THE EFFECTS OF TRACE METALS

ON JUVENILE COCKLES

(*Austrovenus stutchburyi*)

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Alex McConway

Abstract

This thesis investigated the population structure and spatial distribution of the cockle *Austrovenus stutchburyi* in estuaries and bays around the Canterbury coastline. Surveys investigated population attributes (average density and density of small cockles (<10mm)) of *Austrovenus stutchburyi* in relation to physical environmental characteristics such as trace metals (copper, cadmium and zinc), sediment particle size, nitrogen and phosphorus levels, pore water and percentage organic matter of the sediment. Surface cover of flora and number of fauna present was also correlated with the density of cockles and small cockles <10mm. MDS and PCA ordination showed that the biota was similar at 14 sites but differed significantly at the Pleasant Point Yacht Club (PPY) site. There was a positive correlation between fine sand (125 µm) and the average density of cockles and small cockles < 10 mm. High population densities of *Austrovenus stutchburyi* were also positively correlated with phosphorus levels, and percentage cover of Sea Grass (*Ulva* sp). However, *Austrovenus stutchburyi* density was negatively correlated with cadmium and zinc concentration, and percentage of mud present. The density of small cockles < 10 mm was negatively correlated with copper and cadmium concentration in the sediment and positively correlated with Topshell (*Diloma subrostrata*) numbers, Sea grass (*Zostera muelleri*) percentage cover, Sea lettuce (*Ulva* sp), percentage cover, and sediment particles sizes of <63 µm (mud), 63 µm (very fine sand), and 125 µm (fine sand).

Survival and behavioural changes of juvenile *Austrovenus stutchburyi* were investigated in relation to increased levels of copper, cadmium, and zinc in aqueous solution and sediment in the laboratory, and artificially increased levels in the field. In laboratory experiments in contaminated seawater it was found that, over time, copper and zinc had a detrimental effect on the percentage of juvenile cockles with their siphons extended as did copper concentration. Cockles 10 – 12 mm shell length exposed to different concentrations of copper had the lowest survival rate (25%) whilst cockles that were 5 – 7 mm in length had the greatest survival rate (69%). Cadmium did not affect survival or siphon extension in aqueous experiments. In the contaminated sediment experiments in the laboratory, the concentration of zinc (0, 20, 40, 80, 160 mg Zn/kg (dry weight)) and cadmium (0, 1.8, 5.6, 18, 36 mg Cd/kg (dry weight)) both decreased survival and burial of juvenile cockles in higher concentrations. Copper concentration (0, 5, 10, 25, 50 mg Cu /kg (dry weight)) decreased burial rates of juvenile cockles but did not affect survival.

Transfer of juvenile *Austrovenus stutchburyi* within three sites in the Avon Heathcote Estuary during May 2007, found that site and exposure to copper, cadmium and zinc decreased the survival of the juvenile cockles. However, transfer of cockles between estuaries (Takamatua, Saltwater Creek and Avon – Heathcote Estuary) in May 2007 found that exposure to copper, cadmium and zinc had the main effect on survival of juvenile cockles. In July 2007 transfers of cockles between estuaries, site and exposure to copper, cadmium and zinc had an effect of survival on juvenile cockles.

Cockle populations in the present research have shown a strong correlation with environmental variables, which can be used for management and conservation. The research in this thesis is a start to understanding the effects and implications of contaminants on survival, behaviour and recruitment of juvenile cockles. This research will benefit management strategies for increasing population numbers of *Austrovenus stutchburyi*.

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1.1 Estuaries

Estuaries are an important link between freshwater and marine environments. They are globally an important commercial, recreational and aesthetic resource (Hume 1988). The most widely used definition of an estuary is that of Pritchard (1967) who defines an estuary as “A semi–enclosed coastal body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage.” Day (1981) felt that Pritchard’s definition excluded many water bodies such as blind estuaries in southern Africa, western and southern Australia and other arid countries. These are often closed to the sea by sandbars and may be hypersaline in warm summer temperatures. They can burst open during floods after the dry season, such as the St Lucia Estuary in Natal. Day (1981) therefore proposed this definition: “a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of seawater and freshwater derived from land drainage.” Estuaries are limited to river mouths in tidal seas, and they show saline areas; however, this differs depending on the freshwater inflow and the amount of tidal influence from a sea (Caspers 1967).

Estuaries may be classed in many different ways. McLay (1976) classes estuaries by area, population and topography. Another common method of classification is the pattern of salinity distribution. Dyer (1979) and McClusky (1981) classify estuaries as positive, neutral or negative. In a positive estuary, evaporation is less than the volume of fresh water entering the estuary from river and land drainage. This is the most typical type of estuary. In a negative estuary, evaporation exceeds freshwater runoff into the estuary. This type of estuary is most common in the tropics. In a neutral estuary the input of freshwater equals that of evaporation; therefore there is a static salinity regime. This is very rare and almost never occurs. (Dyer 1979, McClusky 1981).

In New Zealand there are five major categories of estuaries based on physiography and origin. Hume (2003) lists these as drowned river valleys; barrier enclosed estuaries and estuarine lagoons; river mouth estuaries; structurally and tectonically influenced estuaries; and glacially excavated fiords. A drowned river valley is formed when the sea has drowned a valley that has been cut by catchment run–off and there is insufficient run–off to fill the estuary and build an alluvial plain, for example, Whangaroa, Northland. Barrier enclosed estuaries and estuarine lagoons are the most common types of estuaries in New Zealand. In a barrier enclosed estuary a spit has been built naturally across a bay or mouth of a drowned river valley to enclose a marine water body, for example, Mangawhai, Northland and the Avon-Heathcote, Christchurch. River mouth estuaries are small water bodies where rivers emerge on the coast, for example the Rakaia River, Canterbury. A structurally and tectonically influenced estuary is very rare. They are structural grabens, tectonically uplifted coastal river valleys, eroded volcanoes and caulderas and rias, for example, the Marlborough Sounds, Marlborough. Glacially excavated fiords are estuaries carved by glaciers. They are long, narrow and very deep. They have sills at the entrance restricting circulation and marked stratification of freshwater, for example, the Nancy Sound, Fiordland (Hume 2003).

Estuaries are fragile ecosystems with a large range of habitats including rocky outcrops, sandy beaches, mangroves, salt marshes and sub–tidal to intertidal areas (Day 1981). They are zones of highly fluctuating environmental conditions due to their position on the border of the land and the sea. They are influenced by oceanic processes, catchment use and in–estuary use (Hume 1988). Estuaries have unique physiochemical characteristics mainly because of their variable salinity and large fluctuations in temperature, pH, dissolved oxygen, redox potential and composition of particles (Chapman 2001). They are rich in nutrients, especially nitrogen and phosphates, as they are continually supplied these from rivers, the sea and the land. For example in the Ythan Estuary, Scotland, the river supplies 70% of the nitrate and 80% of the silicate, whilst the marine environment supplies 70% of the phosphate (McClusky 1981).

Estuaries that interface the land and the sea provide a vital habitat for fish, shellfish, and birds (Zhou 2004). Estuaries are one of the most productive habitats worldwide (McLay 1976). They have high densities of individuals but are poor in species and Day (1981) considers this is because of the challenging conditions. These conditions arise from the mixing of salt and freshwater and challenge the fauna to adapt physiologically (McClusky 1981). Estuaries support a large amount of permanent flora and fauna and often migrating birds and pelagic fish visit for feeding and nurseries (Knox 1973).

According to McClusky (1981) there are four types of estuarine organisms, these are: Oligohaline organisms that do not tolerate salinities greater than 0.1‰, these are the freshwater organisms. Euryhaline marine organisms, these form the majority of organisms in the estuary. Their distribution is from the sea to the central part of the estuary and they tolerate salinities between 2-25‰. Stenohaline marine organisms occur at the mouth of the estuary in salinities as low as 25 ‰ and migrants which spend only part of their life in the estuary as they move from the sea to the freshwater or vice versa (McClusky 1981).

1.2 Estuarine Contaminants

In New Zealand, a very large percentage of the population live on or near coastal areas (Stewart 2005). Estuaries have long been a useful ecosystem for humans, as natural harbours and places to collect food. Because of this use of the coastal ecosystem, many towns have formed around estuaries (Jones 2005). McLay (1976) used census data of the human population around estuaries to estimate anthropogenic pressures exerted on the system. In New Zealand the majority of estuaries have associated population levels of less than 500 and 12% of estuaries within New Zealand have a population of greater than 5000 (McLay 1976). Estuaries receive significant anthropogenic inputs from point and non–point sources upstream, from metropolitan areas and industries near estuaries (Chapman 2001). Non–point pollution comes from diffuse sources such as urban and agricultural run-off, groundwater transport and atmospheric transport. Point pollution sources are derived mainly from dumping and discharge of industrial waste and municipal sewage (Meyer - Reil 2000). It is therefore not surprising that trade and industry has led to the alteration of the natural balance within estuaries (Dyer 1979). The increase of the human population over the last century has dramatically enhanced the pollution pressure on the marine environment (Meyer - Reil 2000).

Sheltered coastal marine and estuarine environments act as sinks for sediments and contaminants (Morrisey 1996), because they allow settling and accumulation of fine sediments (Mills 1999, Cundy 2003). These sediments are rich in organic material. They act as traps for the discharges of organic contaminants (Pridmore 1991) as they become adsorbed on to the sediment particles and carried to the bottom

(Clark 1997). A considerable amount of heavy metal input from anthropogenic sources also accumulates in the bottom sediments (Kennish 1992).

Finer sediment types predominate up-estuary where the river deposits its sediments, compared with coarser sediments which are deposited closer to the mouth of the estuary (Robb 1988). More contaminated sediments are therefore expected up-estuary. Run-off from anthropogenic activities such as industry and agriculture contaminate the coastal marine environment and this has multiple consequences for individuals, communities and ecosystems. Today, organic enrichment is probably the most widespread and severe kind of marine pollution (Saiz - Salinas 1997). There is also a high potential for the continued release of metals and contaminants into the estuary once the contamination has ceased. For example, the Bilbao Estuary, Spain is recognised as one of the most polluted estuaries in northern Spain even though there are now strict environmental policies that have reduced the input of metals into the estuary (Cundy 2003).

Globally, the estuarine environment and costal zone is under increasing pressure because of an increase in anthropogenic activities (Kennish 1992). These multi-use environments are showing modification and deterioration in their condition which is reflecting the increase of human activities within this environment (Robertson 2002). An understanding of ecological effects of contaminants is essential for reducing and managing human impacts on the coastal marine environment (Morrisey 1996). It has been found that relationships between marine organisms, environmental conditions and toxicity of metal ions are extremely complex and interrelated (Morrisey 1996). Pollution by domestic and industrial wastes is an important factor in controlling the distribution of animals in an estuary (Estcourt 1962). Contaminants typically associated with estuarine environments include trace metals, oil, polynuclear aromatic hydrocarbons (PAH's), chlorinated hydrocarbons, for example DDT, organic carbon and pathogens (Kennish 1992). The accumulation of trace metals in estuarine sediments may cause a significant long term contamination problem as well because of the continued release of trace metals into the environment (Rosales - Hoz 2003).

1.3 Trace Metals

Trace metals are defined as those metals that occur in trace amounts in the environment or within organisms. The term "trace metal" can be used synonymously with "heavy metal" (Rainbow 1993); however, for the purpose of this study the term trace metal will be used. Trace metals are natural constituents of water (Robb 1988). They are generally found in very low concentrations, yet can cause many biological effects (Rainbow 1993). Essential trace metals are necessary for an organism's metabolism in low concentrations, for example, copper and zinc, whilst other trace metals are non-essential, for example, cadmium, lead and mercury, and do not play an important role in an animal's metabolism (Kennish 1992). In excess all trace metals are toxic (Marsden 2004b) and at sublethal concentrations they can affect feeding activity, growth, reproduction, behaviour (Marsden 2001), respiration, osmoregulation or disease resistance (McClusky 1981). Flow on effects may occur from individuals to populations, communities and ecosystems (McClusky 1981). Trace metals are of interest because of their known persistence in the environment, their toxicity at certain levels and their ability to accumulate in animal tissue (Kennish 1992). Upon contact with seawater in estuaries, trace metals that are present as a colloid in physiochemical association with humic acids and hydrous iron oxides, flocculate and settle to the bottom (Kennish 1992).

The anthropogenic input of certain metals into estuaries is the same as or greater than the amount released by natural weathering processes (Robb 1988). Some of these contaminants are persistent (that is they do not readily break down) and have a high affinity with the sediments (Nipper 1997). Many of these metals have historically been found in localised areas impacted by point source industrial discharges, for example, tanneries, mines, and boatyards (Mills 1999). Trace metals are produced by a wide range of anthropogenic sources, including zinc from tyre wear and galvanised plumbing, copper from brake linings and lead from vehicle exhaust (Mills 1999). Urban activities also cause stormwater to become contaminated with trace metals such as zinc, lead and copper (Stewart 2005). In the Pearl River Estuary, China, the main source of metal contaminants is industrial wastewater, domestic sewage, marine traffic and runoff from mining sites upstream (Zhou 2004).

Coastal developments of industrial facilities have a significant effect on environmental quality because of discharge of waste effluent (Rosales - Hoz 2003). In the Coatzacoalcos Estuary, Mexico, sediments have shown elevated concentrations of metals caused by the industrialisation of the Coatzacoalcos River (Rosales - Hoz 2003). A combination of biological, physical, chemical and anthropogenic processes affect the way that trace metals are distributed throughout the estuary (Deely 1991).

Zhou *et al* (2004) found that grain size and type, and re- suspension were important factors in controlling redistribution of enriched sediments in the Pearl River Estuary, China.

1.4 Effects of Trace Metals on Organisms

Copper, lead and zinc are the most common contaminants found in estuaries and are also major components of urban runoff (Roper 1995). Trace metals dissolved in the water may be taken up in marine invertebrates via food, obscure routes such as the blood sinuses (Rainbow 1993), or a combination of factors. Food supply and solution are the major uptake routes for trace metals (Furness 1990, Rainbow 1993) with factors such as species, food type, concentrations of metal in the water and physicochemical parameters of the water determining the priority of uptake (Rainbow 1993).

Behaviour modification from exposure to trace metal contaminated sediments has been shown in some studies (Roper 1995). Failure of behavioural systems can cause reduced fitness of individuals and have adverse effects on populations. It is also likely that failure of behavioural response systems will be ecologically relevant (Roper 1994), for example reduced filtering in bivalves due to increased trace metal levels in time will become harmful to their condition (Furness 1990).

Generally, high temperatures and low salinity act synergistically with metals to increase mortality of organisms (Furness 1990); therefore in the estuary metals may show a greater effect because of the lowered salinity and ability of the water to heat up rapidly where it is shallow. Fertilization and embryogenesis are an important part of reproduction and ultimately determine the survival of a population. It has been shown that trace metals effect juvenile polychaetes by reducing fecundity at concentrations lower than levels that effect adult polychaetes (Furness 1990).

Organisms vary in their ability to regulate metal content and those metals that cannot be excreted remain in the tissues of the animal. Over time these metals build up in the tissues of the animals, which is known as bioaccumulation (Clark 1997). Overseas studies have shown that many organisms can concentrate metals to excessive levels leading to all kinds of problems (Millhouse 1977). Accumulation varies widely between species depending on their accumulation strategies, for example, the caridean decapod *Palaemon elegans* regulates its uptake of zinc and therefore retains an almost constant tissue concentration of zinc. This is compared with the barnacle, *Elminius modestus* that has high uptake rates of zinc, yet no significant zinc excretion. It therefore accumulates zinc in the body in a detoxified form (Rainbow 1993). Molluscs are very good accumulators of trace metals and are generally regarded as excellent indicators for trace metal pollution of their environment (Kennish 1992). *Mytilus edulis* (the blue mussel), is a very good accumulator of trace metals through time. They are often used as biomonitors of contaminated habitats, by placing these indicator organisms along a pollution gradient and examining their accumulation response to different contaminants (Kennish 1992). Physiological and toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer capable of matching uptake rates (Furness 1990).

All marine benthic communities depend on recruitment for replacement of organisms (Fraschetti 2002). Post-settlement juvenile survival is important in determining adult populations and is linked to the surrounding environmental conditions. Metals can delay burrowing and settlement (Furness 1990). In contaminated estuaries, trace metal contaminants may regulate population and community organisation because of their effect on the fauna. Roper *et al* (1995) found that copper and zinc affected burrowing, crawling and drifting in *Macomona liliana*. *Macomona liliana* drifted away from increased trace metal contamination which could potentially effect the distribution of this species (Roper 1995).

Zinc and cadmium are more bioavailable to invertebrates at lower salinities (Furness 1990), this may be very important in estuary conditions because of the fluctuating salinity conditions. The biological activity of a trace metal is influenced by the speciation of the metal which affects the transport of the trace metal across the organism's membranes (Phillips 1972). A wide range of environmental factors can influence the uptake and toxicity of trace metals in organisms (Kennish 1992).

1.5 Study Systems

1.5.1 Avon- Heathcote Estuary

The Avon-Heathcote estuary is a bar-built estuary formed by sediment from the Waimakariri River being transported by long-shore currents creating a sand spit to the south, cutting off the bay from the open sea (Knox 1973). The estuary lies to the north of Banks Peninsula (Knox 1973), and to the east of Christchurch City. It is situated at latitude 43^{o}33' south and longitude 172^{o}44' east (Fig 1.1) (Voller 1973).

The estuary is roughly the shape of an equilateral triangle and about 8 km^2 in area. The Avon River enters the estuary in the northern corner and the Heathcote River enters in the southwest corner (Knox 1973). The outlet of the estuary lies between the end of the sandy spit and Shag Rock (Voller 1973). The estuary is shallow with a mean depth of 1.4 m on a spring tide (Purchase 1986), and is mostly intertidal (Estcourt 1962). Approximately 85% of the area is intertidal mudflat (Stephenson 1981). The estuary is micro-tidal with a spring tide range of 2.1 metres (Stephenson 1981). An estimated 8.5×10^6 m^3 of seawater enters the estuary on each flood tide (Wong 1999). Stephenson (1981) estimates a 56% tidal exchange per tide.

The three sides of the estuary have completely different geomorphology: to the west lies swampy land which is now the site for Christchurch City; to the south lies the Port Hills which are part of the volcanic mass of Banks Peninsula; and to the east lies a 5 km long sandy spit which separates the estuary from the Pacific Ocean (Knox 1973, Voller 1973). Both the Avon and the Heathcote Rivers are spring fed, slow-flowing and meandering (Knox 1973). They collect storm water which drains from the Christchurch City streets from within the catchment (Estcourt 1962). The catchment basin of the estuary is 200 km^2 (Stephenson 1981). Tidal and river mixing occurs up the Avon River to the Wainoni Road Bridge (8 km from the river mouth) and up the Heathcote River as far as the Radley Street Bridge (11 km from the river mouth) (Voller 1973).

The estuary may be divided into three intertidal zones. The first, a zone which is narrow with steep banks resulting in short intertidal zones, occurs from the limits of saline water influence to the Bridge Street Bridge on the Avon River and the Ferrymead Bridge on the Heathcote River. The second, mudflats, are downstream from these bridges. The mudflats are uncovered at low tide except for main channels from the two rivers. The third zone is from the mouth of Moncks Bay to the Outlet Channel of the estuary. Sediments in this zone are mainly sand (Knox 1973). The composition of the sediments in the estuary is different throughout. Heavier, coarser particles are dropped by the tide near the mouth of the estuary, while near the river mouths the particles are much finer and have been carried downstream by the rivers. Once these particles make contact with the salt water they stick together and fall to the bottom, therefore the fine sediment is trapped near the river mouths (Harris 1992).

Waste water from the Christchurch Wastewater treatment ponds is released into the estuary on the western side near the mouth of the Avon River (Estcourt 1962).

Around 80% of the nitrogen and 94% of the phosphorus entering the estuary is caused by the effluent (Jones 2005). Over time there has been an increase in total ammonia-nitrogen, total nitrogen, and total phosphorus (Bolton-Ritchie 2005b). Historically, the Avon-Heathcote Estuary has been known as a fragile, dynamic and changeable place and susceptible to the influence of tides, rivers, wind and anthropogenic activity (Harris 1992).

1.5.2 Saltwater Creek Estuary

Saltwater Creek Estuary is a highly productive 170 ha tidal flat at the mouth of the Ashley River, 26 km north of Christchurch City (Fig 1.1). It is bounded on the east by a long narrow unstable sand spit and surrounded by saltmarsh and farm land. Two rivers discharge into this estuary, the Ashley River in the south-western corner and Saltwater Creek in the north-western corner (Fenwick 2006). The estuary, which has been considerably modified by the erosion of the fore-dunes at the southern end of the spit and the movement of the Ashley River to the north, discharges into the Pacific Ocean (Robb 1988). The Ashley River is a foothills river and carries substantial sediment loads from farmlands and the foothills. Saltwater Creek is a lowland stream originating from groundwater discharges (Fenwick 2006), with a flow of approximately $1.7m^3$ per second (Wong 1999). The community of Waikuku is situated on the southern shore of the estuary (Fenwick 2006). In 1859 the estuary was used as a port as the mouth of the estuary was two metres deep at low tide. The Ashley River shifted course in the 1860s and the channel was around five metres deep allowing ships to navigate the entrance easily (Deely 1991). Farming activities upstream caused the silt to slowly fill up the channel at the mouth of the estuary and by 1870 it was impossible for ships to enter this area safely (Deely 1991).

The estuary is divided into two parts, each decided by the characteristics of the discharge of the contributing river (Fenwick 2006). The Saltwater Creek Estuary differs from that of the Avon-Heathcote Estuary because it is a major river mouth estuary that has been formed by a change in position of the Ashley River (Deely 1991).

1.5.3 Takamatua

Takamatua is an inlet of the Banks Peninsula (Fig 1.1). It is an embayment of Akaroa Harbour which was formed by the collapse of the seaward margin of the volcanic complex that forms the Banks Peninsula (Bolton-Ritchie 2005a). The intertidal flats are gently sloping (Bolton-Ritchie 2005a) and consist of pebbly substrate at high tide and shell and mud at low tide (Pickering 1989). The extent of the intertidal flats at Takamatua Bay is ~860 m along the shore and ~600 m seaward (Bolton-Ritchie 2005a). Takamatua is a holiday destination with many baches at the head of the bay (Pickering 1989).

