
Longitude (deg.min)	21.06	-64.20 ± 0.14	-63.63 ± 0.12	-64.04 ± 0.27
Latitude (deg.min)	7.46	45.31 ± 0.12	45.36 ± 0.04	45.25 ± 0.12
Gravel bottom (% by weight)	3.32	3.5 ± 5.5	0 ± 0	7.5 ± 11.5
Distance from shore (m)	2.28	392 ± 335	275 ± 270	355 ± 341

Seventy-five percent of Group 1 reference sites, 100% of Group 2 reference sites, and 50% of Group 3 reference sites were correctly classified using longitude, latitude, gravel bottom, and distance from shore as predictor variables. The overall cross-validated (jackknifed) model accuracy was 68%.

Table 4.11. The number and percentage of 40 reference sites predicted to each community group using discriminant function analysis with selected environmental variables (% correct in parentheses).

Reference site community group	**Number of sites predicted to Group 1**	**Number of sites predicted to Group 2**	**Number of sites predicted to Group 3**
1	9 (75%)	0	3
2	0	8 (100%)	0
3	4	6	10 (50%)
Total	**13**	**14**	**13**

Predicting Group Membership of Test Sites

The output of complete discriminant function analysis in SYSTAT is a spreadsheet detailing the probability that each site will belong to each group (test site predictions shown in Table 4.12). Based on the four environmental predictor variables, six sites were predicted to belong to Group 1, two sites were predicted to belong to Group 2, and three sites were predicted to belong to Group 3. Predictions for test sites T6, and T8 – T11 were considered weak (all <75% for the highest prediction), and the habitat data at these sites were examined in more detail to determine if they were misclassified.

Table 4.12. Probabilities of each test site belonging to one of three reference community groups based on discriminant function analysis using longitude, latitude, gravel bottom, and distance from shore as predictor variables for the 1 mm mesh size biota. Shading indicates the highest probability value.

Site	Predicted Group (based on habitat)	Probability Group 1	Probability Group 2	Probability Group 3
T1	1	0.989	0.000	0.011
T2	1	0.928	0.011	0.061
T3	1	0.963	0.004	0.033
T4	1	0.970	0.003	0.027
T5	3	0.135	0.024	0.841
T6	2	0.200	0.529	0.271
T7	2	0.003	0.830	0.167
T8	3	0.002	0.269	0.729
T9	3	0.344	0.014	0.643
T10	1	0.708	0.002	0.290
T11	1	0.669	0.002	0.329

4.2.2 Assessment of Test Sites

Test sites were assessed for the coarse mesh size data set using the BEAST and RIVPACS methods, outlined in detail below.

The BEnthic Assessment of SedimenT (BEAST)

BEAST assessment was carried out using the four environmental predictor variables listed above. Each test site was plotted in ordination space against the reference sites of the Group to which it is predicted to belong (from Table 4.12) using the MDS routine in PRIMER. These plots were re-drawn in SYSTAT and probability ellipses were constructed.

Each test site was compared to the predicted reference group and assessed accordingly (Table 4.13). The habitat descriptors at the test sites with low prediction values were examined in greater detail, and one test site was thought to be misclassified (T9). The habitat characteristics of site T9 are much more similar to reference Group 1, whereas the other test sites with low prediction values were all considered correctly classified based on their habitat characteristics (summarized in Table 4.13). Thus, six test sites fell within reference (Band 1), one test site was found to be possibly different from reference (Band 2), one test site was found to be different from reference (Band 3), and three test sites were found to be very different from reference (Band 4).

Table 4.13. BEAST assessments for all test sites using the 250 μm mesh community data. Shading indicates sites with weak predictions that were compared to both reference groups.

Site	Predicted Group (Probability)	Habitat Similarities of Test Site Compared to Predicted Reference Group	Biological Comparison of Test Site to Predicted Reference Group	Band
T1	1 P = 0.989	Westerly location, no gravel	Biologically similar to reference but no maldanids or nephtyids	1
T2	1 P = 0.928	Westerly location, no gravel	Not similar to reference - no maldanids or nephtyids, very high *Corophium*, *Macoma,* and total individuals	3
T3	1 P = 0.963	Westerly location, low gravel	Not similar to reference - no maldanids, very high *Corophium*, *Macoma*, and total individuals	4
T4	1 P = 0.970	Westerly location , no gravel, far from shore	Not similar to reference - no maldanids or nephtyids, low spionids number of taxa, higher *Corophium*	4
T5	3 P = 0.841	Westerly location, high gravel	Biologically similar to reference	1
T6	2 (correct) P = 0.529	Easterly location, no gravel, far from shore (grp 1)	Biologically similar to reference but higher *Corophium* and total individuals	1
T7	2 P = 0.830	Easterly location, no gravel, close to shore	Biologically similar to reference but higher *Corophium* and total individuals	1
T8	3 (correct) P = 0.729	Easterly location, high gravel	Biologically similar to reference but lower capitellids and *Corophium*	1
T9	3 (possibly misclassified) P = 0.643	Habitat more similar to Group 1: westerly location, no gravel, far from shore	Differs from Group 1 with low capitellids, taxa, and total individuals, high *Corophium*	4
T10	1 (correct) P = 0.708	Westerly location (grp 1), medium gravel (grp 1)	Biologically similar to reference but higher capitellids, low maldanids and spionids,	2
T11	1 (correct) P = 0.669	Westerly location (grp 1), medium gravel (grp 1)	Biologically similar to reference but higher capitellids	1

Similar to the fine mesh analysis all but one test site (T10) fell within reference when ordinated with Group 3 reference sites (example in Figure 4.9a and b). The Group 3 reference site community is comprised of a dominance of *Corophium* and high numbers of individuals at each site. Again, it is possible that the Group 3 reference sites consist of areas that are inherently more disturbed and further study is required to test this hypothesis.

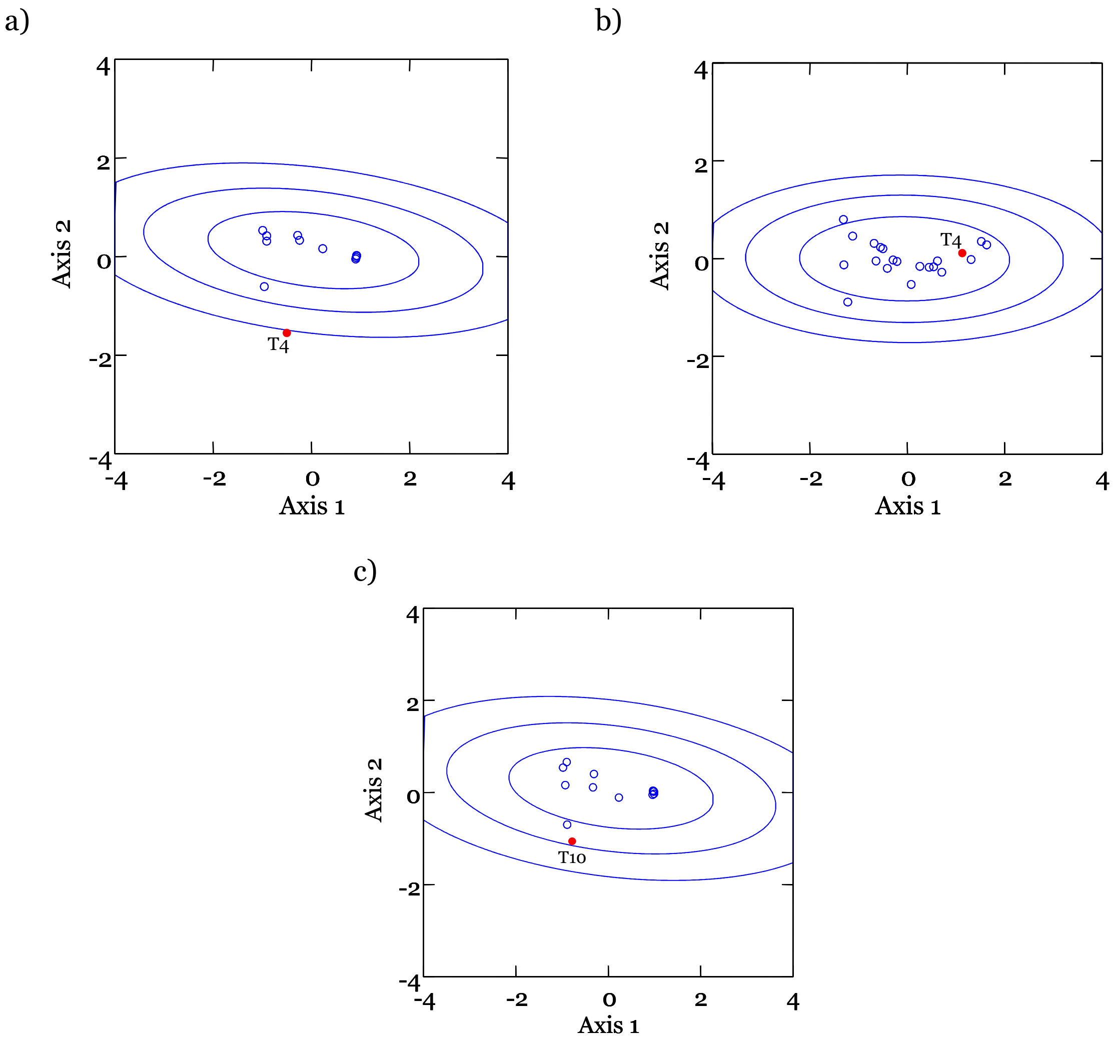

Figure 4.9. Ordination of reference sites (open circles) and seleted test sites (shaded and labelled) based on cluster analysis of the 1 mm mesh size faunal community: (a) test site T4 falls within Band 4 when plotted with Group 1 (correct assessment), (b) the same test site plotted with Group 3 reference sites, and (c) test site T10 falls within Band 2 when ordinated with Group 1. Probability ellipses around the reference sites represent 90, 99, and 99.9% (moving away from centre).

The three test sites that were disturbed by the author before sampling (T1, T7 and T8) are examined again in detail. Site T1 was again predicted to belong to Group 1, and fell within Band 1 when compared to reference. It had lower densities of capitellids and spionids than the Group 1 reference sites, but still had a similar biological composition and relative abundances. Sites T7 and T8 were also correctly predicted to the appropriate reference groups (Group 2 and 3 respectively) and fell within Band 1. Similar to the fine mesh size data, sites T7 had the same taxa present as the reference site 24 (a few meters away) but generally more individuals of each taxon. Site T8 had the same taxa present as reference site 25 (a few meters away) plus another four. Compared to site 25, site T8 had three times the density of *Macoma* (56 compared to 17), about half the density of *Corophium* (146 compared to 310), and the second highest gravel content (23% compared to zero).

The River InVertebrate Prediction And Classification Scheme (RIVPACS)

Observed number of taxa (O) and the expected number of taxa (E) for each reference site were plotted (Figure 4.10), allowing one to immediately compare the range of values for all references sites. A perfect model would result in the observed taxa and expected taxa at each site to be equal (Figure 4.11, dashed line, R^2=1). The model for the 1 mm data set finds a moderate degree of variability in the distribution of observed and expected values (Figure 4.10, R^2=0.40), implying a reasonably strong model comparable to other models described in the literature (Bailey, 2003).

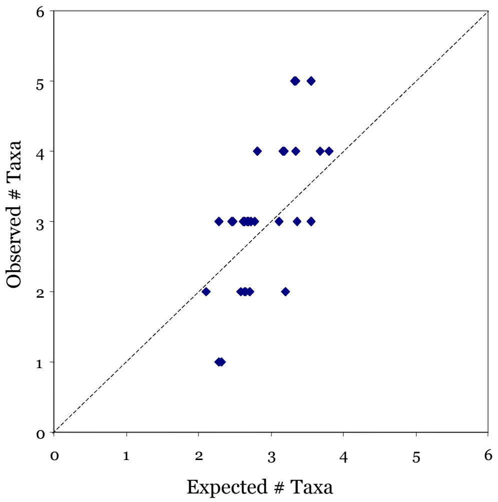

Figure 4.10. Observed number of taxa plotted against expected number of taxa for reference sites from the Minas Basin tidal flats using the 1 mm mesh size data set (R^2<0.40). Dashed line represents the ideal model O/E distribution.

All reference sites were used for RIVPACS analysis and the average O/E ratio was 1.025 ± 0.303. Despite the higher R^2 value, there was still extreme variation in the reference site O/E values, which ranged from 0.26 (site 3) to 1.50 (sites 28 and 31). For any site to fall outside of Band 1, the natural variability of the reference groups, it would have to have an O/E value less than 0.419 or greater than 1.632. This resulted in reference site 3 and test site T10, to fall in Band 2, or possibly different from reference. A summary of all test site O/E values is provided in Table 4.14. Although the model was comparable to others in the literature, the low number of taxa present in the study likely caused the large range of O/E values, thereby reducing the ability of the model to detect variation from reference.

Table 4.14. Number of observed taxa, expected taxa, and O/E values for test sites based on three reference groups for the 1 mm mesh fauna. Shading indicates sites that fall within Band 2, possibly different from reference.

Site	Location	Observed Taxa	Expected Taxa	O/E Ratio
Ref 3	Parrsboro harbour	1	3.82	0.26
T1	Parrsboro harbour	3	3.56	0.84
T2	Five Islands	3	3.45	0.87
T3	Five Islands	2	3.52	0.57
T4	Economy	2	3.53	0.57
T5	Economy	4	3.26	1.23
T6	Saints Rest	3	2.25	1.33
T7	Noel Bay	3	2.64	1.13
T8	Moose Cove	4	2.82	1.42
T9	Avonport	2	2.66	0.75
T10	Houston Beach	6	3.69	1.63
T11	Houston Beach	4	2.66	1.50

Reference site 3 had only 3 taxa present (296 *Corophium*, three gammarids, and one nereid worm). Of the 3.8 taxa predicted by RIVPACS to be present at the site, only *Corophium* was present, resulting in a very low O/E value. Conversely, site T10 was a very rich site with 11 taxa present. Of the 3.7 taxa predicted by RIVPACS to be present at this site, six taxa had a greater than 50% probability of occurring at the site and all were observed in the T10 sample, resulting in a very high O/E value.

Again, as reference site O/E values were quite variable, O/E averages and standard deviations were calculated for each of the three reference groups to better understand where the weakness of the model lay (Table 4.15). All three reference groups had high O/E standard deviation values with Group 3 having the highest, and again this is suspected to be a result of the low number of taxa in the study area.

Table 4.15. Comparison of RIVPACS model average O/E values and standard deviations based on three reference groups identified by cluster analysis of the 1 mm fauna.

	Group 1	Group 2	Group 3
Mean O/E value	1.040	1.053	1.044
Standard deviation	0.227	0.195	0.346

4.2.3 Comparison of BEAST and RIVPACS (1 mm)

RIVPACS classified reference site 3 as possibly different from reference, whereas the BEAST classified it as the same as reference. Reference site 3 was quite far up in Parrsboro Harbour where there were many houses lining the banks of the tidal flat and it is possible that the site was strongly influenced by freshwater, nutrient inputs, or other variables not encountered at any other reference sites. Capitellids were predicted to be present at reference site 3. Although no capitellids were retained by the 1 mm mesh, the 250 um mesh retained 310 individuals. The BEAST and RIVPACS assessments both classified site T10 as "possibly different from reference".

As found in the 250 um analyses, RIVPACS was generally not very powerful (i.e. there was high variance of O/E values) with the 1 mm faunal community using three reference groups. This lack of power is again thought to be a result of low taxa diversity and/or insufficient taxonomic distinctness between groups (Table 4.16), particularly within Group 3 reference sites that were geographically widely spread and had little faunal dominance other than *Corophium*.

The geographical variance of Group 3 is particularly evident in the high standard deviation of longitude values compared to Groups 1 and 2 (Table 4.10). Thus, with longitude being the primary predictor of reference site grouping, Group 3 reference sites had the lowest accuracy rate (only 50% of all Group 3 reference sites were correctly classified, Table 4.11). *Corophium* were present in 100% of the Group 3 reference sites. The percent dominance by *Corophium* ranged from a low of 34% to a high of 100% with an average of 82%. These results suggest *Corophium* distribution and abundance is primarily influenced by environmental factors other than those collected for this study (e.g. grain size, distance from shore), and is widespread throughout the Basin. Therefore, the community structure of reference Group 3 (primarily *Corophium*, capitellids and nereids) is poorly modeled by the RIVPACS approach.

4.3 Discussion

To improve on this RCA study in the Minas Basin, a combination of more reference sites, more relevant environmental descriptors, and refined taxonomic identification are needed. At the family level of identification there were few taxa in the study area and possibly not enough taxonomic distinction between reference groups. Many genera have very specific habitat preferences. For example, the spionid worms in particular have different preferences for grain size for tube construction (Hilbig and Blake, 2000; Guieb *et al.*, 2004).

Table 4.16. Frequency of occurrence of all taxonomic groups found in three biological reference communities. Groups based on cluster analysis of the 1 mm mesh fauna.

Taxonomic listing	Percentage of reference sites with taxon present (%)		
	Group 1	Group 2	Group 3
Phylum Mollusca			
Class Gastropoda			
Family Pyramidellidae	50	0	10
Hydrobiidae	8	0	0
Naticidae	25	0	0
Nassariidae	25	0	15
Buccinidae	0	0	5
Columbellidae	0	0	5
Class Bivalvia			
Family Mytilidae	0	0	5
Anomiidae	8	0	0
Pholadidae	8	0	0
Myidae	0	38	30
Tellinidae	42	100	55
Phylum Annelida			
Class Polychaeta			
Family Sabellidae	25	0	0
Cirratulidae	8	0	10
Maldanidae	67	13	15
Orbiniidae	33	0	0
Capitellidae	67	0	70
Glyceridae	8	0	5
Spionidae	100	25	50
Nephtyidae	67	0	30
Phyllodocidae	50	13	30
Nereidae	25	88	60
Syllidae	8	0	10
Phylum Arthropoda			
Class Crustacea			
Order Copepoda	0	0	10
Order Cumacea			
Family Diastylidae	33	0	15
Order Isopoda			
Family Janiridae	17	0	0
Order Amphipoda			
Family Gammaridae	25	0	15
Corophiidae	42	88	100
Caprellidae	8	0	0
Order Mysidacea			
Family Mysidae	17	13	10
Order Decapoda			
Family Hippolytidae	33	38	30
Order Diptera			
Family Chironomidae	17	0	0
Phylum Rhyncocoela	58	25	30

4.3.1 Overall Comparison of BEAST and RIVPACS

A summary of the BEAST and RIVPACS methods for both faunal analyses is shown in Table 4. 17. The BEAST assessments were fairly consistent between the fine and coarse mesh community analyses, with test sites T3, T4 and T10 showing some variation from reference in both data sets. Both data sets found a total of five test sites different from reference using the BEAST, however the coarse mesh community appeared to be more sensitive in detecting ecological change, as the variation was larger.