1.5.4 Port Levy

Port Levy is a long narrow inlet of the Banks Peninsula close to Lyttelton Harbour (Fig 1.1). It is an extremely sheltered long bay with extensive mudflats. The intertidal habitats that occur at Port Levy are similar to those of the Banks Peninsula region (Marsden 2005). The rocky headlands have boulders of various sizes and are dominated by filter feeding organisms in the intertidal region. The rocky shore has communities that can tolerate exposure to silty turbid conditions and species diversity increases towards the low tide region (Marsden 2005). Cockles are the dominant species at Port Levy, although they have a low mean density of $40.7/m^2$. They do, however, have a large mean length of 40.8 mm (Marsden 2005). The Koukourärata cockle bed is the main cockle bed at Port Levy and is situated opposite the Koukourärata settlement (Voller 2003). The cockle bed was closed for harvesting in late 1995 and annual surveys have been carried out since 1997 (Voller 2003, Marsden 2005). The Koukourärata cockle bed has great cultural significance to Mäori (Marsden 2005). The cockle bed is in a state of equilibrium and not improving. Recruits are occurring most years; however, they are probably only replacing the cockles that were lost through natural mortality (Voller 2003). The bed may be gradually silting up because of land erosion or other fine sediments (Voller 2003), therefore the cockles are becoming buried and cannot escape.

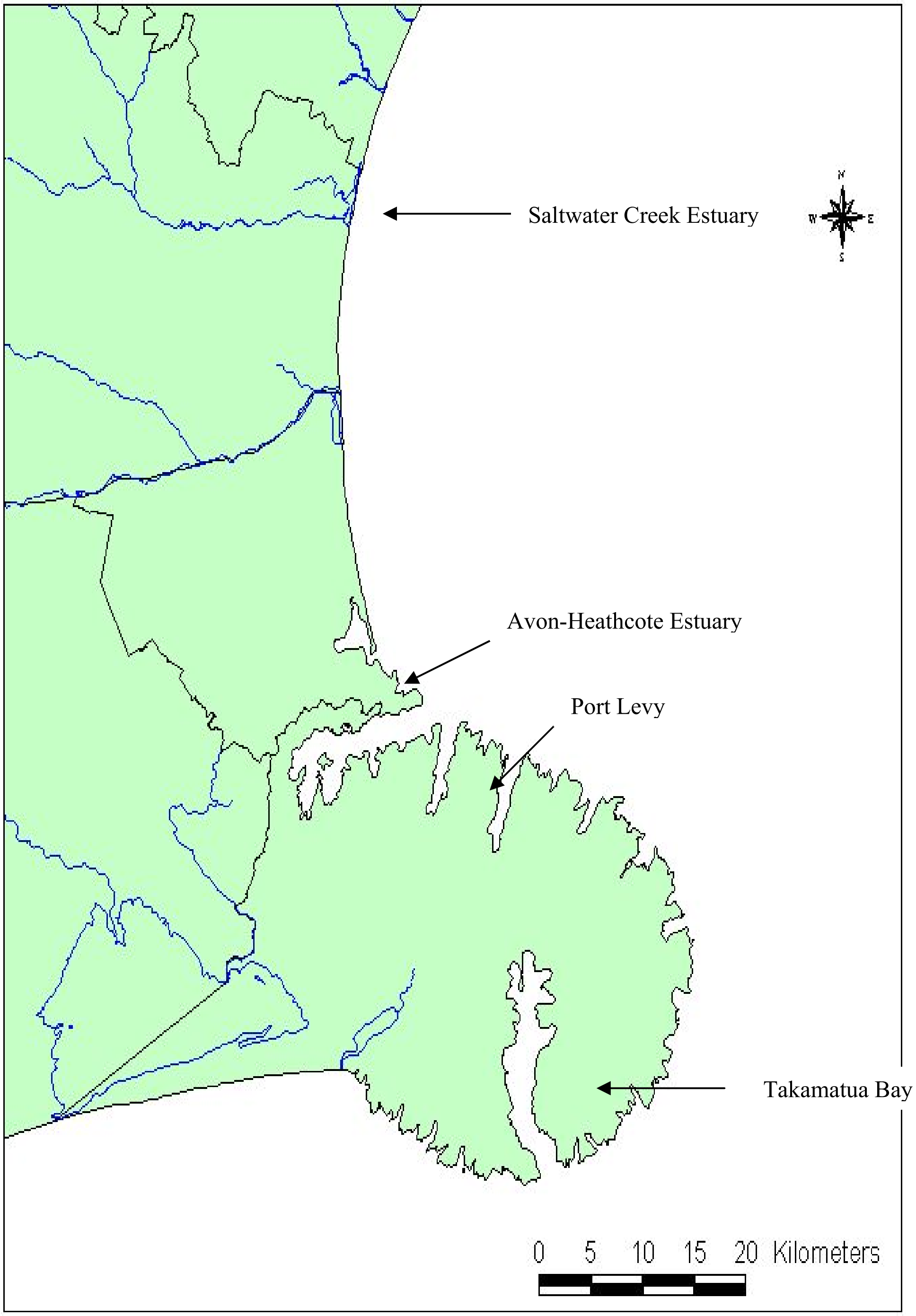


Figure 1.1 Image showing Saltwater Creek Estuary, Avon-Heathcote Estuary, Port Levy and Takamatua Bay.

1.6 Historical Contamination

The Avon River has historically been kept free of industrial contamination but the Heathcote River has been badly abused for 100 or more years by industrial waste dumping from the Woolston district (Robb 1988). Since 1883 there has been a sewage farm at Bromley. This was established by the Drainage Board because the Avon River was becoming more polluted by drains running into the river and estuary (Deely 1992). Around 170,000 m^3/day of waste water was being discharged into the estuary in 2004/2005 Christchurch City Council 2007. Industrial wastes entered the estuary between 1860 and 1900 as Woolston and Sydenham were the main iron working and industrial areas of New Zealand (Deely 1992). In 1903 over 4.5 million litres of industrial waste, such as acids, sulphur compounds, tars, oils, and heavy metals, were being discharged into the Heathcote River (Deely 1992). Many industries have now closed down or stopped discharging wastes straight into the rivers. All industrial and domestic wastes are now being diverted to the Bromley sewage works and the sediment in the estuary is slowly beginning to improve (Deely 1992).

Much of the Avon-Heathcote Estuary is enriched in at least four heavy metals: copper, chromium, zinc, and lead (Robb 1988). Concentrations of heavy metals vary considerably in the Avon-Heathcote Estuary and some elements are in concentrations as high as 195 parts per million (Jones 2005). The finer sediments around the Heathcote and Avon River mouths contain the highest concentrations of metals (Deely 1992). Storm-water runoff and waste water treatment pond effluent are the two main sources of heavy metals entering the estuary (Deely 1992). An influx of clean marine sediments from the estuary mouth accounts for the seaward decline in the trace metal content of the estuary (Kennish 1992).

Copper, chromium, nickel, zinc and lead are present in remarkably consistent proportion throughout the Saltwater Creek Estuary tidal flats and are highly intercorrelated (Robb 1988). Saltwater Creek is a relatively unimpacted estuary compared with the Avon-Heathcote Estuary (Mills 1999) which contains on average over twice the amount of copper, chromium, and zinc and three times the concentration of lead (Deely 1992).

1.7 *Austrovenus stutchburyi*

The endemic New Zealand little-neck clam *Austrovenus stutchburyi* (Wood 1828), known as the cockle or tuangi (Marsden 2004a) is a molluscan bivalve of the Veneridae family (Jones 2005). Both adult and juvenile stages burrow in the surface sediments (top 5 cm) (Hewitt 1997) and are commonly distributed in sheltered intertidal habitats such as estuaries and protected sand flats throughout New Zealand (Stewart 1999, Marsden 2004a). They are a dominant bivalve of communities in sheltered bays (Marsden 1995) often with populations of up to 3000/m^2 (Stephenson 1979). Both adult and juvenile stages are found in the mid to low tide region (Marsden 2004a). They occur in the North, South, Stewart and Chatham Islands (Estcourt 1962). In New Zealand the cockle is commercially harvested and has a high cultural value; moreover numerous people collect cockles for themselves (Stephenson 1979, Marsden 2004a). As habitat conditions have declined around New Zealand, cockle numbers have declined in certain areas (Marsden 2004a).

Cockles have a large, solid, rotund and inflated shell with numerous rounded radial ribs. They are whitish in colour and tinged with a pale purplish – brown colour at the posterior end (Powell 1979). Large *Austrovenus* are relatively sessile and their movement is very sporadic and over short distances (Stewart 2005). They are indiscriminate or non-selective (Jones 2005) suspension feeders (Pridmore 1991). To feed they use two short siphons (Stewart 2005). *Austrovenus* grows up to 60 mm in length (Hewitt 1997); however, maximum age and growth rate are highly variable (Stephenson 1979). Gonadal development of *Austrovenus* peaks in January and spawning occurs in late summer for around four months (Larcombe 1971).

Benthic suspension feeding bivalves are exposed to temporal and spatial variation that can affect all aspects of behaviour, physiology, energetics, population biology and growth (Marsden 2004a). Growth and reproduction can be affected by many factors including: “body size; temperature; tidal level; emersion time; density; salinity; and food availability” (Marsden 1995). Not only are cockles affected by these environmental variables, but they are also exposed to trace metals and contaminants that can affect growth and reproduction (Marsden 1995). Cockles have been impacted on by changes in habitat, such as pollution and sedimentation related to coastal and urban development (Stewart 1999).

Austrovenus stutchburyi is the most abundant bivalve found in the Avon-Heathcote Estuary (Wong 1992), with up to 3,000 m^2 in McCormack's Bay and along Beachville Road. About 200 – 300 cockles per m^2 are found on the mudflats near the low-tide river channels (Jones 2005). Cockle density decreases towards the high tide mark which may be because of their preference for water coverage of more than nine hours (Wong 1992). Density of *Austrovenus stutchburyi* also decreases away from the estuary mouth where the salinity decreases and the sediments become muddier (Jones 2005). *Austrovenus stutchburyi* will die if subjected to salinities below 4‰ for any length of time and a decrease in feeding will occur at salinities below 18‰ (Larcombe 1971).

Post – settlement dispersal is a key process affecting population dynamics (Lundquist 2004) and has many influences on the dynamics of populations and is crucial for the ability to disperse and escape from long-term environmental changes (Norkko 2001). Dispersal of organisms has profound influences on the dynamics of populations and the ability to disperse and escape from environmental changes is critical (Norkko 2001). Cockles show passive transport as their movement is correlated with bedload transport. This, however, does not exclude the possibility of active dispersal processes being important (Turner 1997). *Austrovenus stutchburyi* relies on food that is contained in suspension in the overlying water, most of which is of marine or terrestrial origin (Robb 1988). Stephenson (1980) found using carbon isotopes that *Austrovenus stutchburyi* living up-estuary derive much more food material from terrestrial sources via the rivers and the Christchurch Treatment Works outfalls than those living closer to the mouth of the estuary which derive more of their food from marine sources. They are therefore an indiscriminate filter feeder depending upon its position within the estuary (Stephenson 1980). They are therefore more likely to be exposed to trace metal concentrations (Robb 1988).

1.8 Objectives

Behaviour modification from exposure to trace metal contaminated sediments has been shown in many studies (Roper 1995). Failure of behavioural systems can cause reduced fitness of individuals with adverse effects on populations. It is also likely that the failure of behavioural response systems will be ecologically relevant (Roper 1994). All marine benthic environments depend on recruitment for replacement of organisms (Fraschetti 2002). Post-settlement juvenile survival is important in determining adult populations and is linked to the surrounding environmental conditions. In contaminated estuaries, it is therefore important to know how juvenile cockles are affected by trace metal contaminants to determine population regulation and community organisation.

The purpose of this study is to determine the effects of trace metals on juvenile cockles. The research questions, therefore, are:

1. What factors determine the distribution and habitat preferences of juvenile cockles?
2. What are the tolerance ranges of trace metal contaminants of juvenile cockles?
3. How is the behaviour of juvenile cockles affected by trace metals?

Chapter 2: *Austrovenus stutchburyi* Population Survey

2.1 Introduction

Intertidal estuarine flats are known to be very productive areas that support a wide diversity of fauna that are adapted to cope with variable environmental conditions (McClusky 1981), and therefore marine soft sediments provide a wide range of habitats for marine benthic organisms (Thrush 1991). Ecological systems are naturally heterogeneous (Thrush 2000), and are characterized by many spatial and temporal variations (Thrush 1997). Environmental gradients as well as anthropogenic influences, structure the benthic community (Malloy 2007). The estuarine habitat is an important transition zone for many species, with ecological processes that link terrestrial, freshwater and marine ecosystems (Winberg 2007). Populations and communities are distributed along different gradients that reflect the environmental characteristics (Edgar 2002). Spatial patterns may be due to a number of different biotic and abiotic factors such as predator behaviour, larval settlement, competition, sediment characteristics and food resources (Thrush 1989). A community structure should be based on the regulation of the component species in the community by environmental conditions and interactions with other species in the community (Dayton 1971).

Environmental variables affect the community structure in the estuarine environment (Malloy 2007), and the interactions between the density and size of bivalves is highly complex (Stewart 2005). Variation in year classes is a common occurrence in bivalves and there are often more year classes that are poorly represented within a population than those which contribute markedly to a population (Larcombe 1971). *Austrovenus stutchburyi* population densities are variable, even within the same shore. Population structure may be deficient of smaller size classes because of the unavailability of space for settlement and growth (Larcombe 1971). *Austrovenus stutchburyi* is subjected to many biotic and physical variables within estuaries and its distribution may be limited by salinity, exposure time and substrate composition (Stephenson 1981). Larcombe (1971) found that recruitment of *Austrovenus stutchburyi* can be highly variable in successive years.

Marine soft sediments provide a wide range of habitats for marine benthic organisms (Thrush 1991). The patchy nature of the environment of marine benthic fauna is linked with the behaviour of the species and will determine the spatial arrangement of the individuals (Thrush 1989). Blanchard and Bourget (1999) showed that community structure was modified by three attributes: spatial scale; topographical heterogeneity; and interaction among scales of heterogeneity.

Bivalves that live in estuaries are exposed to many fluctuating environmental variables, but may also be exposed to trace metals and other pollutants that are known to affect settlement, growth and reproduction (Marsden 1995). Anthropogenic disturbances such as shipping and effluent dumping have caused ecological changes in estuaries worldwide. For example cessation of shipping and associated activities such as channel maintenance in the Ythan Estuary, Scotland may have caused silt to accumulate in the estuary and therefore caused an increase in macro – algae that have changed the invertebrate abundance and shorebird counts (Raffaelli 1999). Compared with epifauna some benthic infaunal species are sedentary and show relatively low mobility. They need to adapt to environmental pollutant stress (Bilyard 1987), low dissolved oxygen, limiting nutrient levels and physical disturbances (Chainho 2006) or they will perish.

Examining how communities are influenced by the environmental conditions and scales is considered necessary to better understand community processes (Blanchard 1999) and structures (Thrush 2000). In the Wash, east England, Yates *et al* (1993) tested for the assumption that bird numbers may be predicted directly from invertebrate densities such as *Cerastoderma edule, Macoma balthica, Mytilus edulis,* polychaetes and sediment characteristics. To be able to generalise between different habitats we need to understand how local ecological processes interact with processes with broader scales (Thrush 2000). Many species occur over a very wide range of estuarine habitat whilst others are confined to a narrow habitat, depending on their tolerance to environmental variables (Chainho 2006). Fresh water flow is a main factor influencing spatial variability within communities. Fluctuations of river flow have an important effect on erosion and depositional cycles which influences the sediment composition. This can influence the colonization of some benthic species (Chainho 2006). Testing different physical and chemical environment variables may allow us to find an association (Yates 1993) between the variables and the population structure of *Austrovenus stutchburyi*.

Recruitment and settlement patterns can also influence the patterns of bivalve distribution (Stark 2003). The settlement-development phase of marine invertebrate recruitment is considered to be one of the most critical times in their life history (Watzin 1997, Stark 2003). Sediment contamination is a major concern in marine environments because many invertebrates spend the majority of their life living on or in the sediments (Olsgard 1999, Roach 2001). Trace metals are important during the recruitment stage of individuals in the estuarine environment (Olsgard 1999) because of their close contact with the sediment. Sediment contamination by trace metals could result in differential site selection or mortality (Watzin 1997, Roach 2001). Watzin and Roscigno (1997) found that zinc contamination of the sediment significantly altered the composition and abundance of benthic recruits in the Gulf of Mexico, although it was not clear whether site selection behaviour or post-recruitment mortality was responsible for the observed effects. Olsgard (1999) found that abundance of recruits was reduced in experiments with increased copper content in the Oslofjord, Norway. This may have been the result of early mortality of recruits, active avoidance, migrating adults or post settlement emigration (Olsgard 1999).

Juvenile *Austrovenus stutchburyi* are an important lifestage of the cockle population because they help to keep the population viable to produce next year's recruits. Surveying the cockle population in estuaries in the Canterbury area will allow us to determine the structure of the population and the extent of the juvenile population present. A correlation of the physical variables within the estuaries may be able to predict the optimal conditions not only for populations of *Austrovenus stutchburyi* but optimal conditions for recruitment of juveniles.

2.2 Aims of the Survey

1. Evaluate the current status of the *Austrovenus stutchburyi* population in the Canterbury area: Avon-Heathcote Estuary, Saltwater Creek Estuary and Port Levy and Takamatua Bay on the Banks Peninsula.

2. Examine if the population structure of *Austrovenus stutchburyi* is determined by the physical and chemical environmental variables, with particular emphasis on juvenile cockles (< 10 mm).

2.3 Methods

2.3.1 Population Survey

A preliminary investigation on the distribution and abundance of *Austrovenus stutchburyi* was carried out around the Avon-Heathcote Estuary, Saltwater Creek Estuary and Port Levy and Takamatua Bay on the Banks Peninsula. At each site a plot of 10 x 15 metres was measured out in the mid to low tide. This plot was divided into 10 subplots of 3 x 5 metres. Within each of these subplots a 25 x 25 cm quadrat was randomly placed. The percentage cover of flora and a count of the fauna on the surface were recorded within the quadrats. The quadrats were dug to a depth of 5 cm and sieved with a 0.5 mm mesh. All *Austrovenus stutchburyi* were measured using electronic calipers to the nearest 0.01 mm. Other bivalves present in the sample were also measured and recorded.

Three subplots were randomly chosen at each site and two sediment cores were taken within 300 mm of the quadrat as recommended by the Ministry for the Environment (Robertson 2002). A 50 mm diameter, 60 mm deep plastic pipe was used to take a sediment sample. Sediments were stored in air tight plastic containers and kept in a temperature controlled room at 4^{o}C until analysis was carried out. The sediment samples were analysed to determine organic content of the sediment, pore water, particle size of the sediment, and nitrogen, phosphorus and trace metal content.

2.3.2 Sediment particle size

The sediment cores from each of the 15 sampling sites were used to determine the particle size of the sediments. This was done by coning (making the sediment sample into a ball) and quartering (cutting the sediment into quarters and removing two of the opposite sides) the wet sediment from the sample to gain an even distribution of the particles in the sample. The final amount of sediment was washed through a 1 mm mesh sieve with distilled water to remove any large unwanted debris and large sediment particles. The large sediment particles were kept for further sieving. The sieved sediment was transferred into smaller ceramic bowls where 50% hydrogen peroxide of 5% by volume was added until effervesancing no longer continued. This dissolved some of the organic matter, for example, animal flesh, however did not dissolve shells and debris. Once this reaction finished, the samples were put in the fume cupboard until the water evaporated off. This took between four and six days, depending on the amount of liquid present. Once dry, the samples were ground with a rubber bung to break up sediment particles that were clumped together. The sediment was then coned and quartered to reduce the sample size to ~10 mL. The sediment particle size was analyzed using a Saturn DigiSizer 5200^{TM} which has a high resolution detection system to allow subtle differences in particle sizes. Each sample was analysed three times. The DigiSizer analysis was used as a previous study concluded that greater accuracy is achieved than with manual methods such as sieving and pipetting (Murphy 2006). Particles were grouped according to the Wentworth Scale (Table 2.1) (Wentworth 1922). Particles greater than 1 mm were sieved in larger sieves to determine their size and were weighed to determine their percentage composition of the sample. Grain sizes of the sediment were divided into five categories as in Stewart (2005), to make comparsions between sites and other variables easier. The categories were silts and clay (< 63 μm); very fine sand (63 – 124 μm); fine sand (125 – 249 μm); medium sand (250 – 499 μm); coarse sand (500 – 999 μm); and very coarse sand, granule and pebble (> 1000 μm).

Table 2.1: Wentworth size class and grade limits.

Wentworth Size Class	Grade Limits
Boulder	>256 mm
Cobble	256 – 64 mm
Pebble	4 – 64 mm
Gravel	2 – 4 mm
Very coarse sand	1 – 2 mm
Coarse sand	500 – 999 µm
Medium sand	250 – 499 µm
Fine sand	125 – 249 µm
Very fine sand	63 – 124 µm
Coarse Silt	32 – 62 µm
Medium Silt	15 – 31 µm
Fine Silt	7.8 – 14 µm
Very Fine Silt	3.9 -7.7 µm
Clay	<3.8 µm

2.3.3 Trace metal content of sediments

Sediments that were collected from each of the 15 sampling sites were sent to R. J. Hill Laboratories in Hamilton for analysis of total recoverable phosphorus, total nitrogen and the trace metal levels of, arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) (mg/kg dry weight). Trace metals were analysed using nitric / hydrochloric acid digestion, ICP-MS (low level) US EPA 200.2. Total nitrogen was analysed as g/100g (dry weight) by catalytic combustion, separation, thermal conductivity detector and total recoverable phosphorus was analysed as mg/kg (dry weight) by nitric / hydrochloric acid digestion, ICP-MS (low level) US EPA 200.2.

2.3.4 Pore water and organic content of sediment

From the sediment cores collected at each site a proportion of each sample was weighed to obtain the wet weight of the sediment to the nearest 0.001g. The sample was then dried at 60 ^{o}C overnight. The sample was reweighed to obtain the dried weight of the sediment to the nearest 0.001g. From this, the percentage of pore water (the amount of water that the sediment holds) was calculated as: (Wet weight – Dry weight)/Wet weight x (100). The dried sediment was then ashed at 425 ^{o}C for five hours. The ashed sediment was reweighed to the nearest 0.001g and the percentage of organic content in the sediment at each site was calculated as: (Dry weight – Ashed weight)/Dry weight x (100).