Table 4.17. Overall comparison of the BEAST and RIVPACS assessment results for the fine and coarse mesh size fauna.

Site	250 µm		1 mm	
	BEAST Assessment Band	RIVPACS Assessment Band	BEAST Assessment Band	RIVPACS Assessment Band
T1	1	1	1	1
T2	1	1	3	1
T3	2	1	4	1
T4	2	1	4	1
T5	2	1	1	1
T6	2	1	1	1
T7	1	1	1	1
T8	1	1	1	1
T9	1	1	4	1
T10	4	1	2	2
T11	1	1	1	1

Although geographic descriptors such as longitude have been used to predict fauna in other RCA studies, they have typically covered a much larger study area. To compare with other successful RCA studies, the Fraser River study in British Columbia used 219 reference sites to characterize 239,000 km^2 (Reynoldson *et al.*, 2001), the RIVPACS III program in the UK used 614 reference sites to characterize all of Great Britain (Clarke *et al.*, 2003), and the Australian-wide AusRivAS program uses 1,500 reference sites (Department of the Environment and Heritage, 2005).

The BEAST approach assesses faunal communities based on presence-absence of taxa as well as their relative abundances, which is quite appropriate for the Minas Basin intertidal community. Many test sites showed relative changes in taxon abundances such as increases of capitellids, *Corophium*, and nematodes, and decreases in other taxa such as maldanids and spionids. For example, *Corophium* was present in 73% of all coarse mesh reference sites with densities ranging dramatically, between approximately 16 and 29,950/m^2. Discrimination between reference sites based on presence-absence data alone is therefore applicable to some degree, but including relative abundances provides the additional detail about community structure required for assessment purposes. Of the two approaches, the BEAST seems better suited for the Minas Basin intertidal environment, given the homogeneous taxonomic composition and highly variable densities of the fauna.

Reference site 3, found by RIVPACS to be impaired using coarse mesh fauna, provides a solid case for using both meiofauna and macrofauna when assessing impacts in marine benthos. The inability of the coarse mesh to retain the (very small) capitellids effectively led to a Type I error, as capitellids were present (and in high numbers) but too small to be retained. It would be useful for the BEAST and RIVPACS assessment to be conducted with combined fine and coarse mesh size data, and may provide a more complete picture than what was gathered in this study. Meiofauna is often overlooked in bioassessment studies, as sample sorting is time consuming and taxonomically difficult (Sherman and Coull, 1980; Leguerrier *et al.*, 2003).

Whereas the BEAST method responds to quantitative changes in community composition, the RIVPACS method does not respond until a taxon is absent (Reynoldson *et al.*, 1997). RIVPACS was initially developed for and has been used in freshwater systems (Wright *et* *al.*, 1984). In terms of biota and environmental characteristics, upland rivers are very different from lowland streams. In comparison, the Minas Basin tidal flat communities were found to be relatively homogeneous with respect to both the meiofauna and the macrofauna. Additionally, environmental differences in the Minas Basin tidal flats are few, aside from some regional variation in turbidity, currents, salinity, and grain size. The RIVPACS approach may not be suited to perform adequately in such a uniform environment. It is also plausible that the family level identification of the fauna was not fine enough (i.e., reduced the sensitivity and precision of the approach).

It is unfortunate that there were limited environmental variables measured for this study. Other variables such as current strength, amount of available chlorophyll *a*, and length of inundation time per tidal cycle have all been shown to have significant correlations with benthic community structure (Lopez Gappa *et al.*, 2001; Bursey and Wooldridge, 2003; Rodriguez-Villanueva *et al.*, 2003). Inclusion of these variables would have certainly increased the accuracy of the predictive model. Despite the lack of measured descriptors, however, models were built to predict fauna based on the environmental descriptors available, and performed reasonably well (73% with the fine mesh fauna and 68% accuracy with the coarse mesh). These models both used latitude and longitude as the most important faunal predictors, which were believed to be surrogates for other environmental variables (previously discussed).

4.3.2 Biological Interactions

A major disadvantage of using only habitat data to predict classification of test sites is that it does not take biological interactions into account. This was particularly evident when only 50% of the coarse mesh Group 3 reference sites, heavily dominated by *Corophium*, were correctly classified using the predictor variables. This is problematic in the Minas

Basin tidal flats, as most benthic species are infaunal and their very presence often has direct effects on habitat. Benthic invertebrates play a major role in the physical, chemical, and biological properties of the marine water–sediment interface, primarily a result of sediment reworking and bio-irrigation (Rhoads and Young, 1970; Pearson, 2001).

Species diversity is usually low in an area immediately following a disturbance (Figure 1.1). As species re-establish populations, taxa diversity and organism density both increase quickly. Diversity is usually maximal at some intermediate stage of succession (Connell, 1978), declining after one or a few species come to dominate the assemblage and block the recruitment of other species (Sousa, 2001).

Harpacticoid copepods and nematodes, in that order, are usually the first invertebrates to become established after a disturbance causing defaunation, either as a result of physical disturbance (Varon and Thistle, 1988) or experimental biocide treatments (Underwood, 1993). Variation in recolonization rates of the larger meiofaunal species is likely due to current velocities large enough to passively horizontally transport them. Harpacticoids are most active near the sediment-water interface and are therefore most rapid recolonizers of disturbed patches (Probert, 1984). Riemann and Schrage (1978) found nematode abundance returned to control levels 7 days post-disturbance. Sherman and Coull (1980) observed 91% recolonization of nematodes after only 12 hours of a 9 m^2 being dug over and raked.

The concept of facilitation is thought to be very important in soft sediment communities – opportunistic species oxygenate sediments, alter sediment dynamics, and create biotic structures such as tubes, mounds, and fecal pellets. This alteration allows other species to colonize the patch that may be intolerant of more unstructured sediment (Probert, 1984).

Microorganisms (e.g., bacteria and diatoms) prepare the sediment for the larger meiofauna (e.g., harpacticoids and nematodes), which in turn prepare the sediment for the macrofauna. Microorganisms are largely responsible for attractiveness of sediments to macrofaunal larvae, which can delay metamorphosis until suitable substratum is found (Probert, 1984). Conversely, however, several studies have shown that the activities of some infauna may also inhibit, rather than facilitate the occurrence of potential competitors for space (Rhoads and Young, 1970; Woodin, 1974; Snelgrove and Butman, 1994). This is a possible explanation of the inverse relationship seen between *Corophium* and polychaete dominance in the coarse mesh fauna, and between Corophium and mud snails (*Ilyanassa obsoleta*) in some locations (Hamilton *et al.*, in press).

McLusky *et al.* (1983) found extreme reductions of populations in the gastropod *Hydrobia ulvae* (80-100%) and the bivalve *Macoma* (100%) after hand digging in the Forth Estuary in Scotland, but normal population levels after 15 days. The longer-lived, more infrequently occurring species such as the large bivalve *Mya arenaria* are reported to take much longer to become re-established from habitat damage caused by digging (Ambrose *et al.*, 1998). Probert (1984) reported a return of infaunal density to control levels within 40 days. As the disturbance parameters at the large-scale test sites in this study (all but the three disturbed by the author) are unknown, it is not possible to speculate on recovery rate or the recovery process with any degree of certainty.

Over the past few decades, many studies have correlated infaunal invertebrate distributions with sediment grain size, leading to the generalization of distinct associations between animals and specific sediment types (Rhoads, 1967; Grassle and Sanders, 1973; Gray, 1974; Rhoads, 1974; Risk *et al.*, 1976; Yeo, 1977; Cahoon *et al.*, 1999). When these data are compiled and reviewed critically, however, animal-sediment relationships are much more variable than traditionally purported (Snelgrove and Butman, 1994). There is, in fact, little evidence that sedimentary grain size alone is the primary determinant of infaunal species distributions (Snelgrove and Butman, 1994), an observation this study supports. In addition to observed variability in animal-sediment relationships, a clear mechanism by which grain size per se limits distributions has not been demonstrated in any study. Most importantly, however, is that sediment grain size is usually determined on completely disaggregated samples which may have little relevance to what an organism actually encounters in the field. Likewise, patterns have been documented using primarily sediment and biological samples that were not integrated over the same vertical scales within the bed, or on samples that were integrated over much larger vertical scales than those relevant to most organisms. Thus, grain-size distributions described for a given habitat may be very different from those within the actual realm of the organism. In addition to grain size, other proposed causative factors include organic content, microbial content, food supply and trophic interactions, but no single mechanism has been able to explain distributional patterns observed across many different environments (Snelgrove and Butman, 1994).