2.3.5 Statistical Analysis

The relationship of variables to site was analysed using a one way ANOVA. Data that failed to comply with all the assumptions of ANOVA, because it violated the assumption of homogeneity of variances, was transformed. However, in all cases, transformation did not improve homogeneity of variances and analysis was performed on the original data. The validity of this robust test (ANOVA) is not affected significantly by violations of homogenous variances, which is particularly true for balanced data with a large number of samples (Underwood 1997). Tukey HSD tests were used to determine where the significance difference lied and letters above each histogram bar were used to show similarities and differences. Similar letters have no significance difference whilst different letters denote sites are significantly different to each other. A correlation matrix was used to analyse the relationship between the density of the population of cockles at each site and those cockles at each site < 10 mm against the different variables at each site.

Non-metric Multi-Dimensional Scaling (MDS) ordination was used in PRIMER v6.1.5 to examine the differences in community assemblage and environmental variables between sampling sites. Data were averaged and means were square root transformed to scale down any effects of very abundant species (Clarke 2006). MDS represents the samples as points in a low-dimensional space such that the relative distances of the points are similar to dissimilarities of the samples. Therefore points that are close together represent sites that are similar in composition and points that are far apart represent sites that are dissimilar (Clarke 2006).

Relationships between environmental and biotic variables were analysed using the BIOENV procedure of PRIMER v6.1.5. The main rationale for this procedure is to find the best match between the multivariate among-sample patterns of an assemblage and that from environmental variables associated with those samples (Clarke 2006). The level of correlation is shown by the Rho-value (rank correlation coefficient) closer to 1 = high correlation, closer to 0 = low correlation (Clarke 2006).

Principal components analysis (PCA) was used in PRIMER v6.1.5 to analyse environmental variables. For environmental data this procedure is appropriate as they have complex measurement scales such as sediment particle size (%), total nitrogen (g/100g) and trace metals (mg/kg dry weight) (Murphy 2006). Data were square root transformed and normalized to give comparable scales (Clarke 2006).

2.4 Site Descriptions and Locations

Sites were chosen based on previous studies of the *Austrovenus stutchburyi* population in the Canterbury area.

2.4.1 Avon-Heathcote Estuary

Table 2.2 Sampling sites and characteristics of the Avon-Heathcote Estuary.

Symbol	Site	Area of estuary	Residential properties	Defining features
BR	Beachville Road	South side	Yes	Artificial seawall
TS2	Tern Street Sea grass	East side	Yes	Dense Zostera beds
TS1	Tern Street Non-Sea grass	East side	Yes	Low tide channel of Avon River
HS	Heron Street	East side	Yes	Low tide channel of Avon River
PPJ	Pleasant Point Jetty	East side	No	Extensive area of rushes at high tide
PPY	Pleasant Point Yacht Club	West side	No	North of oxidation pond 6 Current construction of outlet pipe
HC	Heathcote River Mouth	South side	No	Permanent Heathcote River channel
HD	Humphrey's Drive	West side	No	Surrounded by man-made seawall
OX	Wastewater Treatment Outfall	West side	No	Discharge point of oxidation ponds
SP	Sandy Point	West side	No	Popular area for water-sports
MB	McCormacks Bay	South side	Yes	Connected to main estuary by two small culverts through the causeway

2.4.2 Saltwater Creek Estuary

Table 2.3 Sampling sites and characteristics of the Saltwater Creek Estuary.

Symbol	Site	Area of estuary	Residential properties	Defining features
SC1	Saltwater Creek Site 1	South side	No	Small channels of water Close to Ashley River Channel
SC2	Saltwater Creek Site 2	East side	No	Further up the estuary Close to Saltwater Creek Channel

2.4.3 Banks Peninsula

Table 2.4 Sampling sites and characteristics on the Banks Peninsula

Symbol	Site	Area of estuary	Residential properties	Defining features
TK	Takamatua Bay	North side	Yes	Small stream, pebbly at high tide Shell and mud at low tide
PL	Port Levy	East side	Yes	No shellfish collecting zone Small outlet drain

Figure 2.1 Image of the Avon – Heathcote Estuary showing locations of sampling sites. Humphreys Drive (HD), Heathcote (HC), Sandy Point (SP), McCormacks Bay (MB), Beachville Road (BR), Tern Street non sea grass (TS1), Tern Street, sea grass (TS2), Heron Street (HS), Pleasant Point Jetty (PPJ), Pleasant Point Yacht Club (PPY), Wastewater treatment pond outfall (OX), the mouth of the Avon and Heathcote Rivers and the wastewater treatment ponds.

Figure 2.2 Image of Saltwater Creek Estuary showing locations of sampling sites, Saltwater Creek 1 (SC1), Saltwater Creek 2 (SC2), Saltwater Creek and the Ashley River.

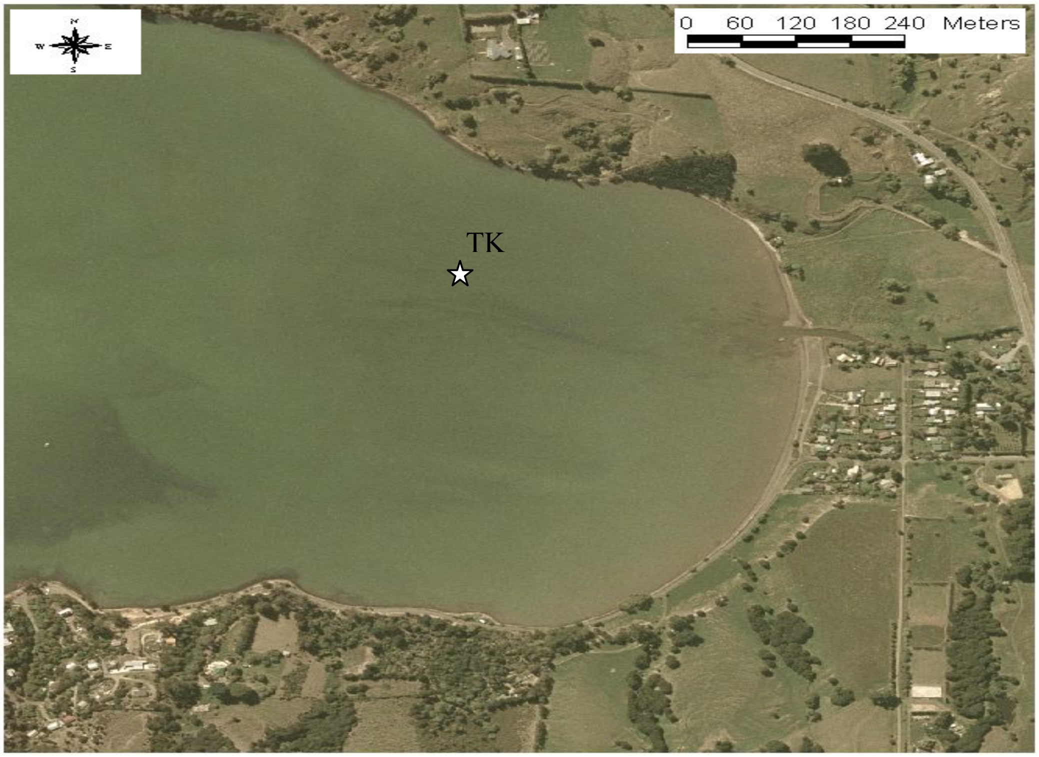


Figure 2.3 Image of Takamatua showing the location of the sampling site Takamatua (TK).

Figure 2.4 Satellite image of Port Levy showing the location of the sampling site Port Levy (PL).

2.5 Results

2.5.1 Austrovenus stutchburyi *population structure.*

The population structure of *Austrovenus stutchburyi* varied considerably between all the sampling sites (Fig 2.5). Populations were generally unimodal or bimodal (Fig 2.5). The site TS1 was dominated by *Austrovenus stutchburyi* in the larger length groups compared to TS2 where there were a greater number of juveniles. Sites SC1 and SC2 have very different population structures with the majority of the SC1 population between 15 – 20 mm, whilst SC2 had a more even size range of *Austrovenus stutchburyi.* There were no juvenile *Austrovenus stutchburyi* in the length range of 0 – 5 mm at sites PPY, TK, and PL whilst a small percentage of *Austrovenus stutchburyi* in this size range were seen at all other sites. A large percentage of the population was between 0 – 10 mm at sites HC, HD, PPJ, HS, TS1, and SC1 (Table 2.5). McCormacks Bay (MB) had a relatively even distribution of length groups. Sites BR, OX, and SC2 all had cockles above 35 mm in size, although they only represent a small percentage of the population distribution at these sites. HD was the only site with missing length groups in the population structure with no *Austrovenus stutchburyi* in the 20 – 25 mm length group. Site PPY showed a very unusual population structure with only one size class and one animal present in this size class (Fig 2.5, Table 2.5).

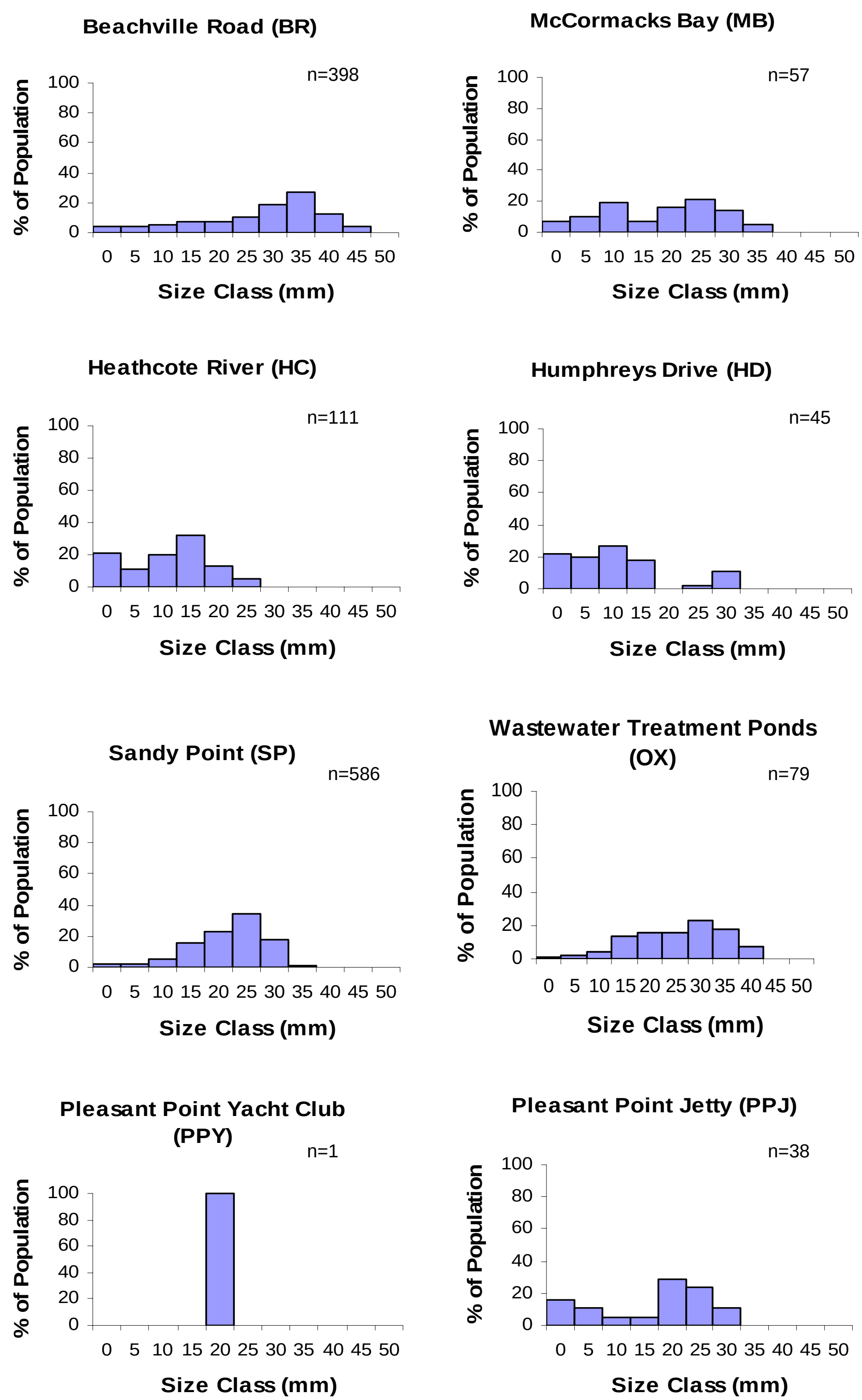


Figure 2.5 Population structure of Austrovenus stutchburyi at each sampling site.

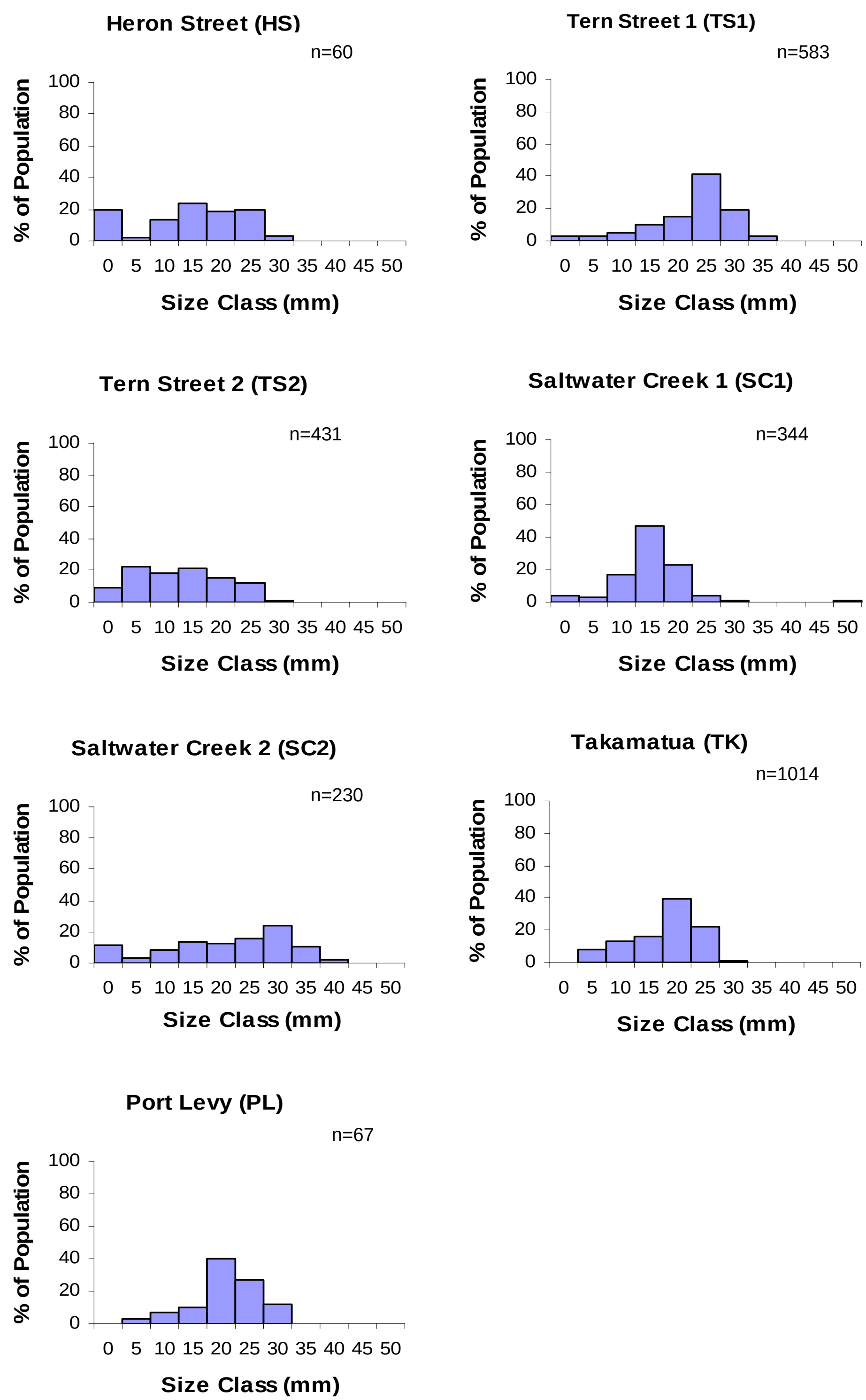


Figure 2.5 Population structure of Austrovenus stutchburyi at each sampling site.

Table 2.5 Percentage population structure of *Austrovenus stutchburyi* broken down into three length classes (juvenile, medium and large), at each sampling site (Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL)).

Site	Juvenile (< 10 mm)	Medium (< 30 mm)	Large (< 50 mm)
HS	21.67	75	3.33
TS1	5.83	72.04	22.13
BR	8.04	29.65	62.31
TS2	32.02	67.05	0.93
TK	8.68	90.34	0.99
HC	31.53	68.47	0
PPY	0	100	0
HD	42.22	46.67	11.11
OX	3.8	48.1	48.1
SP	4.1	77.47	18.43
MB	17.54	63.16	19.3
PL	2.99	85.07	11.94
SC2	14.78	49.57	35.65
SC1	7.56	91.28	1.16
PPJ	26.32	63.16	10.53

2.5.2 Cockle density

Density varied greatly between sites and ranged from 1.6/m^2 at site PPY to 1622.4/m^2 at site TK (Fig 2.6). The number of cockles per m^2 was compared between sites without transformation of data because it did not improve homogeneity of variances. The number of cockles per m^2 was significantly different between sites ($F_{0.05(14,135)} = 1.96$, $F = 34.970$, $p < 0.001$), with the number of cockles per m^2 at site TK being significantly greater than all other sites. Cockle density at sites BR; TS1; TS2; SP; SC1; and SC2 were similar in cockle density (Fig 2.6).

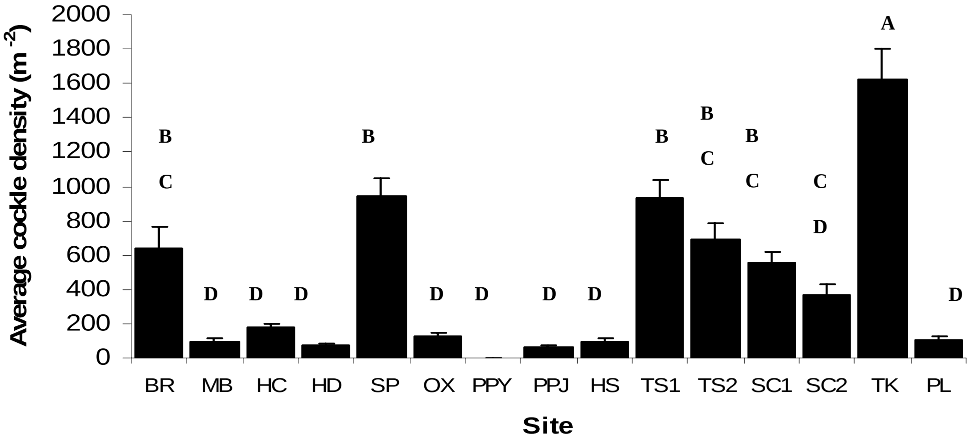


Figure 2.6 Average Austrovenus stutchburyi density (m2) (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL).

Density of juvenile cockles (<10 mm) ranged from 0/m^2 at site PPY to 220.8/m^2 at TS2. Juvenile *Austrovenus stutchburyi* density varied between sites ($F_{0.05(14,135)}$ = 1.96, F = 15.07, p < 0.001), with TS2 significantly higher than all other sites (Fig 2.7).

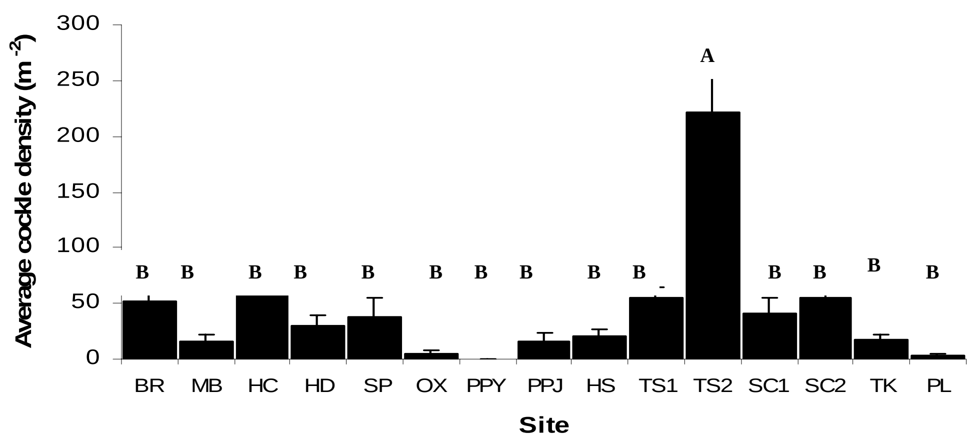


Figure 2.7 Average juvenile (<10mm) Austrovenus stutchburyi density (m2) (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL).

2.5.3 Sediment particle size

There was a significant difference in percentage silt and clay (< 63 µm); very fine sand (63 µm); fine sand (125 µm); medium sand (250 µm); and very coarse sand, granule and pebble (1000 µm) between the sites. All sites had a similar percentage of coarse sand (500 µm) (Table 2.6). The largest sediment particles were found only at Takamatua and Port Levy, where the sample was made up of 0.48% and 25% respectively (Fig 2.8). Fine sediment particles (125 µm) were found at all sites, McCormacks Bay had the highest silt and clay fraction (73%) and Port Levy the least silt and clay fraction of 5% (Fig 2.8). Average cockle density was negatively correlated with percentage silt and clay ($R=0.371$, $F_{0.05(1, 43)} = 4.08$, $F = 6.877$, $p = 0.012$). Small cockles < 10 mm were positively correlated to silt and clay percentage ($R=0.321$, $F_{0.05(1, 43)} = 4.08$, $F = 4.954$, $p = 0.031$) (Table 2.8). Fine sand (125 µm) was significantly positively correlated with both average cockle density ($R=0.610$, $F_{0.05(1, 43)} = 4.08$, $F = 25.441$, $p = < 0.001$) and density of cockles < 10 mm ($R=0.396$, $F_{0.05(1, 43)} = 4.08$, $F = 8.013$, $p = 0.007$) (Table 2.8).