Corophium spp.

Corophium is a generalist feeder with the ability to switch from surface deposit feeding to active suspension feeding. They feed primarily on epipilic diatoms, bacteria and particulate organic matter, preferring to ingest particles 4-63 μm (Gerdol and Hughes, 1994). *Corophium* have no dispersive larval phase. Embryos mature in the female's brood pouch and are released directly as morphologically developed juveniles.

In Minas Basin, *Corophium* live in irrigated burrows which increases the surface area for exchange between the water column and sediment. The activities of *Corophium* (as well as nereid worms) have been found to enhance oxidation of sediments, increase microbial activity, and reduce the levels of active sulphate-reducing bacteria (Meadows *et al.*, 1990; Mermillod-Blondin *et al.*, 2004). Meadows *et al.* (1990) found *Corophium* and nereid burrows to be densely packed in upper 2-3 cm of sediment in the Clyde Estuary in the UK. Both species produce mucus to bind particles together when constructing burrows, and the presence of *Corophium* increased sediment stability by 50% (and the presence of nereid worms increased sediment stability by 39%). Stabilization of sediments by *Corophium* tube construction reduces particles available for erosion (Grant *et al.*, 1986). Resuspension of particles, however, is enhanced by the grazing activities of *Corophium* on mucus-producing diatoms (Grant *et al.*, 1986; Gerdol and Hughes, 1994). Sediment stability may also change as a result of changes in water content and porosity, but the role of *Corophium* is still unclear (de Deckere *et al.*, 2000). The effects of the U-shaped *Corophium* burrows, which protrude about 1 to 1.5 mm above the sediment surface (Meadows and Reid, 1966) was recently studied by de Deckere (2000). His study found that protruding tubes result in an increase in micro-relief and therefore micro-turbulence resulting in scour around the tube as the boundary layer is disrupted. The overall effect was found to be an increase in the erosion rate as a result of an increase in surface roughness, and significantly higher interstitial water content when in high densities.

Studies by Yeo and Risk (1981) and Wilson (1989) reported that *Corophium* preferred sandier substrates and that clays and silts are dominated by polychaete worms. This study found that *Corophium* was not linked to any environmental variable measured, including grain size, and was actually negatively correlated with polychaete worms. Further study on the dynamics, interactions, and influences of *Corophium* on the intertidal ecosystem is required.

4.3.3 General Conclusions

It appears that the fine mesh (250 µm) community did exhibit signs of an initial colonization community (Probert, 1984; Bolam *et al.*, 2002; Zajac and Whitlatch, 2003). Total organism abundances, nematodes and harpacticoids, were all present in high numbers, and the tube building community (primarily maldanids and spionids) were present in low numbers. Based on reference site data, harpacticoids and nematodes may be displaced as *Corophium* is the next to become established in a disturbed area. Once *Corophium* has become established and removes some of the fine grained sediment via feeding and tube irrigation (de Deckere *et al.*, 2000), the tube building polychaetes may begin to select the area for settlement and growth. Over the long-term in stable conditions, spionids may be the better competitor and out-compete *Corophium* for space and food, thereby explaining the negative relationship between amphipod and polychaete community dominance; however, time series data are required to adequately explore this hypothesis. Any time series study examining post-disturbance tidal flat recovery should include both the meiofauna and macrofauna as this study did.

The selection of reference groups is an iterative process (Reynoldson *et al.*, 1995), and is a function of the method, as discriminant function analysis requires more than one group in order to perform. The degree to which the number of reference groups reflects the actual community structure can only be assumed. Uncertainty in making a decision about passing or failing a test site depends on the degree of uncertainty about the characterization of the distribution of the reference condition and the biota and their environment at the test sites (Bailey *et al.*, 2003). Unfortunately the weak performance of the RIVPACS model somewhat limited the assessment of the approaches in an intertidal estuarine environment, however insight into the effects of intertidal harvesting in the Minas Basin has been gained.

Ultimately, there is no right answer when it comes to field bioassessments, and the BEAST approach showed greater potential as a useful tool in the Minas Basin. The two major drawbacks of the reference condition approach are that it is statistically complex and very time consuming (and therefore potentially hard to understand and expensive). The 'beauty of the BEAST' is the visually appealing end product that conveys messages easily for both resource management purposes and public education.

CHAPTER 5: SUMMARY AND CONCLUSIONS

Although the immediate effect of disturbance on Minas Basin tidal flats related to harvesting activity is a reduction of biomass and abundance, the net effect is to increase local abundances of numerous species that would be less abundant or absent altogether in the absence of disturbance (Sousa, 2001). Many test sites in this study were found to have higher than reference numbers of total organism densities, supporting this claim. It is important to note, however, that the long-term effects of continued intertidal harvesting are still largely unknown and need scientific attention. This study provided only a snapshot, and the scientific and resource management communities are in dire need of time series data on tidal flat recovery rates in the Minas Basin.

Species react differently to disturbances. Some species are strict fugitives, meaning they would be otherwise extinct by competitive exclusion if it weren't for disturbed patches (Hutchinson, 1951). Other species increase in abundance in disturbed areas but their persistence does not depend on it (e.g. *Corophium*). Finally, species that thrive in more stable environments are negatively impacted by disturbances (e.g. maldanids).

The results of this study suggest that most of the larger meiofauna, such as harpacticoid copepods and nematodes, are a major part of the colonization community, rapidly increasing in abundance post-disturbance. *Corophium* and capitellids also substantially increased in density at several test sites, suggesting they may thrive in recently disturbed areas. The tube builders (spionids and maldanids) tended to react negatively to physical disturbance at most test sites, suggesting they prefer more stable surroundings.

If a disturbance is small, rare, or weak, the biological recovery processes will likely be fast, and can be beneficial to many species. Conversely, if a disturbance is large, intense, or frequent, the biological recovery process is usually much slower. Large-scale disturbance can create large-scale patchiness in community composition, patterns of zonation and ecological changes over large areas (Sousa, 2001). The areas of large-scale, long-term disturbance in the Minas Basin include Five Islands (T2 and T3), Economy (T4 and T5), and the Avonport area (T9). (Department of Fisheries and Oceans, 1997)

Based on this study and others discussed above, it is clear that intertidal invertebrate harvesting has effects on sediment stability and the faunal community. However, the impacts are difficult to quantify in exact terms, as benthic recovery is site-specific and depends on the frequency and intensity of disturbance. More studies are needed on the post-disturbance biotic and abiotic recovery processes specific to the Minas Basin tidal flats.

Suggestions for Future Work

There are many ways in which this investigation of the applicability of the reference condition approach in an intertidal estuarine environment could be expanded:

- identification of meiofauna and macrofauna to the genus or species level,
- defining the reference condition and assessing test sites using functional groups or biomass,
- inclusion of more relevant environmental variables such as sediment cohesiveness, inundation time, interstitial water content, organic carbon, chlorophyll *a*, redox potential, bacterial population density, local current speeds, and critical shear stress,
- inclusion of a seasonal element,

- inclusion of post-disturbance time series data, and
- addition of other areas such as the Cumberland Basin.

Overall, this study illustrates the usefulness and applicability of the Reference Condition Approach, previously untested in a marine intertidal ecosystem. This study has provided a substantial stepping-stone for the development of a more robust reference condition database for the Minas Basin, which should be expanded on in the future.

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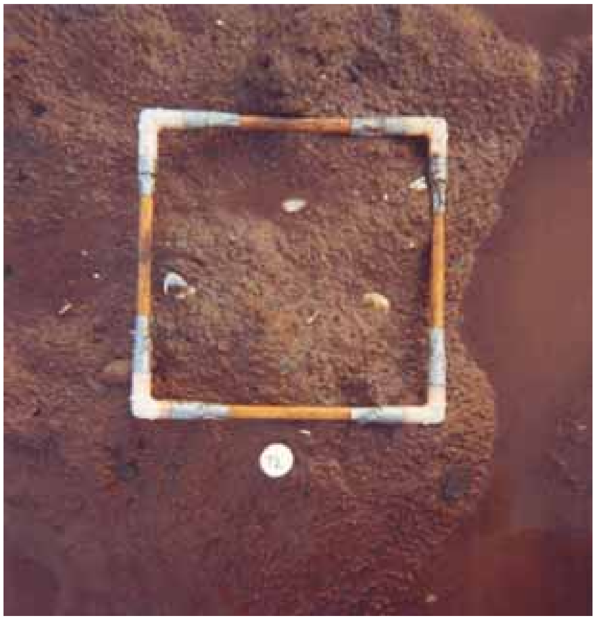
Appendix A. Photograph showing the extent of clam harvesting at Five Islands (test sites T2 and T3).

Appendix B. Photographs of all test sites, locations shown on page 25.