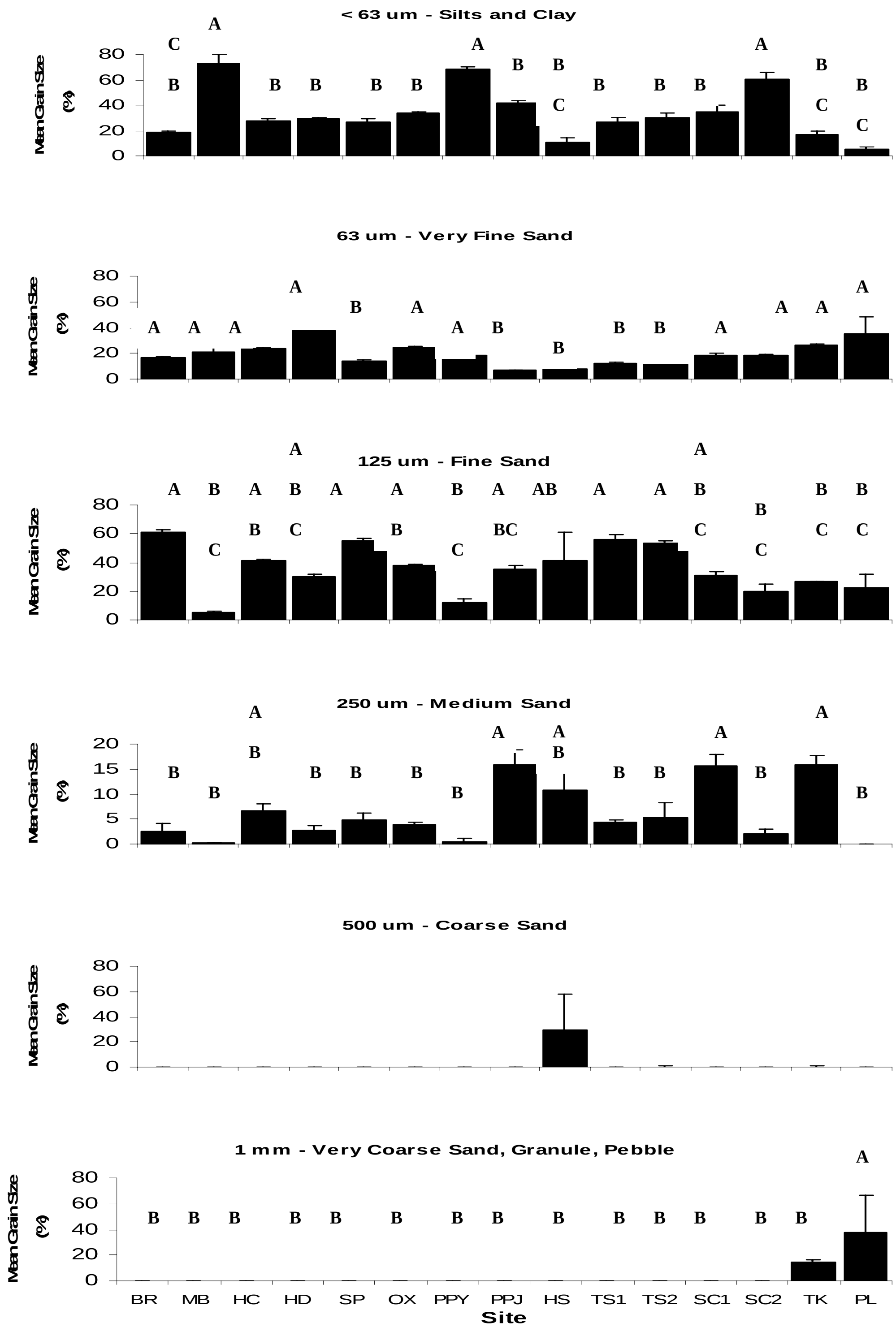


Figure 2.8 Mean percent size grain for each site (± 1 S.E.). Each graph represents one size class of sediments (note the different scale for medium sand).

Table 2.6 Results of analysis of variance comparing the percentage of sediment grain size between sites. Values in bold indicate significance where $p < 0.05$.

Source	SS	*df*	MS	*F*	p
< 63 µm					
Site	16590.9	14	1185.1	46.56	**< 0.001**
Error	763.65	30	25.46		
63 µm					
Site	332.46	14	237.39	5.713	**< 0.001**
Error	1246.54	30	41.55		
125 µm					
Site	11623.7	14	830.26	7.745	**< 0.001**
Error	3216.12	30	107.2		
250 µm					
Site	1359.78	14	97.13	9.292	**< 0.001**
Error	313.59	30	10.45		
500 µm					
Site	2347.68	14	167.69	1.028	0.454
Error	4895.92	30	163.2		
1000 µm					
Site	6354.17	14	453.87	9.188	**< 0.001**
Error	1432.52	30	49.4		

2.5.4 Trace metals, total nitrogen and total phosphorus

Levels of total recoverable nitrogen ranged from < 0.05 g/100g (dry weight) at sites BR, HC, HS, PL and HD to 0.15 g/100g (dry weight) at site PPY (Fig 2.9). There were significant differences between sites and the level of total recoverable nitrogen in the sediment ($F_{0.05(14,30)} = 2.04$, $F = 9.236$, $p < 0.001$), with values at the Saltwater Creek site being significantly different to most other sites. Nitrogen levels at sites PPY and TK were generally higher values compared to other sites. There was no significant correlation between the number of cockles per m^2 and nitrogen levels of the sediment ($R=0.107$, $F_{0.05(1, 43)} = 4.08$, $F = 0.499$, $p = 0.484$), or between densities of small cockles < 10 mm and nitrogen levels ($R = 0.099$, $F_{0.05(1, 43)} = 4.08$, $F = 0.428$, $p = 0.516$) (Table 2.8).

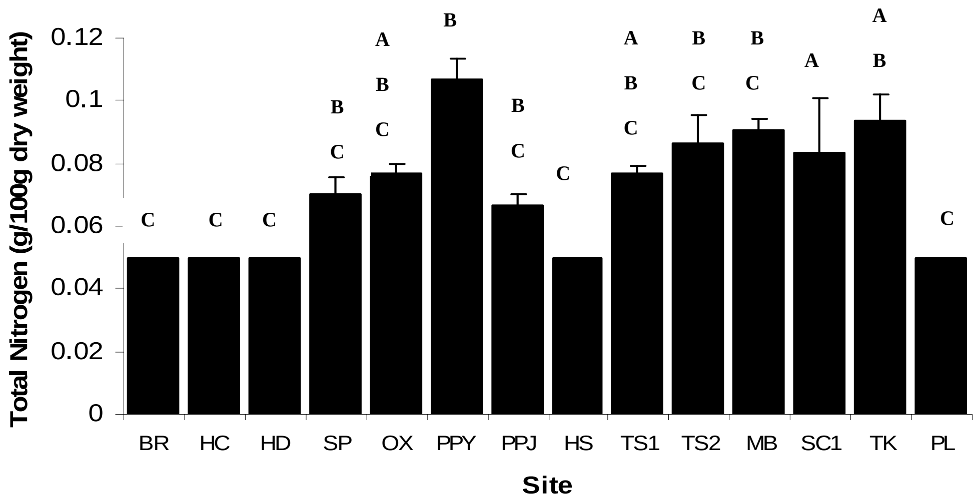


Figure 2.9 Total recoverable nitrogen (g/100g dry weight) in the sediment (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek (SC1); Takamatua (TK); and Port Levy (PL).

Levels of total recoverable phosphorus ranged from 276 mg/kg (dry weight) at site HS to 1190 mg/kg (dry weight) at site TK (Fig 2.10). There was a significant difference between sites and the level of total recoverable phosphorus in the sediment ($F_{0.05(14,30)} = 2.04$, $F = 32.354$, $p < 0.001$) (Fig 2.10). Site TK had phosphorus levels that were significantly different to all other sampling sites, and site PPY and PL, and PPJ and SC1 had significantly similar levels. Cockle density was significantly correlated with total recoverable phosphorus levels ($R=0.324$, $F_{0.05(1,43)} = 4.08$, $F = 5.057$, $p = 0.030$), although there was no correlation between phosphorus and density of small cockles < 10 mm ($R=0.265$, $F_{0.05(1,43)} = 4.08$, $F = 3.235$, $p = 0.079$) (Table 2.8).

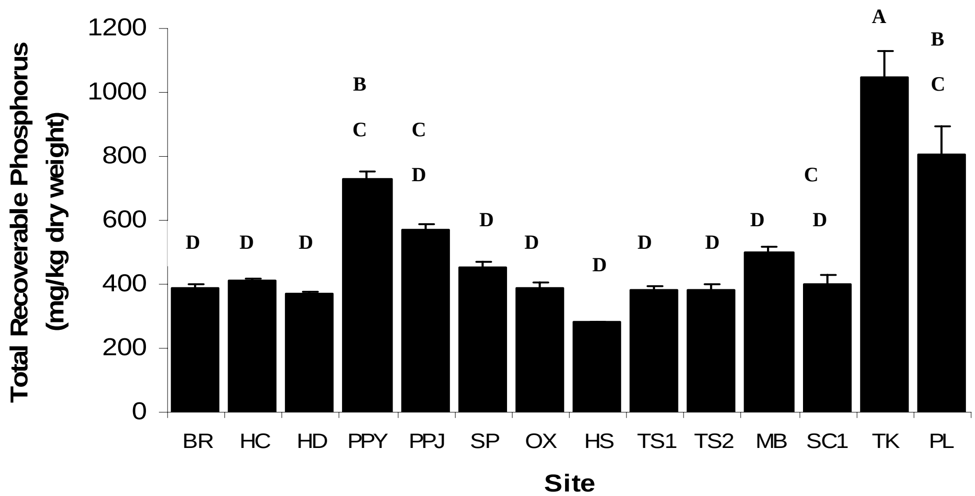


Figure 2.10 Total recoverable phosphorus (mg/kg dry weight) in the sediment (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek (SC1); Takamatua (TK); and Port Levy (PL).

Levels of zinc ranged between 35.1 mg/kg (dry weight) at Heron Street (HS) to 99 mg/kg at Pleasant Point Yacht Club (PPY) (Fig 2.11). There was a significant difference between sites and the level of total recoverable zinc in the sediment ($F_{0.05(14,30)} = 2.04$, $F = 56.617$, $p < 0.001$). Site PPY had significantly different zinc levels than other sampling sites. Site BR was not significantly different to either Tern Street sites (TS1 and TS2) or site HS. The McCormacks Bay site (MB) was similar in sediment zinc levels to sites, HD, SP, OX, PPJ and PL. The two Tern Street sites (TS1 and TS2) were statistically similar in zinc levels to the Saltwater Creek site. There was a negative correlation between average cockle density and zinc levels in the sediment ($R=0.406$, $F_{0.05(1,43)} = 4.08$, $F = 8.504$, $p = 0.006$), and for small cockles of < 10 mm and zinc ($R=0.350$, $F_{0.05(1,43)} = 4.08$, $F = 5.999$, $p = 0.018$) (Table 2.8).

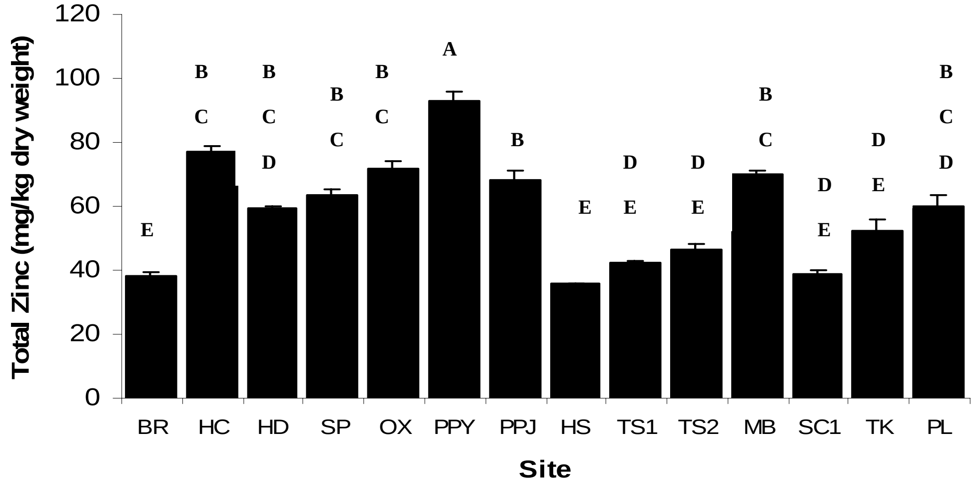


Figure 2.11 Total recoverable zinc (mg/kg dry weight) in the sediment (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek (SC1); Takamatua (TK); and Port Levy (PL).

Levels of copper ranged between 3.2 mg/kg (dry weight) at Heron Street (HS) to 15.4 mg/kg (dry weight) at Pleasant Point Yacht Club (PPY) (Fig 2.12). There was a significant difference between sites and the level of total recoverable copper in the sediment ($F_{0.05(14,30)} = 2.04$, $F = 53.092$, $p < 0.001$). PPY had significantly different copper levels in the sediment to all other sites. Sites BR, HC, HD, SP, HS, TS1 and TS2 had similar copper levels in the sediment. Saltwater Creek was similar in copper levels to sites OX, PPJ, MB, TK and PL. There was no significant correlation between average cockle density and total recoverable copper ($R=0.234$, $F_{0.05(1,43)} = 4.08$, $F = 2.496$, $p = 0.121$), although there was a significant negative correlation between copper levels and densities of small cockles < 10 mm ($R=0.417$, $F_{0.05(1,43)} = 4.08$, $F = 9.077$, $p = 0.004$) (Table 2.8).

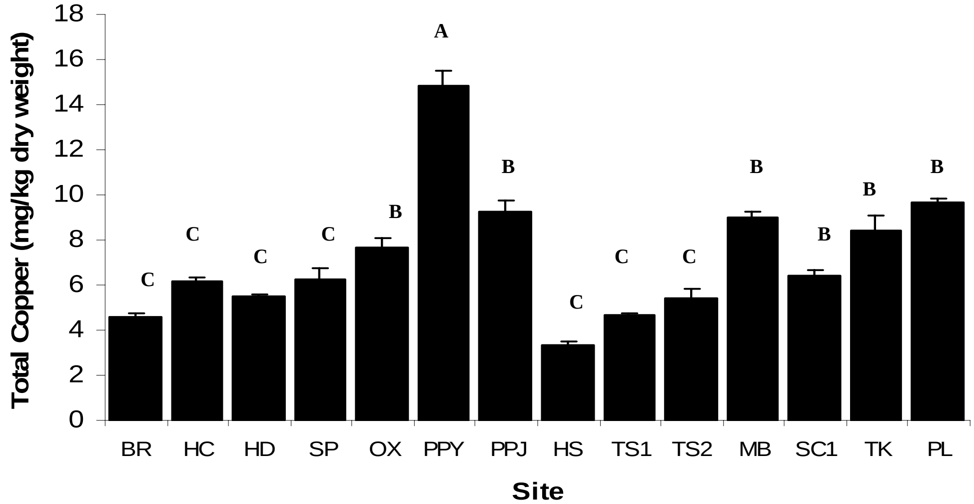


Figure 2.12 Total recoverable copper (mg/kg dry weight) in the sediment (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek (SC1); Takamatua (TK); and Port Levy (PL).

Levels of cadmium were highly site specific and ranged between 0.03 mg/kg (dry weight) at SC1 and TS1 to 0.2 mg/kg (dry weight) at site PPY (Fig 2.13). There was a significant difference between sites and the level of total recoverable cadmium in the sediment ($F_{0.05(14,30)} = 2.04$, $F = 126.13$, $p < 0.001$). Sites BR, HS, TS1, TS2, MB, SC1, TK and PL all had similar cadmium levels. Site SP was different to all other sites except HD and PPJ, whilst site HC was similar in cadmium levels to sites OX and PPY. There was a significant negative correlation between average cockle density and total recoverable cadmium in the sediment ($R=0.329$, $F_{0.05(1,43)} = 4.08$, $F = 5.214$, $p = 0.027$), there was no similar correlation between densities of small cockles < 10 mm and cadmium levels ($R=0.243$, $F_{0.05(1,43)} = 4.08$, $F = 2.702$, $p = 0.108$) (Table 2.8).

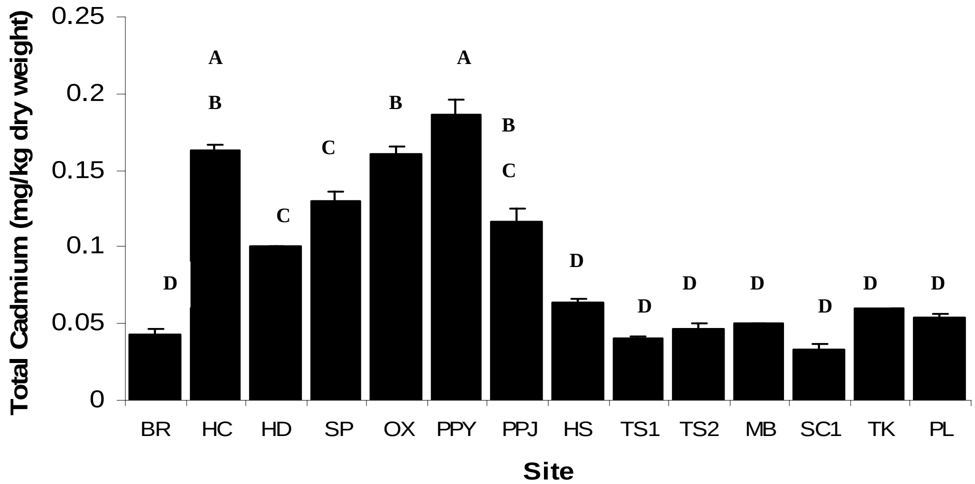


Figure 2.13 Total recoverable cadmium (mg/kg dry weight) in the sediment (+1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek (SC1); Takamatua (TK); and Port Levy (PL).

2.5.5 Porosity and organic content

Percentage pore water of the sediment ranged between 21.16% at site PL to 32.09% at site TS2 (Fig 2.14). All sites were very similar in percentage of pore water of the sediment, however an ANOVA confirmed that there was a significant difference of porosity of the sediment between sites ($F_{0.05(14,30)} = 2.04$, $F = 2.842$, $p = 0.008$). Data did not comply with the assumptions of ANOVA and transformations did not improve the heterogeneity of variances. MB and TS2 had similar high values of porosity whilst site PL was significantly lower than these sites. All of the other 12 sites sampled did not have significantly different porosity of the sediment.

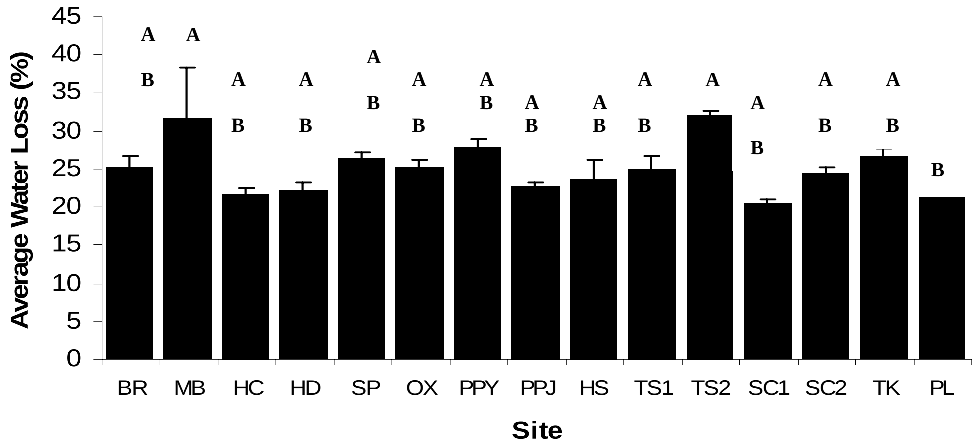


Figure 2.14 Average pore water (%) of the sediment (+ 1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL).

Organic matter in the sediment ranged from 0.73% at site HS to 4.65% at site TK (Fig 2.15). There was a significant difference in percentage of organic matter in the sediment between sites ($F_{0.05(14,30)} = 2.04$, $F = 19.698$, $p < 0.001$). Data did not comply with the assumptions of ANOVA and transformation could not improve the heterogeneity of variances. Sites MB and TK had similar organic content in the sediments, these were however different to all other sites which were similar to each other. The Tern Street sites (TS1 and TS2), had different organic content possibly because one was covered with sea grass and the other was bare. Both Saltwater Creek sites (SC1 and SC2) had similar organic content in the sediment.

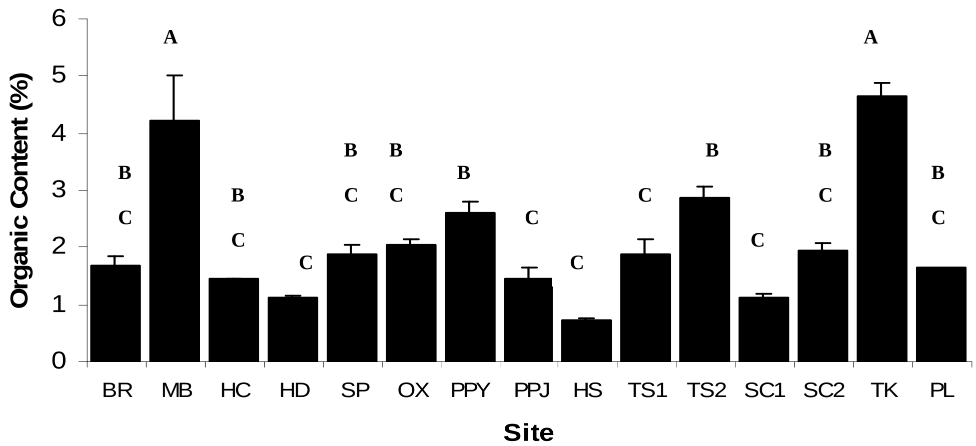


Figure 2.15 Average organic content (%) of sediment (+ 1 SE) at Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL).

Correlation analysis was used to determine the relationship among the environmental variables measured and cockle density. There was no significant relationship between average cockle density and organic matter ($R = 0.115$, $F_{0.05(1,43)} = 4.08$, $F = 0.578$, $p = 0.451$) or average cockle density and porosity ($R = 0.127$, $F_{0.05(1,43)} = 4.08$, F = 0.0.7012, $p = 0.407$). There was also no significant correlation between organic matter and density of small cockles < 10 mm ($R = 0.015$, $F_{0.05(1,43)} = 4.08$, $F = 0.010$, $p = 0.921$), or pore water and small cockles < 10 mm ($R = 0.061$, $F_{0.05(1,43)} = 4.08$, $F = 0.161$, $p = 0.690$) (Table 2.8).

There was a positive correlation between pore water and organic content ($R = 0.71$, $F_{0.05(1,43)} = 4.08$, $F = 43.021$, $p < 0.001$), however there was no significant correlation between pore water and total nitrogen, phosphorus, copper, cadmium or zinc. There was however, a significant correlation between organic content and total recoverable nitrogen in the sediment ($R = 0.377$, $F_{0.05(1,43)} = 4.08$, $F = 7.109$, $p = 0.011$), and between organic content and total recoverable phosphorus levels in the sediment ($R = 0.508$, $F_{0.05(1,43)} = 4.08$, $F = 14.988$, $p < 0.001$). There was a significant correlation between organic content and total copper levels in the sediment ($R = 0.322$, $F_{0.05(1,43)} = 4.08$, $F = 4.978$, $p = 0.031$), however no significant relationship between organic content and zinc or cadmium levels (Table 2.7).

There was a significant positive correlation between sediment grains < 63 µm and pore water (R = 0.365, $F_{0.05(1,43)}$ = 4.08, F = 6.620, p = 0.014), however no other sediment grain size was correlated to pore water. A sediment gain size of < 63 µm also had a positive correlation with percentage organic matter (R = 0.349, $F_{0.05(1,43)}$ = 4.08, F = 5.952, p = 0.019), however there was a negative correlation of a sediment grain size of 125 µm and organic matter(R = 0.335, $F_{0.05(1,43)}$ = 4.08, F = 5.452, p = 0.024). There was a significant negative correlation of 125 µm grain size (R = 0.342, $F_{0.05(1,43)}$ = 4.08, F = 5.711, p = 0.021) and a significant positive correlation of < 63 µm (R = 0.538, $F_{0.05(1,43)}$ = 4.08, F = 17.488, p = < 0.001) to total recoverable nitrogen. Total recoverable phosphorus was correlated with the greatest number of sediment grain sizes. There was a positive correlation with both 63 µm (R = 0.346, $F_{0.05(1,43)}$ = 4.08, F = 5.836, p = 0.020) and 1000 µm (R = 0.398, $F_{0.05(1,43)}$ = 4.08, F = 7.924, p = 0.007) and a negative correlation with 125 µm (R = 0.425, $F_{0.05(1,43)}$ = 4.08, F = 9.465, p = 0.004) (Table 2.7).

There was a significant positive correlation between sediment grain size of < 63 µm and copper (R = 0.564, $F_{0.05(1,43)}$ = 4.08, F = 20.067, p = < 0.001) and between < 63 µm and zinc (R = 0.518, $F_{0.05(1,43)}$ = 4.08, F = 15.738, p = < 0.001). The sediment grain size of 125 µm had a negative correlation with both copper (R = 0.645, $F_{0.05(1,43)}$ = 4.08, F = 30.666, p = < 0.001) and zinc (R = 0.463, $F_{0.05(1,43)}$ = 4.08, F = 11.723, p = 0.001). There were no significant correlations between any sediment grain size and cadmium (Table 2.7). MDS representation of the environmental variables had a low 2D stress of 0.03 (low stress levels reflect high accuracy of the represented data on the ordination (Clarke 2006)) (Fig 2.16). Site PPY is different from all other sites. Sites from the Saltwater Creek Estuary are grouped closely together and sites from the Banks Peninsula are distant from other sites reflecting differences in environmental variables (Fig 2.16).

Table 2.7 Correlation (R) analysis of environmental variables. Bold values have significant correlation of (α) 0.05. (N – total nitrogen; P – total phosphorus; PW - % Pore water; OM - % Organic matter)

	N	P	PW	OM	Cd	Cu	Zn	<63μm	63μm	125μm	250μm	500μm
N												
P	**0.361**											
PW	0.242	0.010										
OM	**0.377**	**0.508**	**0.707**									
Cd	0.116	0.021	0.029	0.097								
Cu	**0.535**	**0.659**	0.088	**0.322**	**0.379**							
Zn	0.166	0.273	0.052	0.175	**0.810**	**0.749**						
<63μm	**0.538**	0.020	**0.365**	**0.349**	0.193	**0.564**	**0.518**					
63μm	0.129	**0.346**	0.187	0.089	0.086	0.172	0.226	0.096				
125μm	**0.342**	**0.425**	0.123	**0.335**	0.061	**0.645**	**0.463**	**0.519**	0.195			
250μm	0.142	0.227	0.287	0.059	0.087	0.069	0.183	0.280	0.251	0.203		
500μm	0.154	0.182	0.118	0.184	0.077	0.228	0.234	0.223	0.256	0.265	0.066	
1mm	0.156	**0.398**	0.216	0.077	0.160	0.174	0.003	**0.375**	0.037	0.293	0.077	0.039

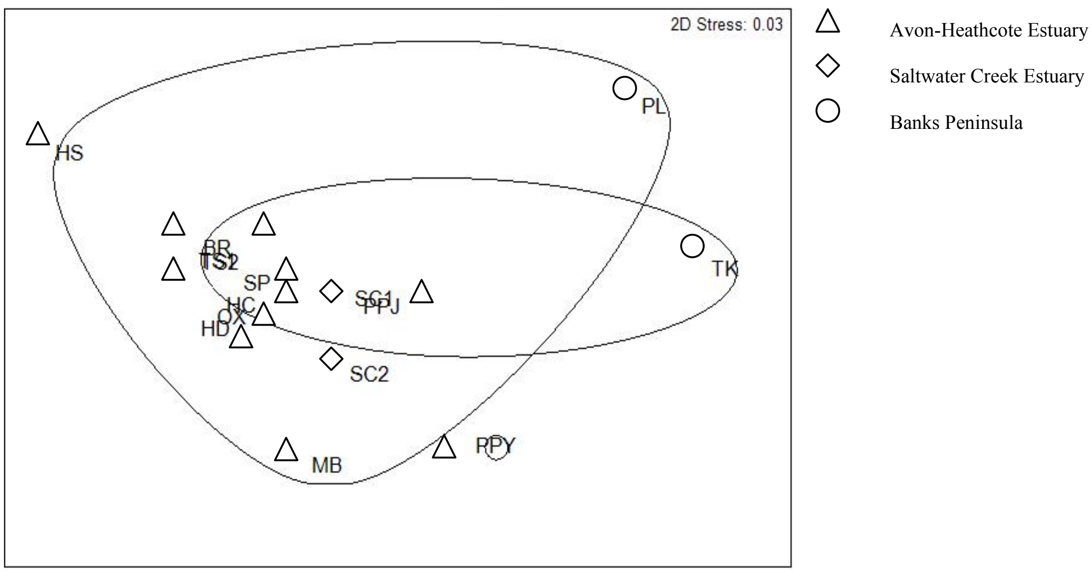


Figure 2.16 MDS ordination of environmental variable similarity between the sites (Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL)).

Results of the PCA ordination were an average representation of patterns of dissimilarities of environmental variables between sampling sites (Fig 2.17). Accuracy was shown by average percentages (54.8%) of variation explained by the PC1 (35.5%) and PC2 (19.3%) axes. Significance of individual environmental variables driving the dissimilarity at each sampling site is represented by the length of the vector line approaching the circle (Fig 2.17). Site PPY is furthest away from other sites on the PC1 axis and similarly in the MDS plot (Fig 2.16) this site is different from the other sites sampled however is related to site MB and SC2 (Fig 2.17). On the PC2 axis sites PL and TK are similarly related as they lie close together (Fig 2.17).

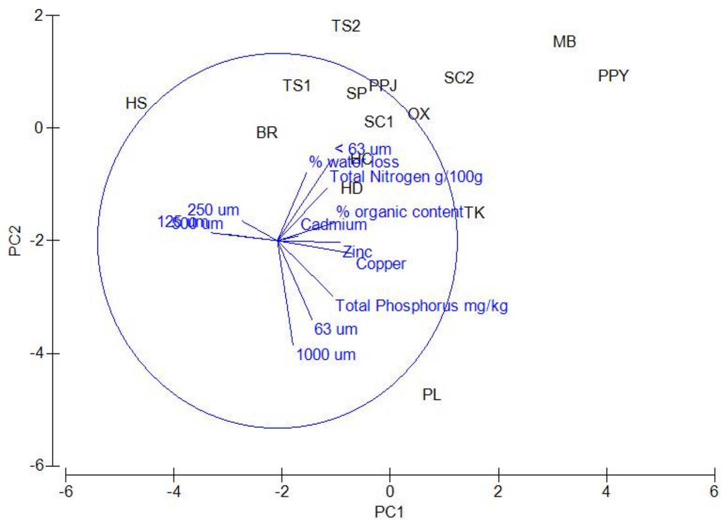


Figure 2.17 PCA ordination of environmental variables and sites. The circle and lined represent the variable vectors and their contribution to dissimilarity of sampling between sites.

2.5.6 Surface flora and fauna

On the mudflat surface at each of the sampling sites there was different fauna (*Amphibola crenata*, *Diloma subrostrata*, *Zeacumantus subcarinatus,* and *Cominella glandiformis*) and flora (*Zostera muelleri* and *Ulva* spp.). There was no significant correlation between *Amphibola crenata, Zostera muelleri*, or *Zeacumantus subcarinatus* and cockle density. In contrast, there was significant correlations between *Diloma subrostrata* density ($R = 0.216$, $F_{0.05(1,148)} = 3.19$, $F = 7.224$, $p = 0.008$), percentage cover of *Ulva* spp. ($R = 0.200$, $F_{0.05(1,148)} = 3.19$, $F = 6.190$, $p = 0.014$), and *Cominella glandiformis* density and cockle density ($R = 0.433$, $F_{0.05(1,148)} = 3.19$, $F = 34.089$, $p < 0.001$).

Fauna and flora were also correlated with juvenile cockles of < 10 mm. *Zostera muelleri* ($R = 0.705$, $F_{0.05(1,148)} = 3.19$, $F = 146.204$, $p = < 0.001$), *Diloma subrostrata* ($R = 0.476$, $F_{0.05(1,148)} = 3.19$, $F = 41.253$, $p = < 0.001$), and *Ulva* spp. ($R = 0.206$, $F_{0.05(1,148)} = 3.19$, $F = 6.542$, $p = 0.012$) were all correlated to the small cockles < 10 mm, although there was no significant correlation between cockles of < 10 mm and *Amphibola crenata, Cominella glandiformis* or *Zeacumantus subcarinatus*

(Table 2.8). MDS representation of the flora and fauna assemblage at all the sampling sites had a low 2D stress of 0.01 (Fig 2.18). Sites were all grouped together except site PPY as the flora and fauna were very different at this site. This difference was because there was no *Austrovenus stutchburyi* found and low abundance of other flora and fauna.

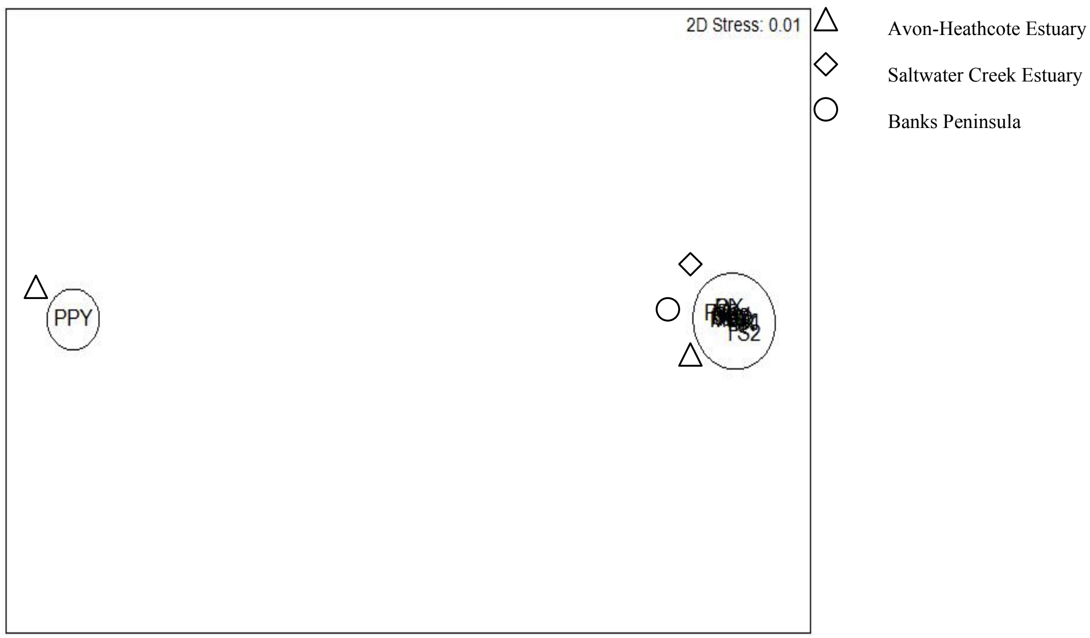


Figure 2.18 MDS ordination of flora and fauna assemblage similarity between the sites (Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL)).

Table 2.8 Correlation matrix indicating associations between environmental variables and mean *Austrovenus stutchburyi* density and juvenile *Austrovenus stutchburyi* (<10 mm). Significant correlations are shown in bold ($p < 0.05$).

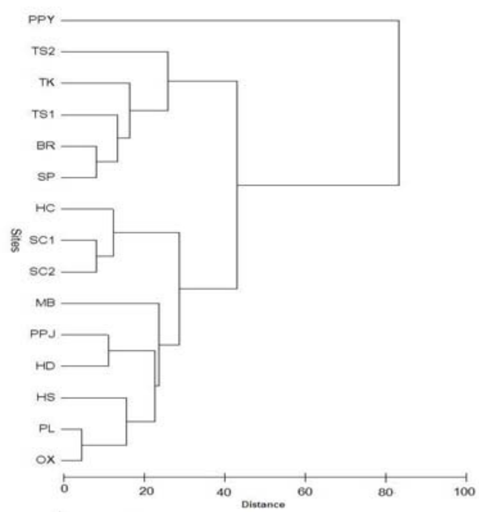
Variable	Density Correlation	*p*	<10 mm Correlation	*p*
Total Nitrogen	0.107	0.484	-0.099	0.516
Total Phosphorus	**0.324**	**0.03**	-0.265	0.079
% Pore Water	0.127	0.407	0.061	0.69
% Organic Matter	0.115	0.451	0.015	0.921
Cadmium	**-0.329**	**0.027**	-0.243	0.108
Copper	-0.234	0.121	**-0.417**	**0.004**
Zinc	**-0.406**	**0.006**	**-0.35**	**0.018**
Amphibola crenata	-0.059	0.475	0.095	0.249
Diloma subrostrata	**0.216**	**0.008**	**0.467**	**< 0.001**
Zeacumantus subcarinatus	-0.115	0.161	-0.061	0.46
Cominella glandiformis	**0.433**	**< 0.001**	0.053	0.519
Zostera mulleri	0.135	0.1	**0.705**	**< 0.001**
Ulva Sp.	**0.2**	**0.014**	**0.206**	**0.012**
Particle Size < 63 µm	**-0.371**	**0.012**	**0.311**	**0.031**
Particle Size 63 µm	0.167	0.274	**0.479**	**< 0.001**
Particle Size 125 µm	**0.511**	**< 0.001**	**0.61**	**< 0.001**
Particle Size 250 µm	0.061	0.689	0.086	0.574
Particle Size 500 µm	0.086	0.573	0.107	0.485
Particle Size 1 mm	0.027	0.859	0.141	0.363

2.5.7 Community composition

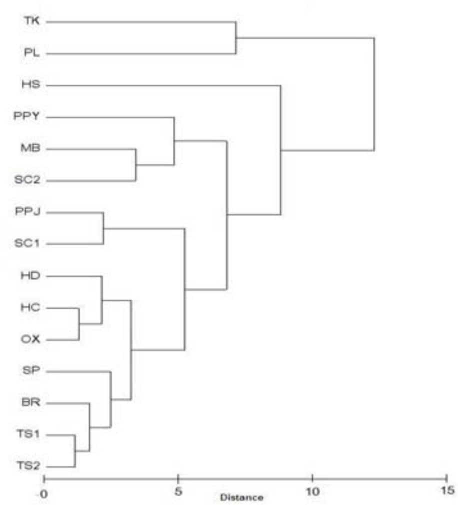
Similarities between abundance of flora and fauna, environmental variables, and community composition (flora, fauna and environmental variables) varied between sites (Fig 2.19). Composition of flora and fauna at site PPY was the most different to all other sites. The OX and PL sites had a similar composition of flora and fauna as did SC1 and SC2, PPJ and HD and BR and SP. The Tern Street sites (TS1 and TS2) did not have similar composition of flora and fauna, mainly due to TS2 being covered in *Zostera mulleri* (seagrass) (Fig 2.19A). The sites grouped for similarities of environmental variables differed from those grouped according to the similarities of the flora and fauna (Fig 2.19). Site TK and PL, which are both open bays on the Banks Peninsula, were closely linked in environmental variables (Fig 2.19B), as were sites TS1 and TS2 which were also closely linked to site BR (Fig 2.19B). These sites are all closest to the mouth of the Avon-Heathcote Estuary. Sites SC1 and SC2, both from the Saltwater Creek Estuary did not have similar environmental variables (Fig 2.19B). Overall community composition differed from abundance and environmental similarities (Fig 2.19). Site PPY was the least similar to all other sites sampled (Fig 2.19C). Both Saltwater Creek Estuary sites (SC1 and

SC2) were closely linked, although both Tern Street sites (TS1 and TS2) were not closely linked (Fig 2.19C). The sites on the Banks Peninsula (TK and PL), were not similar to one another (Fig 2.19C). Sites HC, HD and OX which were on the west-north-west side of the Avon-Heathcote Estuary were similar; however site SP which is also on the west-north-west side was not closely linked to these sites (Fig 2.19C).

A: Abundance of flora and fauna



B: Environmental Variables



C: Community composition

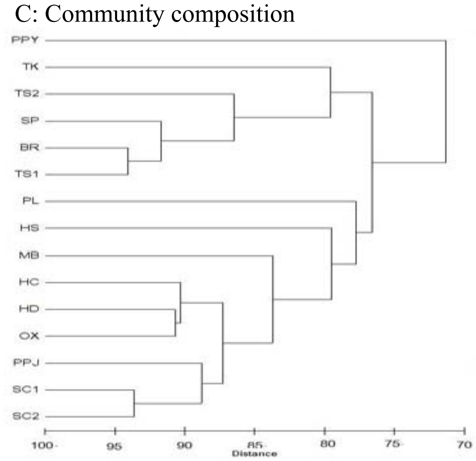


Fig 2.19 Dendograms of similarities for A: abundance of flora and fauna, B: environmental variables and C: community composition between all sites (Beachville Road (BR); McCormacks Bay (MB); Heathcote River (HC); Humphreys Drive (HD); Sandy Point (SP); Wastewater Treatment Pond Outfall (OX); Pleasant Point Yacht Club (PPY); Pleasant Point Jetty (PPJ); Heron Street (HS); Tern Street 1 (TS1); Tern Street 2 (TS2); Saltwater Creek 1 (SC1); Saltwater Creek 2 (SC2); Takamatua (TK); and Port Levy (PL)).

Results of BIOENV analysis showed overall that copper, zinc, pore water, organic matter and a sediment particle size of 125 μm were the most important environmental variables correlated with patterns of flora and fauna assemblages over all sites sampled (Table 2.6). The average Rho-value of 0.456 (Rho is the rank correlation coefficient; closer to 1 = high correlation, closer to 0 = low correlation (Clarke 2006)), showed that this relationship was a weak relationship. Copper, zinc, a sediment particle size of 63 μm and 125 μm were the most important environmental variables correlated with the average density of *Austrovenus stutchburyi* (Table 2.9). This correlation was weaker with a Rho-value of 0.370 (Table 2.9). The most important environmental variables correlated with the density of small cockles < 10 mm were cadmium, copper, pore water and a sediment particle size of 1000 μm. This was the strongest correlation with a Rho-value of 0.492, although it was not a very strong correlation (Table 2.9).

Table 2.9 Results of the BIOENV analysis. The Rho-value indicates the level of correlation of environmental variables that fit best with the patterns in the community. (Community – all flora and fauna; *Austrovenus stutchburyi* – average density of *Austrovenus stutchburyi*; < 10 mm *Austrovenus stutchburyi* – average density of small cockles).

Variable	Environmental Variables	Rho-value
Community	Copper + Zinc + % Pore Water + % Organic Matter + 125 μm particle size	0.456
Austrovenus stutchburyi	Copper + Zinc + 63 μm particle size + 125 μm particle size	0.37
< 10mm *Austrovenus stutchburyi*	Cadmium + Copper + % Pore Water + 1000 μm particle size	0.492

2.6 Discussion

Estuaries are under pressure due to industrial or domestic use and changing environmental condition. The constantly changing conditions affect the distribution of many flora and fauna as they adapt (McClusky 1981). The distribution of benthic invertebrates is thought to be limited by physico-chemical gradients which is also the case for estuarine systems throughout New Zealand (Thrush 1994) and Canterbury. The rapid growth of Christchurch City has lead to significant changes in biological, chemical, and physical characteristics of the Avon-Heathcote Estuary (Deely 1992, Wong 1992). Changes have also occurred in Saltwater Creek Estuary because of farming activities up stream, which have resulted in alteration in the distribution of the macrofauna because of differing environmental conditions. The identification of patterns of densities of marine benthic fauna is fundamental to their correct management (Cole 2000), but dividing a biological continuum is a difficult task (Teixeira 2007) because of all the variables that must be taken into account. Statistical modeling of relationships between habitat and species requires data from numerous locations and many different habitats. In the northern Gulf of Mexico a study of 319 estuaries has shown that variation of macrobenthic assemblages was explained by gradients in salinity, water depth and silt/clay content. Interestingly contaminants were unimportant explaining only residual variation in the fauna (Rakocinski 1997). In New Zealand, Thrush *et al* (2003) used data from 19 estuaries in the North Island to develop a model to predict the occurrence of certain macrofaunal species, including *Austrovenus stutchburyi* relative to sediment mud content.

2.6.1 Community structure

Community analysis in all of the sites except one showed that assemblages of flora and fauna were similar. An ordination of the environmental variables showed that sediments from the sites were different from each another. However, the sites on the Banks Peninsula were clearly differentiated from the sites in the Avon-Heathcote Estuary and the Saltwater Creek Estuary. The Pleasant Point Yacht Club site was very different to all other sites sampled as there was only one cockle and a few *Amphibola crenata* found on the sediment surface at this site.