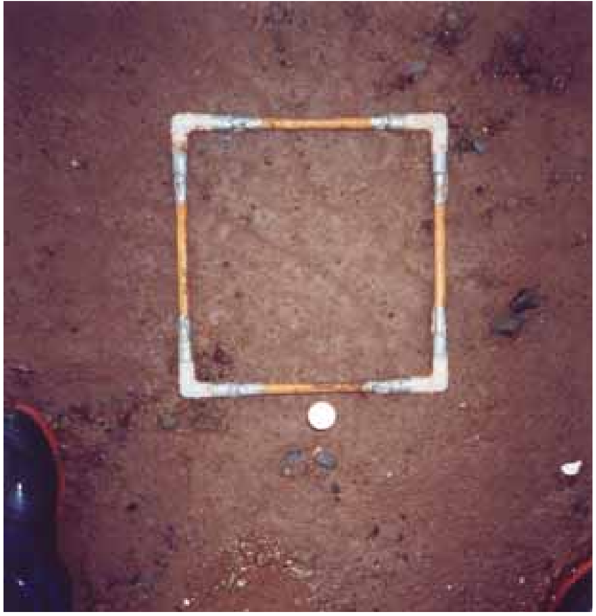
T1

T2



T3

T4

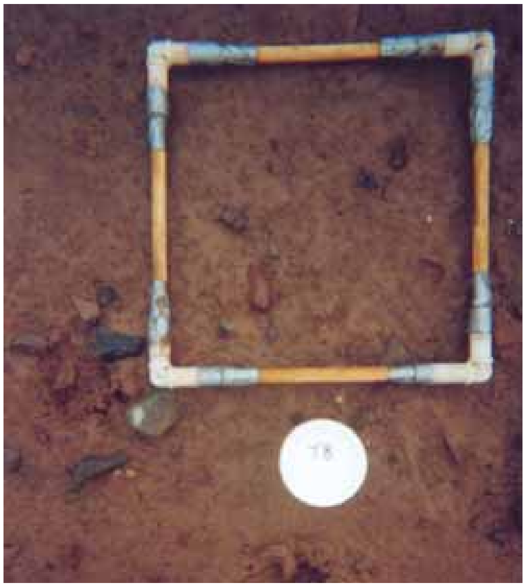


T5

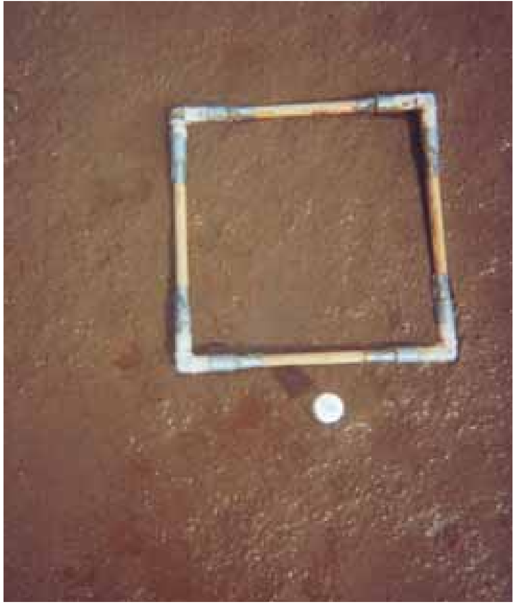
T6

T7

T8

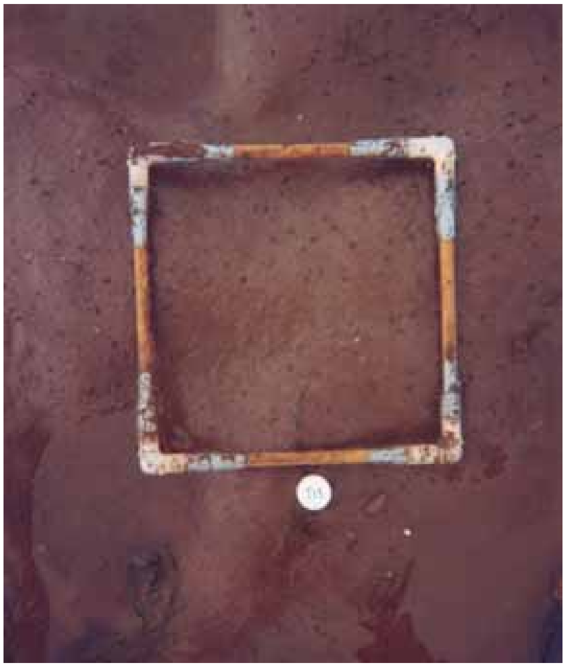


T9



T10

T11



Appendix C. Environmental descriptor values for all reference and test sites.

Station	Long	Lat	Gravel T	Sand T	Silt T	Clay T	Gravel B	Sand B	Silt B	Clay B	TIC	TOC	Anoxic	Distance	Classification
1	64.3236	45.3830	0	26	50	24	0	22	50	28	0.033	0.661	1	150	4
2	64.3230	45.3839	0	38	41	21	0	59	28	17	0.086	0.954	0	150	4
3	64.3262	45.3952	0	32	48	20	22	29	32	17	0.254	0.687	0	150	3
4	64.2420	45.3840	5	73	13	9	32	60	5	3	0.224	0.153	0	250	2
5	64.2405	45.3854	0	82	11	7	0	88	7	4	0.292	0.121	0	300	2
6	64.2392	45.3846	0	76	14	10	0	80	12	8	0.272	0.167	0	350	2
7	64.1198	45.4000	10	78	8	4	10	67	16	7	0.494	0.083	0	200	1
8	64.1180	45.3998	10	81	5	3	13	75	8	4	0.548	0.094	0	150	1
9	64.0608	45.4004	0	9	58	33	0	6	54	40	0.195	0.985	1	200	5
10	63.9583	45.3835	0	71	15	14	32	54	7	7	0.128	0.263	1	1200	2
11	63.8826	45.3528	10	74	9	7	12	57	18	12	0.392	0.147	0	100	3
12	63.8689	45.3715	13	57	26	17	13	57	19	12	0.150	0.242	0	200	3
13	63.8071	45.3910	0	80	11	9	0	68	18	15	0.206	0.122	0	250	3
14	63.7772	45.3939	0	40	36	24	0	50	32	18	0.094	0.238	1	350	3
15	63.7073	45.3896	0	40	35	25	0	41	35	24	0.133	0.425	0	150	3
16	63.7067	45.3882	0	57	24	19	0	46	31	23	0.134	0.240	0	150	3
17	63.7092	45.3866	0	45	28	27	0	40	35	25	0.154	0.375	0	250	3
18	63.5469	45.3746	0	7	50	43	0	7	52	41	0.136	0.984	1	100	5
19	63.5429	45.3719	0	33	40	27	0	45	33	22	0.186	0.557	0	350	4
20	63.5088	45.3671	0	45	35	20	0	54	30	16	0.160	0.526	0	150	5
21	63.4744	45.3396	0	13	52	35	0	12	52	36	0.257	0.803	0	1000	5
22	63.4744	45.3396	0	40	37	24	0	58	28	14	0.146	0.449	0	900	3
23	63.6060	45.3161	0	52	32	16	0	71	21	8	0.278	0.245	0	250	3
24	63.7492	45.3016	0	50	29	21	0	53	25	22	0.448	0.336	1	50	4
25	63.8085	45.2926	0	63	21	16	0	63	21	16	0.440	0.270	0	250	3
26	63.8733	45.2681	0	22	56	22	0	19	55	26	0.300	0.541	0	100	5
27	64.0101	45.2395	0	77	13	10	0	83	9	8	0.281	0.183	1	500	3
28	64.1740	45.1599	11	42	29	18	20	45	19	15	0.388	0.599	1	500	4

Station	Long	Lat	Gravel T	Sand T	Silt T	Clay T	Gravel B	Sand B	Silt B	Clay B	TIC	TOC	Anoxic	Distance	Classification
29	64.1792	45.1645	0	92	4	4	0	92	4	4	0.170	0.091	1	1000	1
30	64.1879	45.1708	0	66	18	15	0	69	17	14	0.235	0.228	1	1200	4
31	64.2059	45.1290	21	71	4	4	21	71	4	4	0.274	0.229	0	350	2
32	64.2046	45.1287	0	42	36	21	0	24	46	30	0.341	0.859	1	350	4
33	64.1853	45.0966	0	77	17	5	0	77	17	5	0.272	0.372	0	50	4
34	64.1750	45.0915	0	45	34	21	0	49	34	17	0.216	0.652	1	50	5
35	64.3229	45.1398	0	18	48	34	0	41	43	16	0.172	0.785	0	350	4
36	64.3196	45.1414	3	82	9	6	6	81	8	5	0.117	0.166	1	550	3
37	64.3203	45.1440	0	50	29	21	0	70	18	12	0.145	0.245	1	900	4
38	64.3752	45.1014	0	33	40	28	0	37	41	22	0.127	0.395	1	50	5
39	64.3752	45.1014	0	47	32	21	0	52	33	15	0.146	0.412	1	50	5
40	64.3640	45.2137	0	85	9	6	0	93	4	3	0.192	0.147	0	300	2
41	64.2595	45.2138	0	86	8	6	11	82	4	3	0.149	0.208	0	400	2
T1	64.3220	45.3835	0	19	53	29	0	16	52	32	0.074	0.555	0	150	5
T2	64.0804	45.4036	0	32	47	22	0	31	47	23	0.106	0.631	0	250	4
T3	64.0804	45.4036	10	51	26	13	0	54	30	16	0.160	0.280	0	450	2
T4	63.9676	45.3816	11	73	9	8	0	64	19	17	0.094	0.155	0	1350	2
T5	63.9548	45.3858	47	46	4	4	43	49	4	4	0.157	0.084	0	850	2
T6	63.7704	45.3939	0	37	40	23	0	40	40	21	0.214	0.173	0	400	3
T7	63.7474	45.2987	0	49	31	21	0	49	31	21	0.497	0.161	0	50	4
T8	63.8085	45.2924	23	61	9	7	23	61	9	7	0.370	0.200	0	250	4
T9	64.2370	45.1126	1	56	30	14	0	41	37	22	0.241	0.344	0	500	5
T10	64.3689	45.2065	5	77	11	7	3	80	11	7	0.300	0.209	1	300	4
T11	64.3689	45.2065	8	80	7	5	4	80	10	6	0.239	0.150	0	300	3

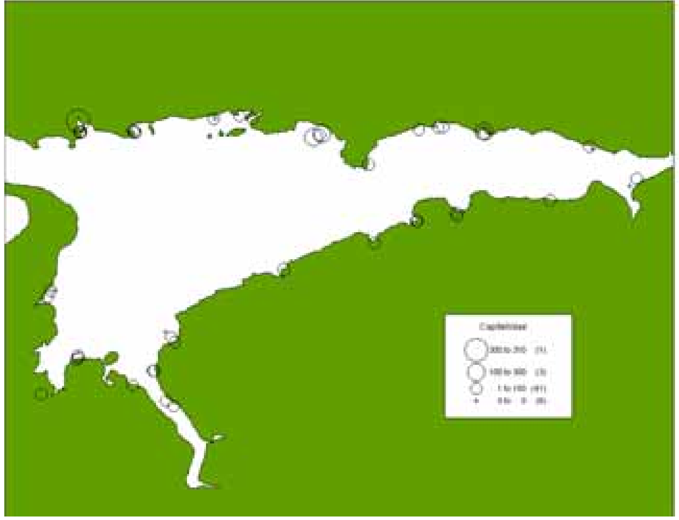
Appendix D. Common biota enumerated in all fine mesh samples (250 µm)

Station	Capitellidae	Corophidae	Harpacticoida	Nematoda	Nephtyidae	Nereidae	Sabellidae	Spionidae	Syllidae	Total # Individ.	Total # Taxa
1	41	1	3	87	0	0	98	38	0	281	9
2	13	4	1	239	0	0	12	82	12	383	10
3	310	88	0	51	0	1	2	1	0	454	8
4	31	156	2	48	0	0	0	13	0	251	7
5	9	0	3	0	0	1	0	53	4	71	7
6	10	0	1	0	1	2	1	84	10	110	9
7	2	0	7	7	0	0	0	5	0	21	5
8	0	0	5	4	0	0	0	2	0	11	4
9	16	4	10	10	0	0	10	10	0	62	9
10	44	162	0	20	0	1	0	40	0	267	6
11	13	127	1	15	0	0	0	3	0	159	6
13	19	141	0	37	0	1	0	2	0	201	7
14	13	49	0	4	0	2	0	0	0	71	6
15	150	325	2	15	0	4	0	0	0	498	7
16	13	22	0	15	0	8	0	2	0	67	7
17	27	42	1	5	0	2	0	15	0	92	7
18	21	59	0	8	0	3	0	0	0	91	5
19	0	89	0	2	0	0	0	0	0	93	4
20	0	3	2	0	0	2	0	0	0	7	4
21	46	64	34	3	1	1	0	0	0	150	8
22	0	12	0	2	0	0	0	0	0	16	5
23	2	0	0	28	0	1	0	6	0	39	6
24	8	75	0	11	0	3	0	2	0	99	6
25	27	78	3	27	0	0	0	0	0	135	5
26	6	216	13	36	0	2	0	0	0	278	7
27	4	84	0	1	0	0	0	4	0	93	5
28	33	21	31	20	0	0	0	0	0	105	5
29	5	19	3	4	0	0	0	0	0	31	5

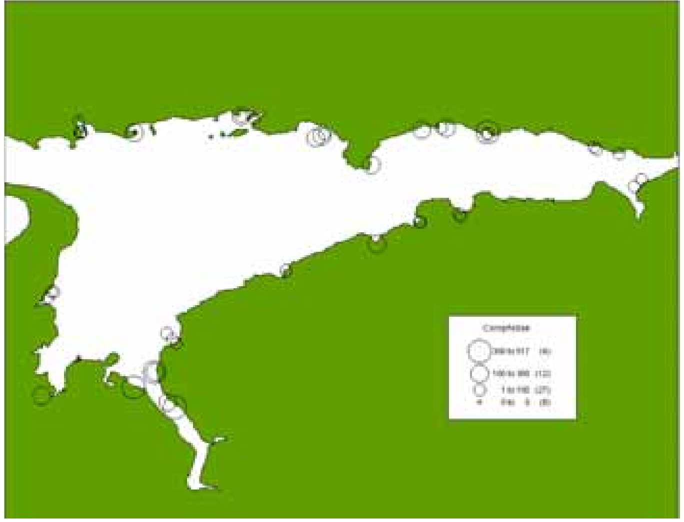
Station	Capitellidae	Corophidae	Harpacticoida	Nematoda	Nephtyidae	Nereidae	Sabellidae	Spionidae	Syllidae	Total # Individ.	Total # Taxa
30	0	1	0	11	1	18	0	27	2	62	8
31	5	336	0	20	0	2	0	1	0	364	6
32	6	244	12	38	0	0	0	0	0	302	7
33	2	175	3	48	0	0	0	0	0	228	5
34	8	307	8	56	0	3	0	0	0	384	8
35	5	0	0	4	64	0	0	53	0	126	5
36	3	0	0	4	8	0	0	8	5	31	7
37	2	0	20	65	29	0	0	25	3	146	8
38	8	214	0	1	0	0	0	1	0	224	5
39	16	244	0	0	0	0	0	0	0	260	3
40	3	4	0	75	0	0	0	6	3	95	8
41	0	80	0	12	3	1	0	19	3	118	7
T1	22	1	13	120	0	0	285	61	0	525	9
T2	23	87	0	40	0	0	0	20	0	172	6
T3	13	169	0	40	1	1	0	5	0	229	7
T4	187	191	1	56	0	0	0	1	0	436	6
T5	159	53	0	14	0	0	0	3	0	229	5
T6	29	259	3	25	0	4	0	0	0	321	7
T7	39	60	8	47	0	1	0	0	0	158	7
T8	92	93	2	52	0	0	0	0	0	240	6
T9	2	517	0	19	5	0	0	0	0	543	5
T10	16	3	2	156	0	0	26	0	0	203	6
T11	34	1	20	268	1	0	68	31	0	423	8

Appendix E. Distribution maps of the common taxa retained with the fine mesh (250 µm).