2.6.2 Cockle density

Austrovenus stutchburyi is an important bivalve of the macrofauna in estuaries around New Zealand. *Austrovenus stutchburyi* density decreases towards the high tide mark (Wong 1992), although populations may be found at tide levels that allow *Austrovenus stutchburyi* to be submerged for only 3.5 hours, however densities here are very low (Larcombe 1971). In the present study sampling was undertaken in the mid to low tide region of the mudflats to avoid obvious effects of tidal level. Stephenson (1981) and Voller (1973) suggest that *Austrovenus stutchburyi* is limited in its distribution because of food availability, salinity, exposure time, and substrate composition. *Austrovenus stutchburyi* densities can be variable even on the same shore (Larcombe 1971). The present study found a maximum density of *Austrovenus* *stutchburyi* within the Avon-Heathcote Estuary of 937.6/m^2 at the Sandy Point site, around half the number previously recorded. This however, may not reflect a decrease in numbers because of variability in sampling sites and methods to previous studies. In the past, density studies of *Austrovenus stutchburyi,* Wong and Thomson (1992) found a maximum density of 1925/m^2 near the mouth of the Avon-Heathcote Estuary and in 1981 Stephenson found a maximum density of 2000/m^2 near the Heathcote River channel in the Avon-Heathcote Estuary.

Wong and Thomson (1992) found that *Austrovenus stutchburyi* numbers increased to the south of the estuary with a maximum at the entry of the Heathcote River however, the present study found a different pattern. Numbers of *Austrovenus stutchburyi* increased towards the mouth of the estuary with the exception at Sandy Point (SP) where high numbers were recorded (937.6/m^2). Also, there was a low density (179.2/m^2) of *Austrovenus stutchburyi* recorded at the mouth of the Heathcote River compared to previous studies of Stephenson (1981) (2000/m^2). It has been suggested that populations of *Austrovenus stutchburyi* near sewage outfalls, rivers and drains may be limited in abundance because of the deposition of fine sediment which, in turn, may clog breathing mechanisms and cause deaths (Larcombe 1971). This may explain low population densities at the mouth of the Heathcote River and extremely limited populations at the Avon River mouth. However on the Banks Peninsula, at Takamatua, a creek runs parallel to the site and the population here has the greatest density over all sites. The high density is possibly explained because there is a low percentage of mud in the sediment compared with the mouth of the Avon and Heathcote Rivers.

2.6.3 Cockle size distribution

Size frequency distributions can provide comparative information about organisms living in different environments (Wenner 1988). Length frequencies of *Austrovenus stutchburyi* populations in the Wellington Harbour, Whangateau Harbour and Te Rauone ‘North’, Otago Harbour, have a single peak (i.e. unimodal structure) at the mean adult length, which is determined by the environmental conditions surrounding the populations (Larcombe 1971). In the present study many of the populations showed bimodal distributions, and some had unimodal distributions with lengths between 0 – 35 mm. At Humphreys Drive cockles of 20 – 25 mm were missing. This may indicate either irregular recruitment or different survival (Wenner

1988). In the Canterbury area most populations had an average length between 20 mm and 25 mm. Maximum length appears to differ between sites; Beachville Road had the highest average length of 29 mm which compares to a maximum cockle length of 23.9 mm at Mangemangeroa (Auckland) (Stewart 2005). Minimum lengths appear to be similar between sites in the North and South Island. The average length of cockles found in the present study is similar with Larcombe's (1971) findings, that the mean adult length of *Austrovenus stutchburyi* reaches a maximum length near the entrance to the estuary and become smaller as the sites become more estuarine. This does not, however, explain the small average length of *Austrovenus stutchburyi* of 14.9 mm at the Tern Street two site, near the estuary mouth. Seaward of this site at the Tern Street one site, (about 100 metres further toward the estuary mouth), the average length was 10 mm greater. The sea grass (*Zostera muelleri*) may affect cockle populations. It may act as a refuge from predators and provide protection against harsh conditions for juvenile *Austrovenus stutchburyi*. Therefore the juveniles may seek protection there, causing a lower average length or better survival of recruits. Another possible reason for the small average length may be explained by a limitation in the suspension feeding mechanism of *Austrovenus stutchburyi*. The sea grass may be an obstacle in obtaining food, therefore decreasing growth rates, causing a small average length. Cockles would however, need to be aged to determine this.

2.6.4 Bivalve/sediment interactions

Relationships between sediment type and benthic organisms have long been recognized (Probert 1984) and this relationship is extremely complex (Gray 1974). Bivalves are often restricted in their distribution because of the composition of the substrate which varies within the estuary and between estuaries.

There has been a long history of attempting to relate macrofaunal distribution and abundance to sediment particle size, which has shown that differences in macrofaunal assemblages in sand and mud habitats are readily apparent (Thrush 2003). The variation in sediment characteristics is used widely to explain the differences in the distribution and abundances of soft sediment fauna (Matthews 2006). Species richness is often affected by differences in sediment particle size; areas with high percentages of silt/clay have fewer species in low numbers compared with areas of high percentages of sand (Yates 1993).

Austrovenus stutchburyi are more likely to be found in sediments with a low mud content, however still have a 75% chance of occurring in 80% mud (Thrush 2003). In the present study the sediment distribution within Canterbury estuaries differs from estuary to estuary, but there was no distinct pattern with geographical location. Sites near the Avon River had a high percentage of silts and clay, as did McCormacks Bay and the Saltwater Creek two site. In contrast, sites near the Heathcote River did not show high percentages of silt and clay, even though the river has the potential to deposit them. Takamatua Bay and Port Levy had a high percentage of larger sediment particles and very little silt and clay fraction. Fine sand dominated sites near the mouth of the estuary. This distribution is in comparison with that of Voller (1973) who found that there was a decrease in particle size from the mouth of the estuary to the head where conditions are the muddiest, except near the Avon River where sediments are sandy. The present study found that there was a more random distribution of particle size throughout the estuary.

2.6.5 Effects of substrate of density patterns of cockles

The substrate composition is determined by many factors including: currents, waves, biological action, runoff from land of fine sediments and alteration by fauna and flora (Probert 1984). Larcombe (1971) surveyed a range of estuaries in New Zealand and found that the only place where substrate was to have an effect on the growth of *Austrovenus stutchburyi* was in the Avon-Heathcote estuary. This was because the composition of the substrate is modified by pollutants that create fine, soft anaerobic mud. Beds of *Austrovenus stutchburyi* have been killed in the past near the mouth of the Heathcote River because of alteration of sediment (Larcombe 1971). Stephenson (1981) hypothesized that sediment composition along with exposure time were the two most important characteristics in limiting distribution of *Austrovenus stutchburyi*.

Cockles are found across a range of sediment sizes, a typical characteristic of a hardy estuarine species (Stewart 2005), and they may survive in a silt/clay fraction of 10.6 – 84.6 % (Voller 1973). Larcombe (1971) concluded that there was no spatial correlation with sediment particle size within the range of sediment particles examined in the Whangateau Harbour. The present study found that there were significant positive correlations between densities of cockles, the density of cockles <10 mm and particles of 125 µm. In the Auckland region Stewart (2005) also found this correlation, however she did not find a significant negative correlation between average cockle density and a particle size of <63 µm or a positive correlation between the density of small cockles and a particle size of < 63 µm. The negative correlation found in the present study allows us to conclude that average *Austrovenus stutchburyi* density is lower in areas where there are smaller particles. This finding is in agreement with Jones and Marsden (2005) who suggest that the density of *Austrovenus stutchburyi* decreases as the sediments become muddier, however no studies have found a positive correlation between small cockles <10 mm and mud (<63 µm).

2.6.6 Effects of contaminants

Larcombe (1971) showed that substrate composition within the Avon-Heathcote Estuary varies within the estuary and attributed this to pollutants from the release of sewage and industrial effluents into the estuary (Larcombe 1971). Voller (1973) found that there was a decrease in organic matter content from the mouth of the Avon-Heathcote Estuary to the head. In the present study, as in Knox and Kilner (1973), there was a significant positive correlation between organic matter in the sediment and total recoverable nitrogen and phosphorus in the sediment. A large amount of nutrients from the land, rivers, and effluent discharge can be retained within the estuary and are not lost out to sea (Knox 1973). The McCormack's Bay site showed high levels of sediment organic content and high levels of nitrogen compared to the other site. Murphy (2006) also found high percentages of sediment organic matter in McCormacks Bay compared to other sampling sites in the Avon-Heathcote Estuary. This may be because of the small water flow in an out of the bay caused by the restriction of the causeway. A high percentage of organic matter may act as a fertilizer to some organisms but can also produce by-products such as H_2S, which may be toxic to some organisms (Larcombe 1971). This could explain the low density of *Austrovenus stutchburyi* in McCormacks Bay. Knox and Kilner (1973) found organic matter was highest at the mouths of the two rivers and lowest at the estuary mouth near Shag Rock however, Murphy (2006) found it was highest at the mouth of the Avon River and in McCormack's Bay. Sites with the lowest organic matter levels were along the spit on the east side of the estuary; however a Tern Street site had elevated organic matter levels compared to the other spit locations. In the present study organic matter content was greatest in McCormacks Bay, and at the sites near the Avon River. Organic content was lower at the mouth of the Heathcote River, but shows high levels towards the mouth of the estuary at the two Tern Street sites which is contradictory to Knox and Kilner's (1973) findings, but similar to Murphy's (2006) findings.

On Banks Peninsula beaches, Takamatua Bay showed extremely high levels of organic content, total recoverable nitrogen, and total recoverable phosphorus compared to all other sites sampled. This was similar to that of Bolton's (2005) findings in Takamatua Bay, who found that organic matter was high, total nitrogen was 1400 mg/kg and total phosphorus was around 500 mg/kg. Takamatua Bay also showed the greatest density of *Austrovenus stutchburyi* in the present study. There was no significant correlation found between the density of *Austrovenus stutchburyi* and organic matter or nitrogen; therefore total nitrogen and percentage of organic matter in the sediment does not pre-determine the density of cockles present. However it was found that density of cockles and total recoverable phosphorus in the sediment were significantly correlated.

2.6.7 Environmental interactions

A positive correlation between a sediment particle size of < 63 μm and percentage of organic matter in the sediment occurred, however there was a negative correlation between organic matter and a sediment particle size of 125 μm. Organic matter content is related to the percent silt-clay distribution; therefore a high silt-clay fraction causes a high organic content. Also distribution patterns of nitrogen and total phosphorus correspond closely with the distribution patterns of organic matter and sediment particle size (Knox 1973), which was also true in this study.

The mixing of fresh and marine water leads to accumulation of fine suspended sediments and these sediments act as a trap for trace metals in estuaries all around the world (Bonnerie 1994, Fernández Caliani 1997). Stewart (2005) found that zinc was by far the most dominant trace metal in estuaries in the Auckland region, and cadmium was found in extremely low concentrations throughout all estuaries, which was also found in the present study. A comparison of trace metal levels from a 1988 study and the present study shows that levels of trace metals have decreased (Robb 1988). Copper levels from sites outside the waste water treatment pond outlets were 24.6 mg/kg and zinc levels were 135.2 mg/kg in 1988, however in the present study, those levels have dropped to 7.66 mg/kg and 71.7 mg/kg of copper and zinc respectively. In 1988 sampling areas at the mouth of the Avon River had 10.4 mg/kg of copper and zinc levels of 73.0 mg/kg and in 2007 in the present study levels were generally similar with 9.26 mg/kg of copper and 68.1 mg/kg of zinc. Levels of copper have dropped at the Heathcote River sampling site however; there has been a slight increase in the zinc concentration. These differences in trace metal levels are possibly due to a difference in analytical techniques as Robb (1988) used a Perkin Elmer 3110 flame atomic absorption spectrometer, after extraction with a mixture of 4% nitric acid and 2.5% perchloric acid for two hours at 90^{o}C, which was different to the methods used in the present study (see methods). Nipper *et al* (1997) found 16.6 mg/kg of copper and 89.0 mg/kg of zinc at the mouth of the Avon River which was higher than the Christchurch Drainage Board study in 1988 and also higher than the present study. The same analytical technique was used in 1997 as in 1988 (Nipper 1997). At the mouth of the Heathcote River in 1997 Nipper *et al* (1997) found 10 mg/kg and 90.2 mg/kg of copper and zinc respectively. Levels of both copper and zinc have dropped in the present study compared to the 1997 levels.

Phillips and Rainbow (1994) suggest that concentrations of trace metals are linked with sediment particle size, with finer particles showing higher concentrations of trace metals (Phillips 1994). The present study confirmed this finding with a positive correlation between sediment < 63 µm and levels of zinc and copper. However, there was a negative correlation between sediment particles of 125 µm and copper and zinc. This is similar to Milne (1998), who found that as the percentage of smaller sediment particles increased the greater the concentration of copper and zinc in the sediment (Milne 1998).

2.6.8 Environment/Cockle interaction

Natural events can alter estuarine habitats by putting populations and communities within these ecosystems at risk and changing their structure (Ferraro 2007). Ecological heterogeneity is a valuable characteristic of a habitat. Factors that drive heterogeneity in tidal flat assemblages may affect recruitment, function and resilience of assemblages (Winberg 2007). Within the sites that have been sampled in this study there were areas that had a higher percentage of juvenile cockles. The present study showed that both the average density of cockles and the density of small cockles <10 mm was related to environmental variables and fauna and flora on the surface of the intertidal flats. Only one environmental variable was positively correlated with the density of small cockles <10 mm (fine sand - 125 µm), however copper, zinc and silts and clay (< 63 µm) were negatively correlated with the density of small cockles < 10 mm. There were also positive correlations between the abundance of *Diloma subrostrata, Cominella glandiformis* and small cockles < 10 mm and between the percentage cover of *Zostera muelleri, Ulva* sp. and small cockles < 10 mm.

2.6.9 Conclusions

It is known that bivalve populations have great variability which has resulted from events, such as recruitment early in life (Cole 2000). It has been hypothesized that for *Austrovenus stutchburyi* the period of exposure and the sediment characteristics are the two most important factors limiting their distribution (Stephenson 1981). Stewart (2005) found that populations of *Austrovenus stutchburyi* do not have high continuous recruitment but low continuous recruitment or trickle recruitment, which may explain low numbers of juvenile cockles at sites compared to the adult population. A unimodal size frequency distribution suggests that recruitment occurs each year into these populations although it may be sporadic. Another explanation for low juvenile density may be because of the negative correlation between juvenile cockles and high levels of zinc and copper. Suitable habitats with low levels of copper and zinc may be hard for juveniles to find and therefore they settle in unsuitable habitat which may cause higher mortalities.

These correlations allow us to determine the most appropriate sites for potential high juvenile abundance and may help to determine possible sites for transfer of populations for management reasons.

Chapter 3: Laboratory Studies

3.1 Introduction

Coastal development, industry and human population increases have led to a significant effect on the environment from the release of pollutants including trace metals into the marine environment (Meyer - Reil 2000, Rosales - Hoz 2003). Bivalves living in estuaries are commonly exposed to trace metals and these are known to affect growth and reproduction (Marsden 1995). Contaminants can potentially affect the health and behaviour of individual organism's, communities and the whole ecosystem (Mills 1999). Sediments are known major reservoirs for toxic chemicals in the marine environment (Mearns 1986). Incorporation of contaminants into sediments has been linked to adverse effects on estuarine organisms (Schlekat 1992); especially to benthic species (Malloy 2007). This does not necessarily result in mortality and sub-lethal effects may modify biological organization (Bilyard 1987).

The toxicity of a trace metal depends on how much enters the organism and its ability to regulate it (Depledge 1990, Amiard 2007). Trace metals are not readily degradable and therefore may persist in ecosystems for long periods of time (Gagnaire 2004). Fortunately in New Zealand trace metal levels in estuaries are lower than many industrialized and populated centers overseas (Mills 1999). Infaunal bivalves are likely to be exposed to elevated concentrations of metals and biological, chemical and physical processes tend to concentrate trace metals within bivalve tissues (Gagnaire 2004).

Copper is a common contaminant found in the marine environment worldwide (Trannum 2004, Cartwright 2006), occurring in sewage sludge, antifouling paints, alloys and algicides (Kennish 1992). It occurs naturally in a wide variety of forms and is very common in industrial operations, pesticides and algicides (Othman 2002). Copper binds with the sediment and is incorporated into estuaries readily and therefore, has a large potential to influence populations and assemblages (Morrisey 1996). To a wide range of marine life, copper is the most toxic trace metal after mercury and silver (Trannum 2004). Molluscs are particularly sensitive with effects such as inhibition of oxygen consumption, mortality and behavioural changes (Roper 1995). In the Auckland region, the median concentration of copper in marine sediments is about 50 mg Cu kg^{-1} (dry weight) (Roper 1995). Compared to New Zealand copper levels in contaminated sites in other parts of the world range from 50 μg g^{-1} in sediment cores in the rias of NW Spain (Rubio 2001), and 30 to 130 μg Cu g^{-1} in San Francisco Bay, USA (Chapman 1987).

Zinc contamination is mainly associated with sewage sludge dumping or secondary industries such as the china clay industry. High zinc concentrations may occur naturally and these can be worsened by historic mining activities (Conradi 1999). Concentrations of zinc in sediments near highly populated areas in the Sydney region reach up to 1000 μg g^{-1} where as in less developed areas concentrations only reach 100 μg g^{-1} (Lindegarth 2002). In the Auckland region the median zinc concentration in the sediment is around 100 mg Zn kg^{-1} (dry weight) (Roper 1995). Zinc toxicity is particularly variable depending on the uptake rate and the animal's ability to detoxify it (Bryan 1984). Zinc and cadmium are more bioavailable to invertebrates at lower salinities (Furness 1990, Hall 1995), and this has important implications in the estuarine environment.

Cadmium is one of the most disruptive trace metals in estuaries (Kennish 1992), and in the ocean occurs at concentrations of 0.02 – 0.12 μg/L (Bryan 1992). Cadmium is released into the environment via a variety of sources including, fumes, dust from lead and zinc mining, iron and steel industries, galvanized coatings containing cadmium, wear of car tyres and sewage sludge (Kennish 1992). Cadmium remobilized from the sediment is more likely to be an important source for organisms rather than the sediment particles (Bryan 1992). A delay in burrowing occurs in the short-neck clam *Ruditapes philippinarum* when exposed to sediments with a high cadmium concentration (Shin 2002). Copper and zinc are essential trace metals that are required in the body to meet metabolic needs, whilst cadmium is a non-essential trace metal and has no required minimum concentration in the body (Rainbow 2002). Benthic invertebrates have the ability to take up trace metals whether essential or not, and they potentially cause toxic effects (Rainbow 2007).

Concentrations and bioavailability of trace metals are readily influenced by several processes once they have entered the estuarine environment (Morrisey 1996). Understanding ecological effects of contaminants on ecosystem processes is essential to reduce the impact of humans on the marine environment (Morrisey 1996). Understanding how benthic species respond to pollution such as trace metals is important in the understanding of how populations cope with their environment. It is important to understand if long term sublethal exposure to contaminants threaten the survival, growth or reproduction of a species (Conradi 1999).

Bivalves are filter feeders and have high water-tissue contact for the uptake of trace metals because of this mechanism of feeding (Kennish 1992). *Austrovenus stutchburyi* is a sedentary organism and is exposed to high fluctuations in environmental conditions and trace metal pollution, with little possibility of juveniles escaping. Many studies have shown that the early developmental stages of invertebrates such as bivalves and sea urchins are more responsive to toxic chemicals than their adult counterparts (Bellas 2001, Beiras 2004). Abnormalities occur in the embryonic and larval stages of tunicates such as in *Ciona intestinalis*, and these have many implications for recruitment and settlement (Furness 1990), for example, copper and mercury inhibit *Ciona intestinalis* larvae attaching to the substrate (Bellas 2001).

Austrovenus stutchburyi is an important shellfish species both economically and culturally in New Zealand (De Luca - Abbott 2001). Juvenile recruitment is important for a sustainable population, and many factors may disrupt their recruitment and settlement. Laboratory experiments permit the identification of effects of contaminants under controlled conditions (Morrisey 1996), and will help determine the tolerance ranges of juvenile *Austrovenus stutchburyi* to the trace metals, copper, cadmium and zinc. The aim of these experiments is to find the tolerance ranges of juvenile *Austrovenus stutchburyi* to copper, cadmium and zinc in aqueous solution and sediments dosed with metals. There has been no previous research of this kind in New Zealand on any marine organism.

3.2 Methods

3.2.1 Collection and storage of Austrovenus stutchburyi *and sediments*

Individuals of *Austrovenus stutchburyi* of 5 – 7 mm, 7 – 10 mm and 10 – 12 mm (length) were collected from Beachville Road on the south side of the Avon-Heathcote Estuary. *Austrovenus stutchburyi* was sieved from the sand collected on a 1 mm mesh sieve, shell length measured once separated from the sand and placed in containers with cool seawater. Animals were brought back to the lab where they were acclimated in water in a 15^{o}C temperature controlled room with aerators and a 12:12 light:dark cycle. All sediment was collected from Shag Rock, Sumner and sieved through a 1 mm mesh sieve to remove any unwanted macrofauna and debris. Sediment was stored in containers at room temperature.

3.2.2 Experimental lab work: Water and sediments

Trace metal solutions and sediment preparation

Stock solutions of 1 g/L of copper, cadmium and zinc were made and stored in a 15^{o}C temperature controlled room (for equations see Appendix 1). Glass jars of 75 mm diameter, 500 mL were acid washed in 10% HCl acid and used for experiments. Aqueous trace metal concentrations were made in 25‰ seawater with the stock solutions and stored in the 15^{o}C temperature controlled room. Around 200 mL of the trace metal solutions were put into each of the glass jars.

Sediment was mixed with water to obtain a wet sand mix of 24% water per kilogram of dry sand. Trace metals were then added to the wet sand from the stock solutions. The sand was mixed continuously for five minutes with a drill and paint stirrer. The trace metal dosed sediments were left to stand overnight in a 4^{o}C temperature controlled room and the following day were remixed for a further five minutes. About 20 mm of sand was placed in the bottom of each acid washed jar and 200 mL of 25‰ seawater overlain the sediment. The water was aerated and stored in a 15^{o}C temperature controlled room overnight. After 24 hours the water was drained off and replaced to stop any immediate effects of leaching.

Experimental design – Aqueous experiments

Varying concentrations of prepared copper (0, 0.1, 0.05 and 0.01 mg/L), zinc (0, 0.2, 0.4 and 0.6 mg/L) and cadmium (0, 0.1, 0.05 and 0.01 mg/L) solutions were used. There were three replicates each of five individual cockles for each size class and each concentration of metal solution. The control with no added trace metals only had two replicates. A second copper experiment of three replicates was undertaken four months after (May 2007) the original experiments (January 2007). The water was aerated and experiments were undertaken in a 15^{o}C temperature controlled room in a 12:12 light:dark cycle as used by Conradi and Depledge (1999). The cockles were not fed over the 10 days.