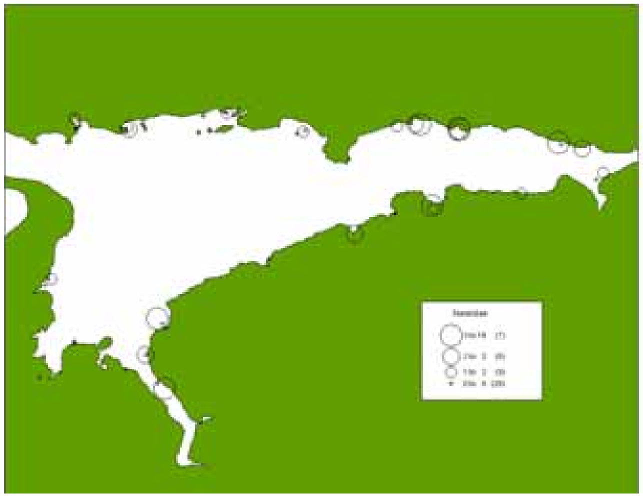
Capitellidae



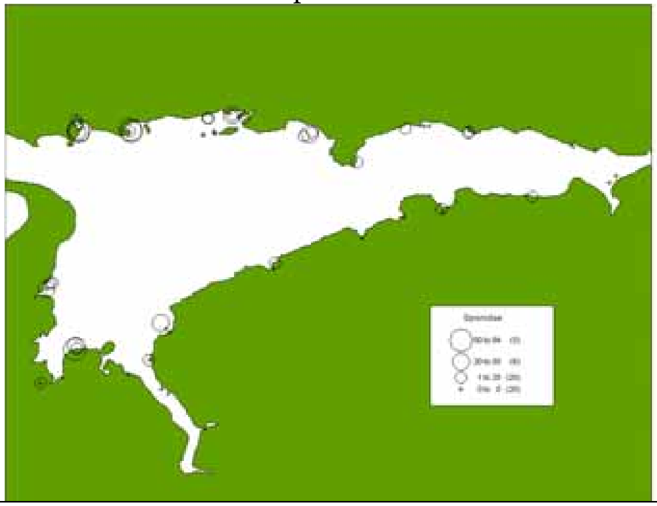
Corophiidae



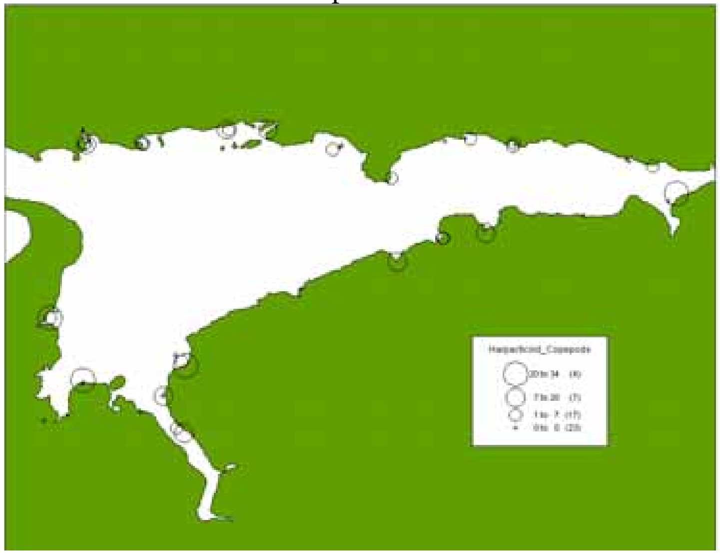
Nereidae



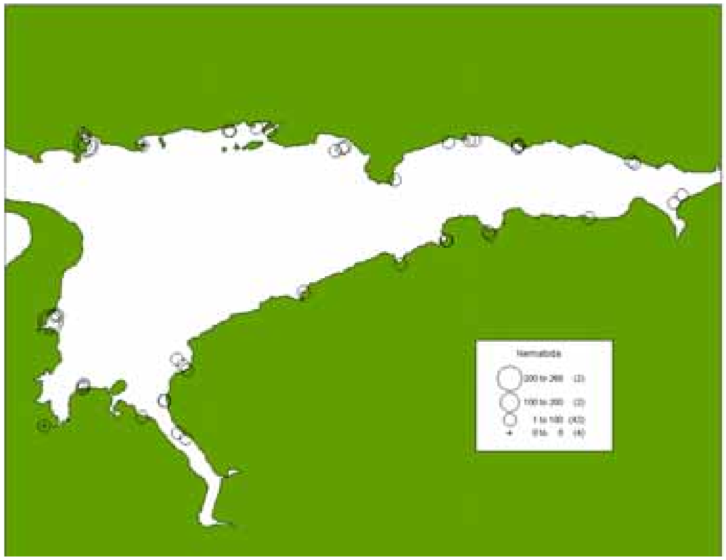
Spionidae



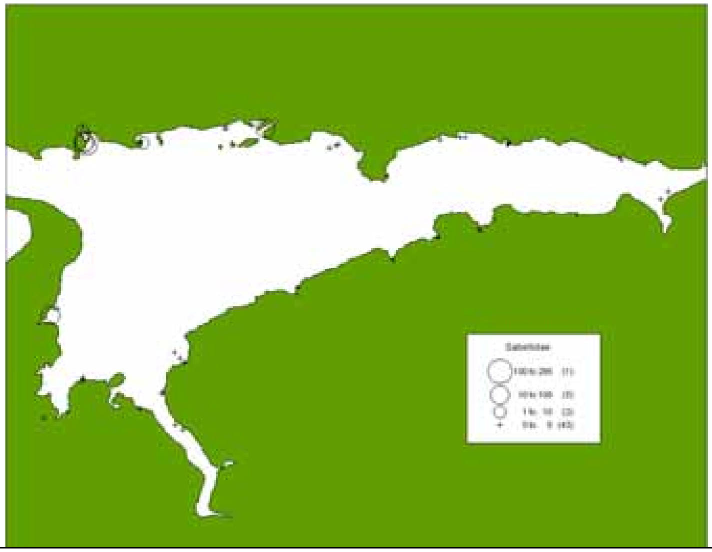
Harpacticoida



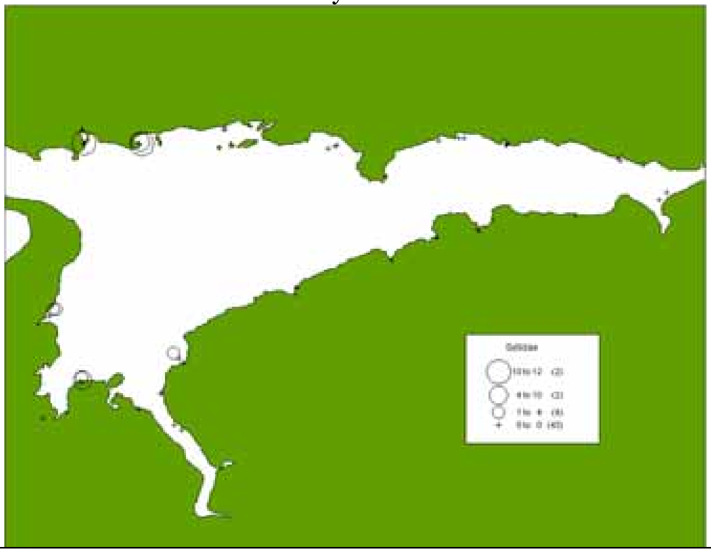
Nematoda



Sabellidae



Syllidae



Appendix F. Common biota enumerated in all fine mesh samples (1 mm).

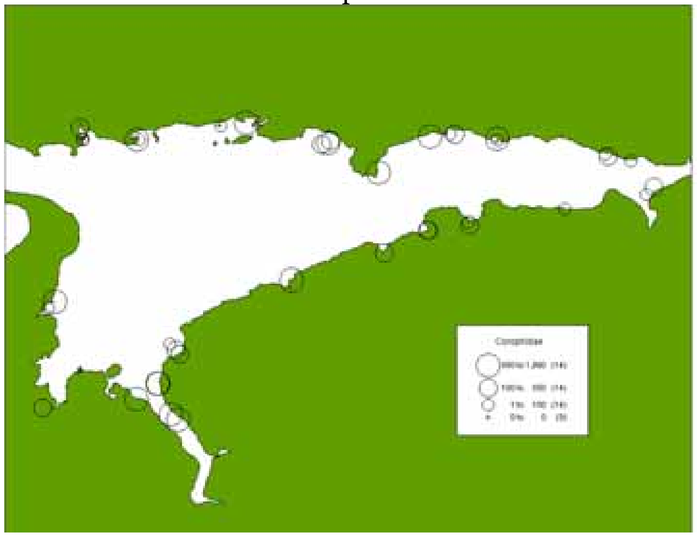
Station	**Capitellidae**	**Corophidae**	**Maldanidae**	**Myidae**	**Mysidae**	**Nemertea**	**Nephtys**	**Nereidae**	**Sabellidae**	**Spionidae**	**Tellinidae**	**Total # Individ.**	**Total # Taxa**
1	5	0	0	0	0	6	0	0	13	16	13	149	6
2	31	1	22	0	0	4	5	0	1	209	3	284	10
3	0	296	0	0	0	0	0	1	0	0	0	297	2
4	5	1325	0	1	0	0	0	7	0	110	5	1453	6
5	0	0	16	0	0	0	5	1	0	435	0	457	4
6	2	1	43	0	0	3	8	2	0	725	0	787	9
7	4	0	0	0	0	0	0	0	0	16	0	20	2
8	0	2	0	0	0	1	0	0	0	11	0	14	3
9	16	0	0	0	0	5	0	0	7	36	25	146	7
10	0	1872	7	2	0	1	0	5	0	42	0	1929	6
11	5	602	0	0	0	0	0	0	0	38	0	645	3
12	0	2	5	0	0	3	1	16	0	51	8	89	8
13	0	837	0	1	0	0	0	0	0	1	0	839	3
14	0	40	0	5	0	0	0	16	0	0	85	146	4
15	0	575	0	15	0	0	0	9	0	0	13	612	4
16	0	5	0	15	0	0	0	23	0	0	45	88	4
17	0	0	5	0	0	6	0	14	0	0	25	50	4
18	0	16	0	0	0	0	0	7	0	0	7	30	3
19	0	267	0	0	1	0	0	0	0	0	41	309	3
20	0	10	0	0	0	0	0	9	0	1	17	37	4
21	20	162	0	0	1	0	0	3	0	0	16	202	5
22	0	51	0	0	3	0	0	0	0	0	3	57	3
23	0	1	0	0	0	3	0	7	0	1	19	32	6
24	0	44	0	8	0	0	0	6	0	0	50	108	4
25	1	310	0	1	0	0	0	10	0	0	17	339	5
26	0	425	0	0	0	2	0	17	0	0	149	593	4
27	2	724	0	0	0	0	2	0	0	155	0	883	4
28	6	393	0	0	0	0	1	1	0	1	7	412	7
29	2	130	0	0	0	0	2	0	0	11	0	145	4
30	0	25	2	0	0	0	8	0	0	534	0	569	4
Station	**Capitellidae**	**Corophidae**	**Maldanidae**	**Myidae**	**Mysidae**	**Nemertea**	**Nephtys**	**Nereidae**	**Sabellidae**	**Spionidae**	**Tellinidae**	**Total # Individ.**	**Total # Taxa**
31	50	985	5	0	0	26	5	2	0	510	1	1612	9
32	296	620	0	2	0	1	0	0	0	3	62	995	7