Each day for the next 10 days, 100 mL (approx. half of the initial vouume) of the water was replaced with new solution of the same concentration using a syringe, so as not to disturb the cockles. After 10 days, cockles were removed from the jars and placed on clean sediment in uncontaminated 25‰ seawater. Those cockles that did not move, bury or extend their siphons or foot within one hour were recorded as dead.

Experimental design – Sediment experiments

Sediments were prepared using different concentrations of copper (0, 5, 10, 25 and 50 mg Cu/kg), zinc (0, 20, 40, 80 and 160 mg Zn/kg) or cadmium (0, 1.8, 5.6, 18 and 36 mg Cd/kg) using a drill and paint stirrer as in Roper *et al* (1995). Five individual *Austrovenus stutchburyi* were placed in each jar using three replicates of each concentration and size class (5 – 7 mm, 7 – 10 mm, 10 – 12 mm). Cockles were placed on the sediment surface and the number that did not bury within two hours was recorded. Cockles were left for 96 hours and 100 mL water was changed every 24 hours with a syringe to minimise the effects of the leaching of the trace metals into the overlying water. The water was gently and constantly aerated. After 96 hours, cockles were sieved out of the sediment and survival recorded. Dead cockles were recognised as individuals with no siphon extension or foot extension within one hour when placed in clean seawater or those with shells that had gaped open.

3.2.5 Statistical analysis

Siphon extension data were tested with repeated measures ANOVA. This type of analysis has the advantage of using siphon extension data through time and testing for size class and concentration effects. Correlation among sampling days may lead to problems of compound symmetry and sphericity and assumptions may become violated. Therefore, within subjects factors (including time) were tested with multivariate analysis of variance using Wilks' Lambda (Pirker 2002). Data were Arc Sin transformed, as it was percentage data, to meet the assumptions of ANOVA. Tukey HSD tests were used to compare responses on different days, between different concentrations and for different length *Austrovenus stutchburyi*.

3.3 Results

3.3.1 Aqueous experiments

Behavioural responses to aqueous copper (Trial 1)

Siphon extension of *Austrovenus stutchburyi* ranged from 0 to 90% over the period of nine days (Fig 3.1). The mean percentage siphon extension of *Austrovenus stutchburyi* decreased over the course of nine days (Fig 3.2). Those animals that were 5 – 7 mm and 7 – 10 mm had a generally constant siphon extension for the first two days and then decreased over the subsequent days; however *Austrovenus stutchburyi* of 10 – 12 mm had a steady decrease in siphon extension from day one (Fig 3.1). *Austrovenus stutchburyi* that were 7 – 10 mm had a greater reduction between days two and three compared to *Austrovenus stutchburyi* of 5 – 7 mm and 10 – 12 mm (Fig 3.1).

There was no significant effect of either the length of cockles or copper concentration on the siphon extension of *Austrovenus stutchburyi* as indicated by a univariate analysis of variance (Table 3.1). However, the effect of copper concentration of the siphon extension of *Austrovenus stutchburyi* almost had a significant effect (p = 0.053) (Table 3.1). Over nine days there was a significant effect of time on the percentage siphon extension of *Austrovenus stutchburyi* as indicated by a multivariate analysis of variance (Table 3.1). There was no significant interaction of time with either length of *Austrovenus stutchburyi* or copper concentration (Table 3.1).

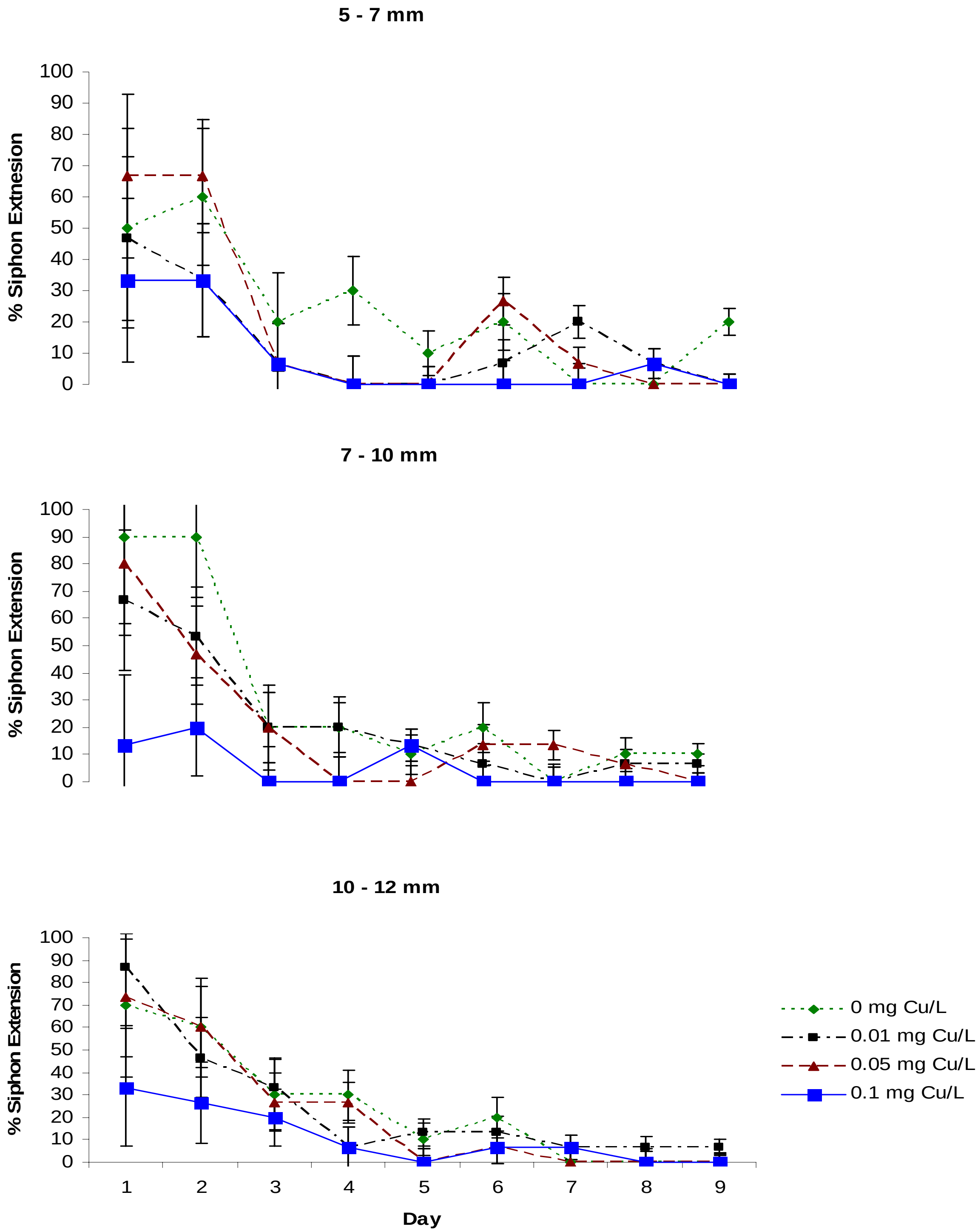


Figure 3.1 Percentage siphon extension (± 1 SE) of Austrovenus stutchburyi per day over 9 days in different concentrations of copper for different sizes (5 – 7 mm, 7 – 10 mm, and 10 – 12 mm).

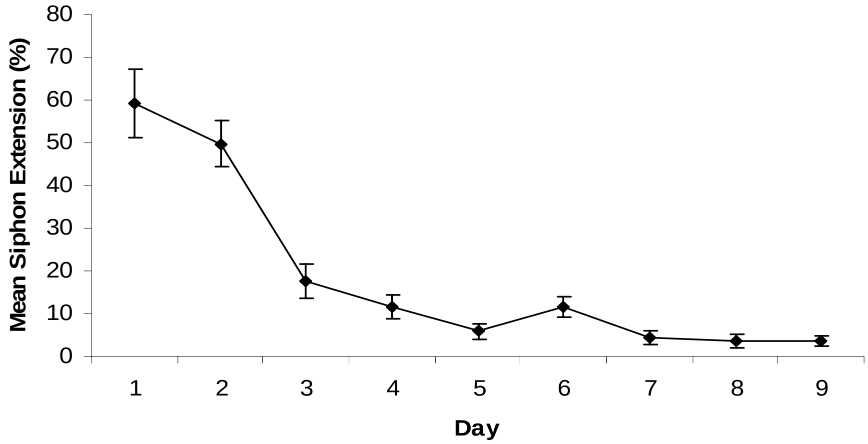


Figure 3.2 Effect of time on mean percentage siphon extension (± 1 SE) per day of *Austrovenus stutchburyi* (over all sizes and concentrations of copper combined).

Table 3.1 Analysis of the effects of copper concentration on siphon extension in three lengths of *Austrovenus stutchburyi.* (A) Repeated measures univariate analysis of variance for effects of different concentrations of copper on three length classes of *Austrovenus stutchburyi*, and (B) repeated measures multivariate analysis of variance of siphon extension within subjects through time, and interactions of length and different concentrations of copper. Values in bold indicate significance where $p < 0.05$.

A

Source of Variation	*df*	MS	*F*	*p*
Size	2	0.04	0.18	0.84
Concentration	3	0.69	3.02	0.05
Size*Concentration	6	0.06	0.28	0.94
Error	21	0.23		

B

Source of Variation	*df*	Wilks	*F*	*p*
Time	8	0.234	5.715	**0.002**
Time*Size	16	0.569	0.57	0.88
Time*Concentration	24	0.171	1.437	0.15
Time*Size*Concentration	48	0.108	0.871	0.693

Behavioural responses to aqueous copper (Trial 2)

A second trial of copper was undertaken four months later in mid May 2007 (as in methods). Siphon extension ranged between 0 to 60 % over the nine days (Fig 3.3). There was a general decrease in percentage siphon extension over nine days of 7 – 10 mm *Austrovenus stutchburyi,* although 0.05 mg Cu/L increased in siphon extension on days five and six (Fig 3.3). *Austrovenus stutchburyi* of 5 – 7 mm and 10 – 12 mm had a more inconsistent pattern of siphon extension. In general the percentage of siphon extension was less than in *Austrovenus stutchburyi* of 7 – 10 mm (Fig 3.3). There was a significant effect of both length and copper concentration on the siphon extension of *Austrovenus stutchburyi* over nine days as indicated by univariate analysis of variance (Table 3.2). There was a significant effect of time (Fig 3.4) and a significant interaction between time and length and between time and copper concentration on the siphon extension of *Austrovenus stutchburyi*, however there was no time, size, concentration interaction effect as indicated by multivariate analysis of variance (Table 3.2).

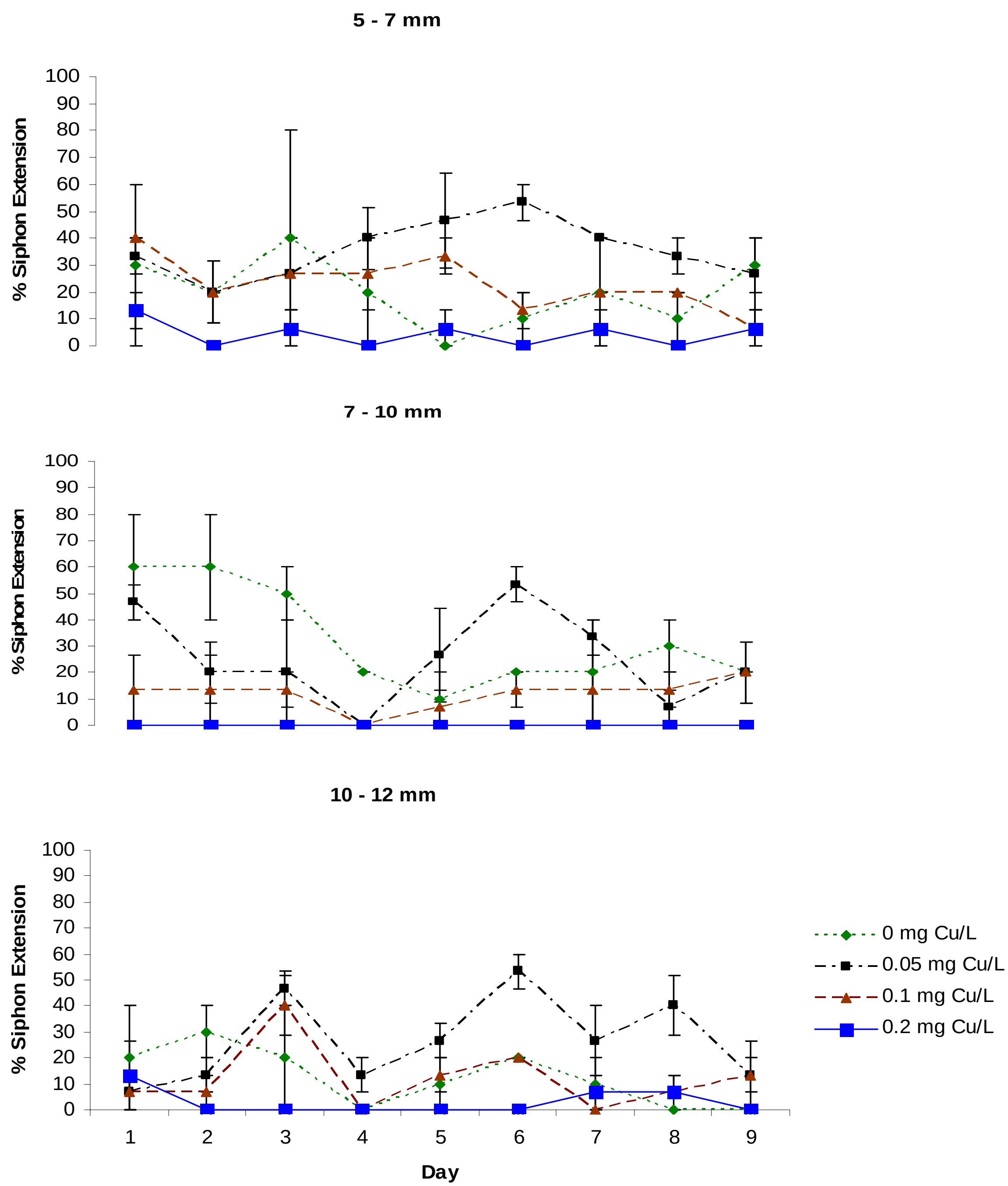


Figure 3.3 Percentage siphon extension (± 1 SE) of *Austrovenus stutchburyi* per day over 9 days in different concentrations of copper for different sizes (5 – 7 mm, 7 – 10 mm, and 10 – 12 mm).

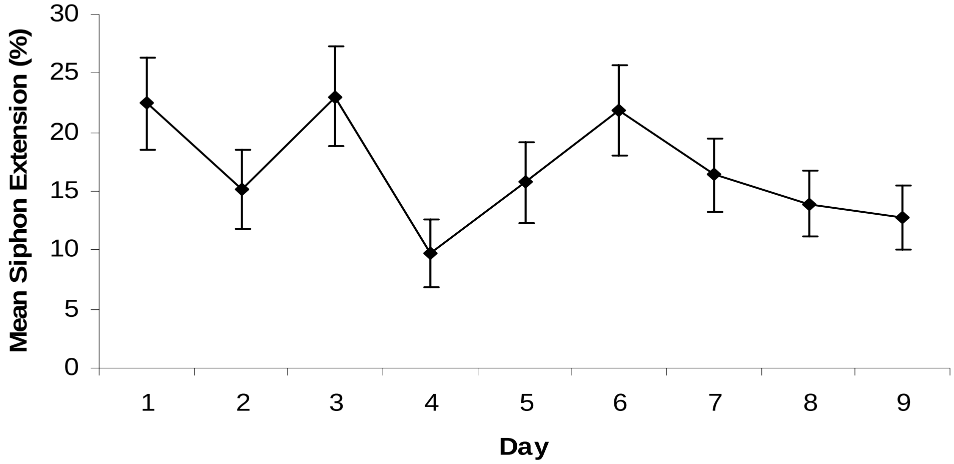


Figure 3.4 Effect of time on mean percentage siphon extension (± 1 SE) per day of *Austrovenus stutchburyi* (for all sizes and concentrations combined).

Table 3.2 Analysis of the effects of copper concentration on siphon extension in three lengths of *Austrovenus stutchburyi.* (A) Repeated measures univariate analysis of variance for effects of different concentrations of copper on three length classes of *Austrovenus stutchburyi*, and (B) repeated measures multivariate analysis of variance of siphon extension within subjects through time, and interactions of length and different concentrations of copper. Values in bold indicate significance where $p < 0.05$.

A

Source of Variation	*df*	MS	*F*	*p*
Size	2	0.297	4.919	**0.018**
Concentration	3	0.812	13.435	**< 0.001**
Size*Concentration	6	0.086	1.406	0.093
Error	21	0.06		

B

Source of Variation	*df*	Wilks	*F*	*p*
Time	8	0.299	4.113	**0.01**
Time*Size	16	0.137	2.976	0.006
Time*Concentration	24	0.029	4.07	**<0.001**
Time*Size*Concentration	48	0.04	1.406	0.093

Behavioural responses to aqueous zinc

The percentage of siphon extension over nine days ranged from 0 to 70% (Fig 3.5). The mean percentage siphon extension was higher in day two and day three compared to subsequent days (Fig 3.6). There was no significant effect of length or zinc concentration on the siphon extension of *Austrovenus stutchburyi* indicated by univariate analysis of variance (Table 3.3).

There was a significant effect of time on average percentage siphon extension of *Austrovenus stutchburyi* (Table 3.3). Siphon extension on day two and day three showed significant differences in *Austrovenus stutchburyi* compared to all other days (Fig 3.6). There was a significant interaction effect between time and length of *Austrovenus stutchburyi* (Table 3.3, Fig 3.5). There was a significant interaction between time and concentration of zinc in the water (Table 3.3). There was no significant interaction between time, length of *Austrovenus stutchburyi*, and the concentration of zinc in the water.

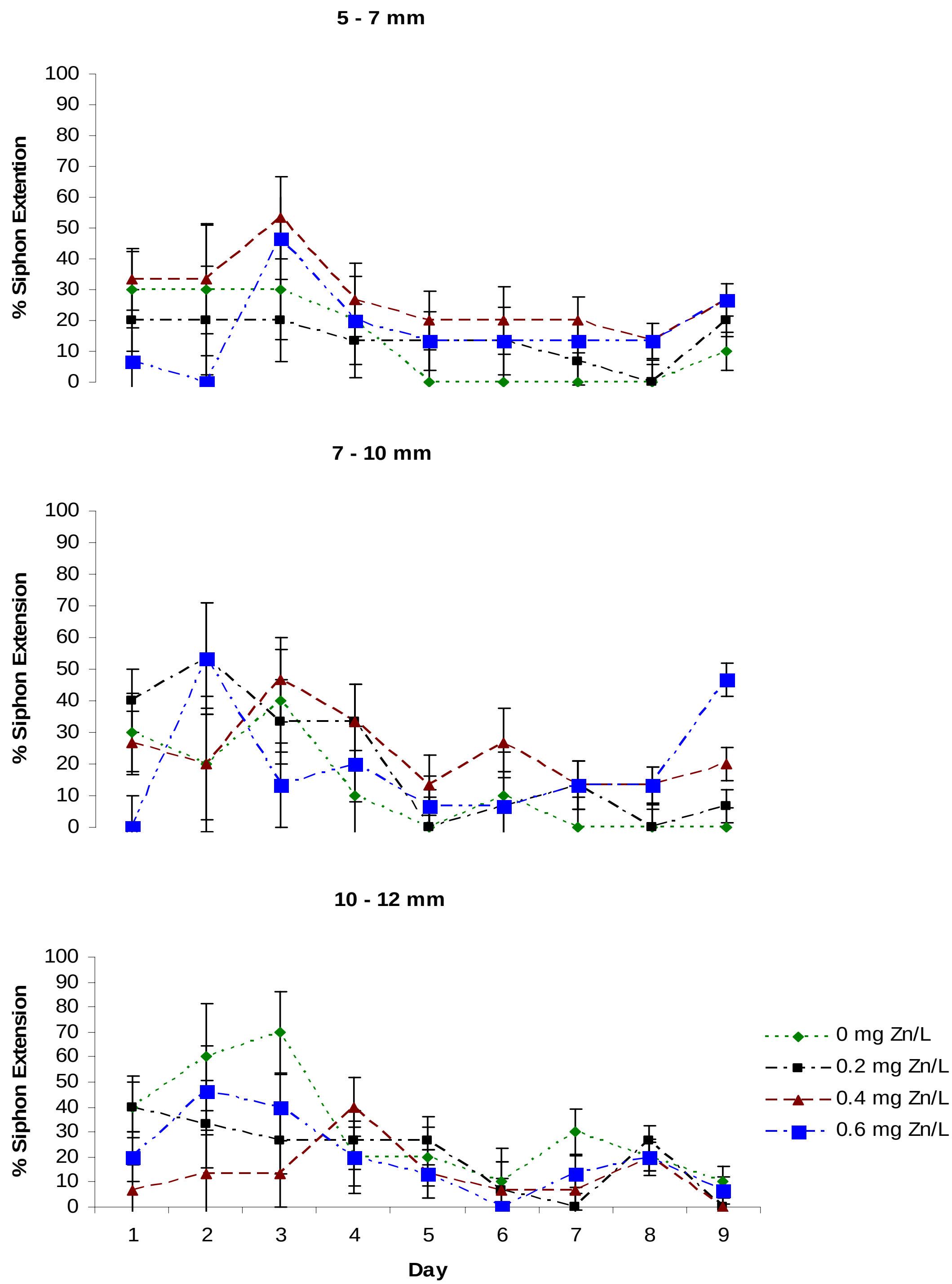


Figure 3.5 Percentage siphon extension (± 1 SE) of Austrovenus stutchburyi each day over 9 days in different concentrations of zinc for different sizes (5 – 7 mm, 7 – 10 mm, and 10 – 12 mm).