33	18	952	0	0	0	0	1	1	0	0	2	975	6
34	57	797	0	0	0	1	0	2	0	0	18	875	5
35	60	0	1	0	0	2	1	0	0	9	0	73	5
36	1	0	13	0	1	0	8	0	0	471	1	496	7
37	11	0	44	0	1	0	6	0	0	582	0	652	6
38	38	117	0	0	0	0	0	0	0	0	0	156	3
39	119	335	0	0	0	1	0	0	0	0	0	455	3
41	1	586	15	0	0	0	6	7	0	969	0	1611	7
T1	10	1	0	0	0	2	0	0	9	7	6	113	7
T2	15	987	0	4	0	4	0	2	0	72	100	1246	9
T3	0	1593	0	0	0	2	0	3	0	2	14	1615	6
T4	4	190	0	0	0	0	0	5	0	2	0	201	4
T5	29	549	0	0	0	0	0	6	0	1	0	585	4
T6	0	359	0	12	0	0	0	25	0	0	45	441	4
T7	0	103	0	12	0	0	0	21	0	0	45	181	4
T8	6	146	0	2	0	0	1	10	0	1	56	223	8
T9	30	1073	4	0	0	1	1	0	0	0	0	1110	6
T10	187	2	1	0	0	2	4	0	71	9	1	282	9
T11	119	1	0	0	0	0	1	0	7	18	1	151	8

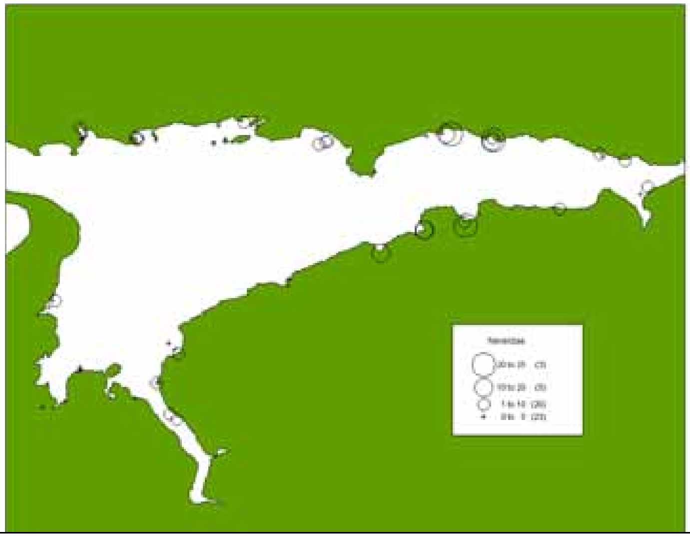
Appendix E. Distribution maps of the common taxa retained with the coarse mesh (1 mm).

Capitellidae

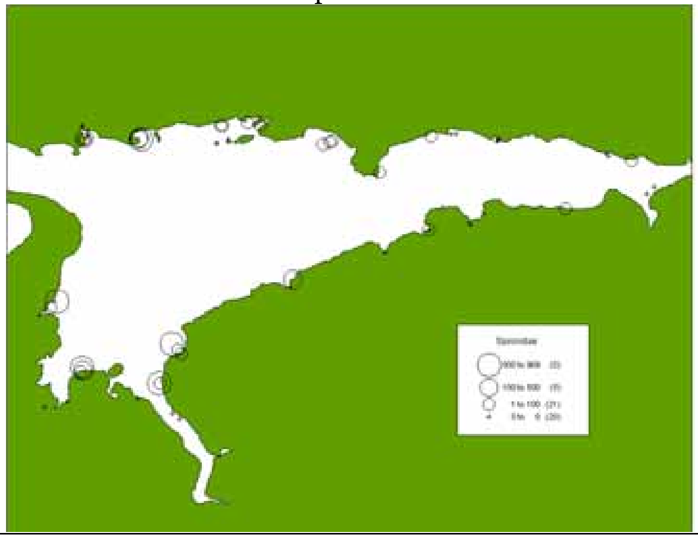
Corophiidae



Nereidae

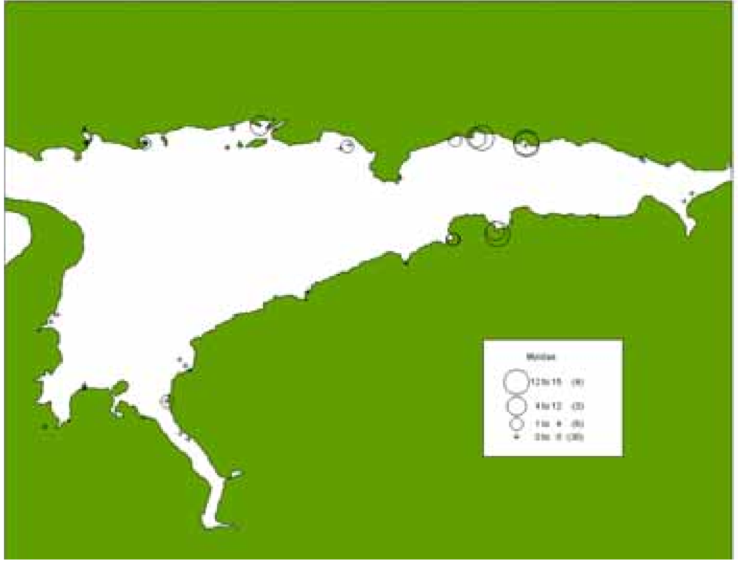


Spionidae

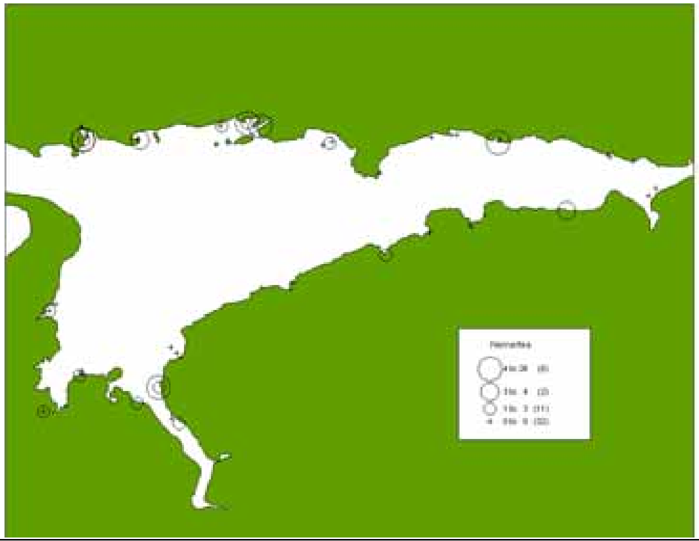


Maldanidae

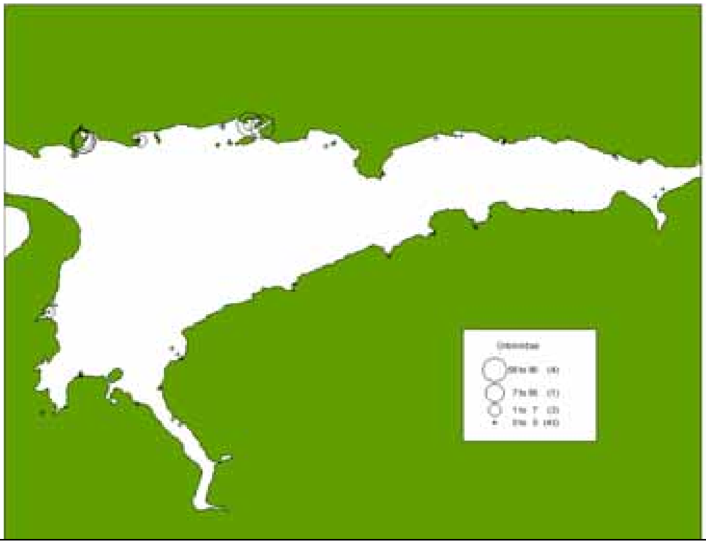
Myidae



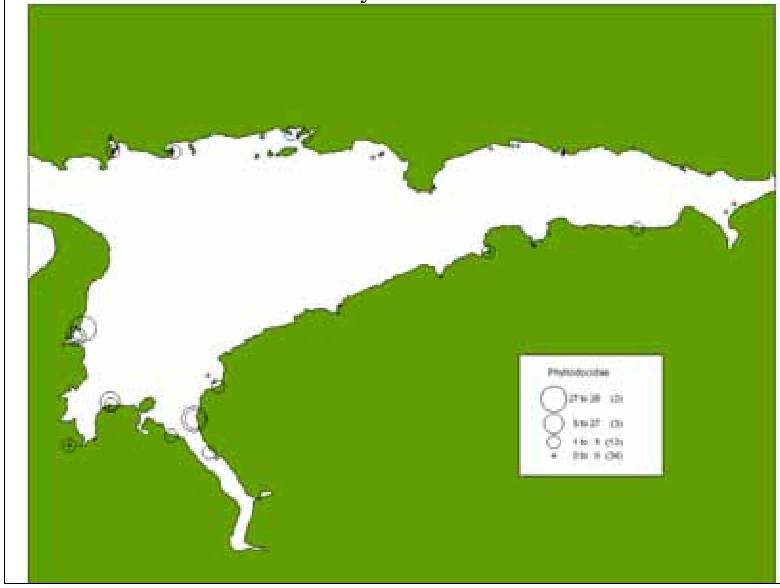
Nemertea



Orbiniidae



Phyllodocidae



Tellinidae