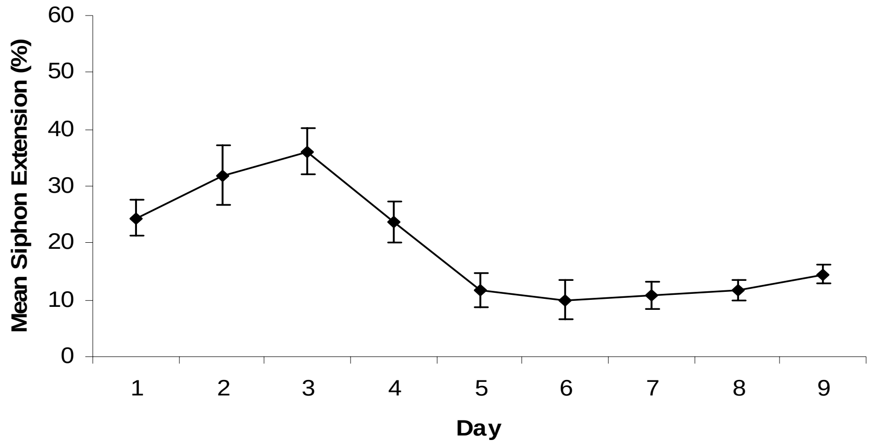


Figure 3.6 Effect of time on mean percentage siphon extension (± 1 SE) per day of *Austrovenus stutchburyi* (over all sizes and concentrations of zinc combined).

Table 3.3 Analysis of the effects of zinc concentration on siphon extension in three length classes of *Austrovenus stutchburyi.* (A) Repeated measures univariate analysis of variance for effects of different concentrations of zinc on three length classes of *Austrovenus stutchburyi*, and (B) repeated measures multivariate analysis of variance of siphon extension within subjects through time, and interactions of length and different concentrations of zinc. Values in bold indicate significance where $p < 0.05$.

A

Source of Variation	*df*	MS	*F*	*p*
Size	2	0.009	0.125	0.883
Concentration	3	0.027	0.367	0.778
Size*Concentration	6	0.158	2.163	0.091
Error	20	0.073		

B

Source of Variation	*df*	Wilks	*F*	*p*
Time	8	0.151	9.111	<**0.001**
Time*Size	16	0.15	2.573	**0.016**
Time*Concentration	24	0.091	2.057	**0.023**
Time*Size*Concentration	48	0.035	1.391	0.104

Behavioural responses to aqueous cadmium

The average percentage siphon extension of *Austrovenus stutchburyi* exposed to different concentrations of cadmium over nine days was similar, ranging from 0 to 40% (Fig 3.7). There was no significant effect of length or cadmium concentration in the water on the siphon extension of *Austrovenus stutchburyi* as indicated by univariate analysis of variance (Table 3.4). There was no significant effect of time or interaction between time, size of the cockles and concentration of cadmium in the water on the siphon extension of *Austrovenus stutchburyi* as indicated by multivariate analysis of variance (Table 3.4).

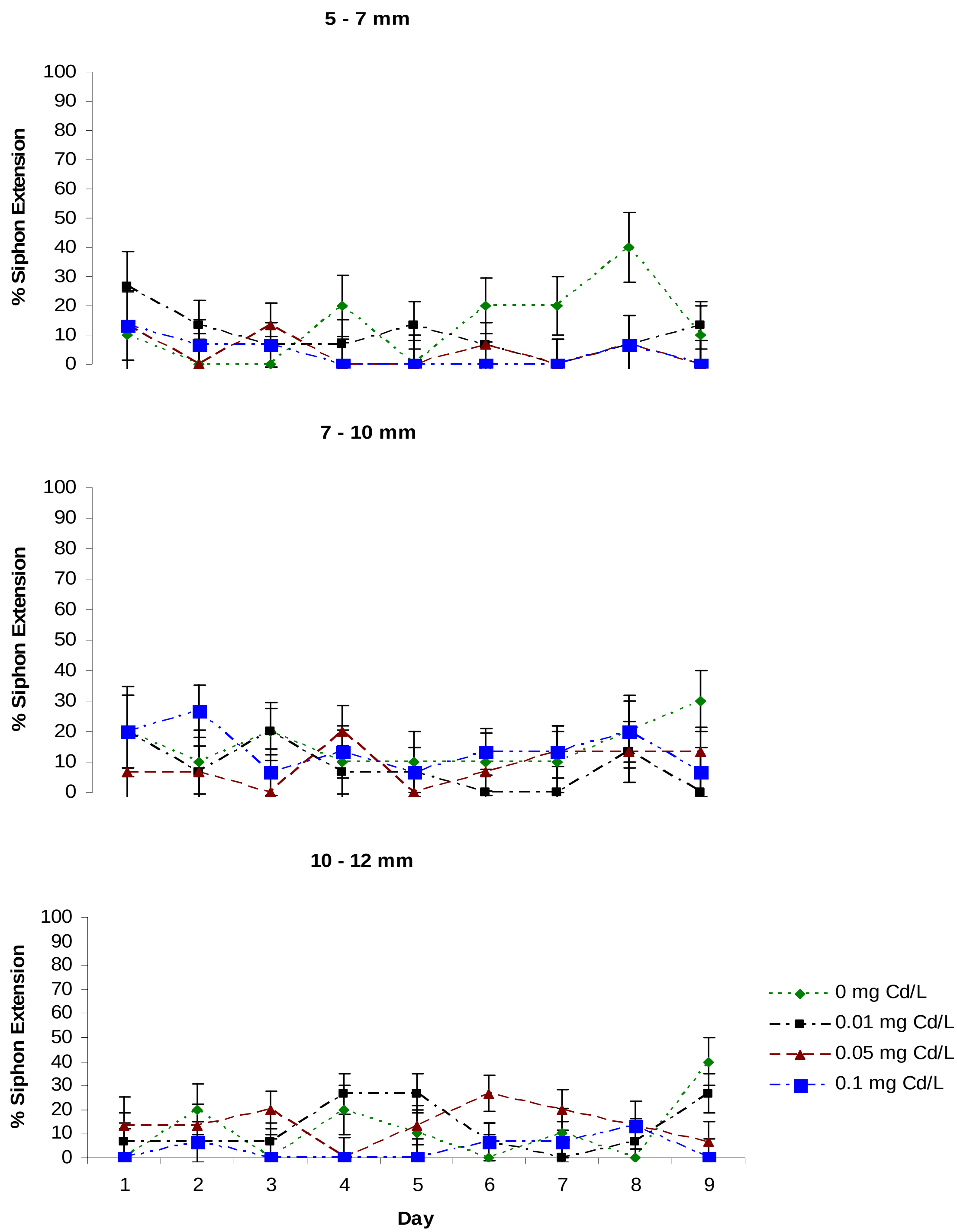


Figure 3.7 Percentage siphon extension (± 1 SE) of Austrovenus stutchburyi each day over 9 days in different concentrations of cadmium for different sizes (5 – 7 mm, 7 – 10 mm, and 10 – 12 mm).

Table 3.4 Analysis of the effects of cadmium concentration on siphon extension in three length classes of *Austrovenus stutchburyi*. (A) Repeated measures univariate analysis of variance for effects of different concentrations of cadmium on three length classes of *Austrovenus stutchburyi*, and (B) repeated measures multivariate analysis of variance of siphon extension within subjects through time, and interactions of length and different concentrations of cadmium.

A

Source of Variation	*df*	MS	*F*	*p*
Size	2	0.033	0.453	0.642
Concentration	3	0.047	0.64	0.598
Size*Concentration	6	0.051	0.698	0.654
Error	21	0.073		

B

Source of Variation	*df*	Wilks	*F*	*p*
Time	8	0.562	1.366	0.291
Time*Size	16	0.38	1.089	0.408
Time*Concentration	24	0.223	1.163	0.328
Time*Size *Concentration	48	0.051	1.265	0.18

Survival responses to aqueous copper (Trial 1)

Survival of *Austrovenus stutchburyi* was poor after 10 days exposure to all concentrations of copper (Fig 3.8). Survival ranged from 0 to 40% (Fig 3.8). Cockles of 7 – 10 mm had an overall percentage survival of 30% whilst cockles of 5 -7 mm and 10 – 12 mm had overall survival rates less than 20%. There was no significant effect of copper concentration on the survival of juvenile *Austrovenus stutchburyi* after 10 days, however there was an effect of size and a significant interaction effect between the length of cockles and copper concentration (Table 3.5). Survival of juvenile cockles in aqueous copper depended on shell length however patterns were inconsistent.

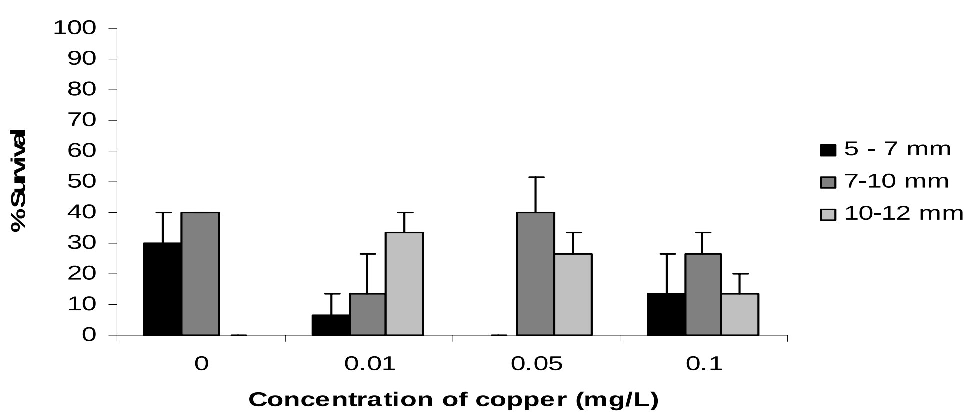


Figure 3.8 Effect of size and copper concentration in water on the percentage survival (± 1 SE) of *Austrovenus stutchburyi* after 10 days.

Table 3.5 Analysis of the effects of aqueous copper on the survival after 10 days in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.184	2	0.092	3.958	**0.035**
Concentration	0.023	3	0.008	0.326	0.807
Size*Concentration	0.416	6	0.069	2.982	0.029
Error	0.488	21	0.023		

Survival responses to aqueous copper (Trial 2)

Survival of *Austrovenus stutchburyi* was very variable after 10 days exposed to various concentrations of copper (Fig 3.9). Survival ranged from 0 to 80% for all length groups. Average survival of juvenile cockles was highest in the smallest length group (69%) and least in the largest length group (25%). There was no significant effect of concentration on the survival of *Austrovenus stutchburyi* after 10 days however there was a significant effect of cockle length (Table 3.6).

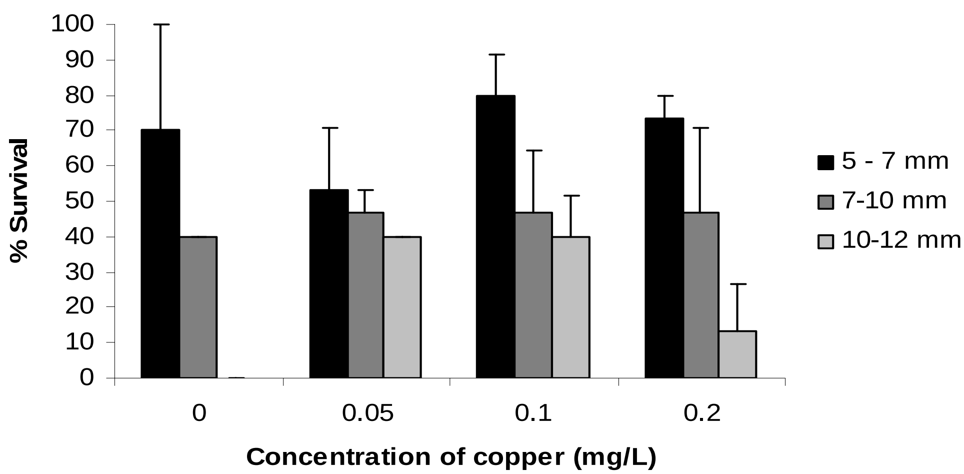


Figure 3.9 Effect of size and concentration of copper in water on the percentage survival (± 1 SE) of Austrovenus stutchburyi after 10 days.

Table 3.6 Analysis of the effects of copper concentration on the survival after 10 days in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	2.108	2	1.054	9.139	**0.001**
Concentration	0.193	3	0.064	0.557	0.649
Size*Concentration	0.508	6	0.085	0.734	0.628
Error	2.422	21	0.115		

Survival responses to aqueous zinc

Survival of juvenile *Austrovenus stutchburyi* subjected to different concentrations of zinc for 10 days was below 50% for all concentrations including the controls (Fig 3.10). The lowest mean survival rate of 6.67% occurred in 5 – 7 mm cockles in a zinc concentration of 0.6 mg Zn/L trials. The highest mean survival rate of 40% occurred in *Austrovenus stutchburyi* of 7 – 10 mm in 0.2 mg Zn/L trials (Fig 3.10). There was no significant effect of cockle length or concentration of zinc in the water on the survival of *Austrovenus stutchburyi* after 10 days (Table 3.7).

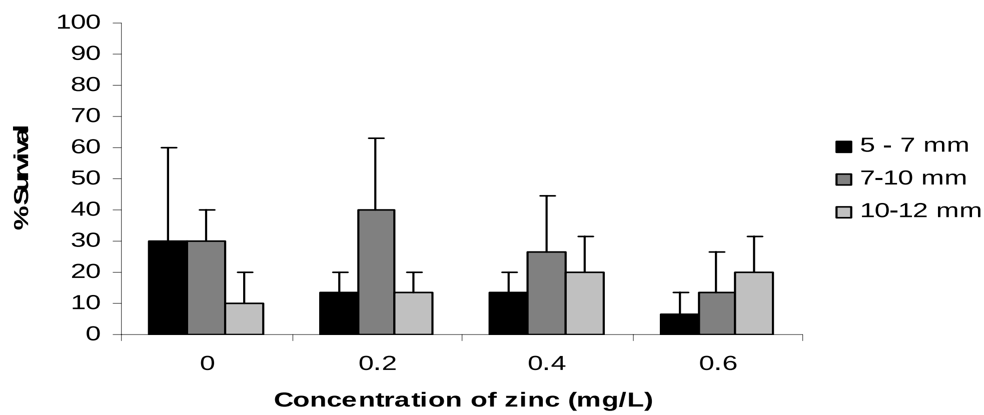


Figure 3.10 Effect of size and zinc concentration in water on the percentage survival (± 1 SE) of Austrovenus stutchburyi after 10 days**.**

Table 3.7 Analysis of the effects of zinc concentration on the survival after 10 days in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.121	2	0.06	0.984	0.39
Concentration	0.061	3	0.02	0.332	0.802
Size*Concentration	0.184	6	0.031	0.501	0.8
Error	1.288	21	0.061		

Survival responses to aqueous cadmium

Survival of *Austrovenus stutchburyi* subjected to various concentrations of cadmium for 10 days was dependent on cockle length and cadmium concentration (Fig 3.11). Overall survival of *Austrovenus stutchburyi* was quite poor. *Austrovenus stutchburyi* of 5 – 7 mm showed a definite decrease in survival as the concentration of cadmium increased (Fig 3.11). After 10 days there was no significant effect of either the length of the cockles or the concentration of cadmium on the survival of *Austrovenus stutchburyi* (Table 3.8).

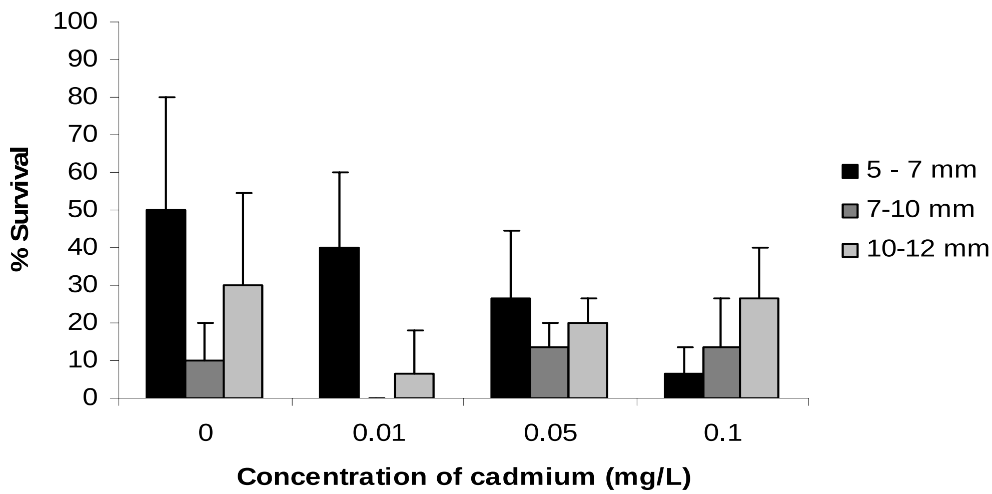


Figure 3.11 Effect of size and concentration of cadmium in water on the percentage survival (± 1 SE) of *Austrovenus stutchburyi* after 10 days.

Table 3.8 Analysis of the effects of cadmium concentration on the survival after 10 days in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.323	2	0.161	2.338	0.121
Concentration	0.122	3	0.041	0.587	0.63
Size*Concentration	0.375	6	0.063	0.906	0.509
Error	1.45	21	0.069		

3.3.2 Sediment experiments

Survival responses to copper dosed sediments

The average survival of *Austrovenus stutchburyi* after 96 hours exposure to different copper concentrations was considerably higher than survival in either zinc or cadmium (Fig 3.12). Survival rates varied between 90 to 100% for the majority of experiments; however the exception was all sizes of cockles in 25 mg Cu/kg (dry weight) which had survival rates between 69 and 80% (Fig 3.12). There was no statistical significance of the effect of cockle length or copper concentration on the survival of *Austrovenus stutchburyi* after 96 hours (Table 3.9).

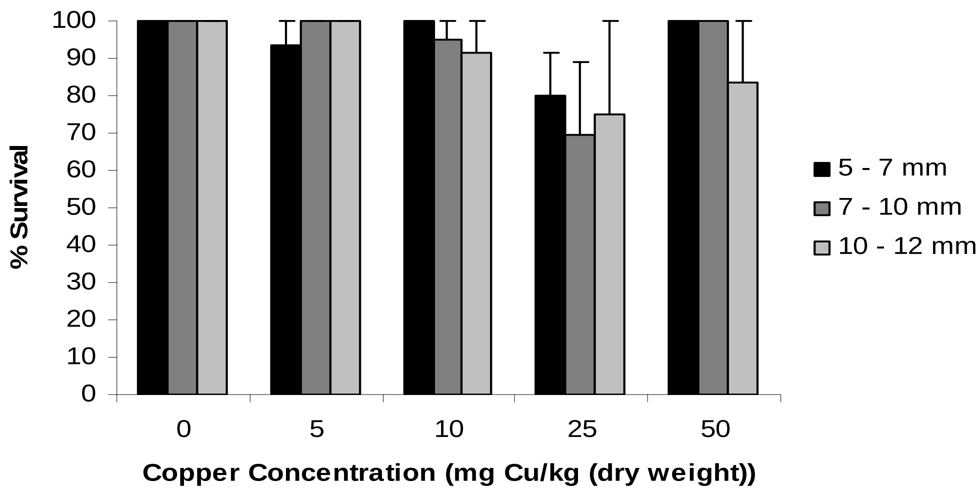


Figure 3.12 Average percentage survival (± 1 SE) of *Austrovenus stutchburyi* exposed to different concentrations of copper dosed sediments after 96 hours.

Table 3.9 Analysis of the effects of copper concentration on the survival after 96 hours in three length classes of Austrovenus stutchburyi. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.024	2	0.012	0.082	0.921
Concentration	1.502	4	0.376	2.577	0.06
Size*Concentration	0.471	8	0.059	0.404	0.908
Error	3.936	27	0.146		

Survival responses to zinc dosed sediments

The average survival of *Austrovenus stutchburyi* after 96 hours exposed to zinc dosed sediments declined with increasing zinc concentration (Fig 3.13). All controls except those with size 10 – 12 mm cockles were still alive after 96 hours (Fig 3.13). Cockles of 7 – 10 mm in the highest concentration of zinc (160 mg Zn/kg (dry weight)) had the least survival (Fig 3.13). Both size of the cockles and the zinc concentration in the sediment had a significant effect on the survival of *Austrovenus stutchburyi* indicated by univariate ANOVA (Table 3.10).

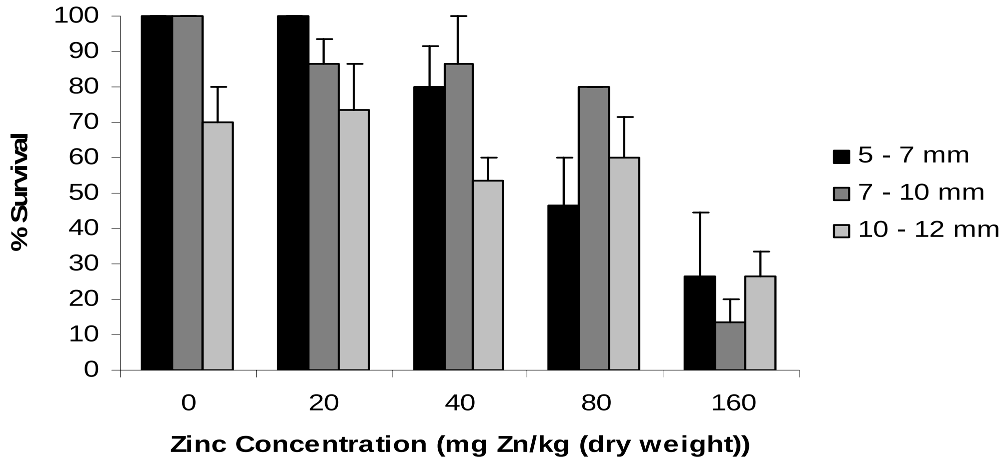


Figure 3.13 Average percentage survival (± 1 SE) of *Austrovenus stutchburyi* exposed to different concentration of zinc dosed sediments after 96 hours.

Table 3.10 Analysis of the effects of zinc concentration on the survival after 96 hours in three length classes of Austrovenus stutchburyi. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	1.134	2	0.567	6.155	**0.005**
Concentration	6.299	4	1.575	17.1	**< 0.001**
Size*Concentration	1.541	8	0.193	2.091	0.073
Error	2.487	27	0.092		

Survival responses to cadmium dosed sediments

The average survival percentage of *Austrovenus stutchburyi* after 96 hours exposed to different concentrations of cadmium dosed sediments declined as the cadmium concentration increased (Fig 3.14). All controls except cockles of 10 – 12 mm survived after 96 hours (Fig 3.14). Concentration of cadmium in the sediment had a significant effect on survival of *Austrovenus stutchburyi* after 96 hours, however there was no significant effect of cockle length or interaction between cadmium concentration and cockle length on survival (Table 3.11).

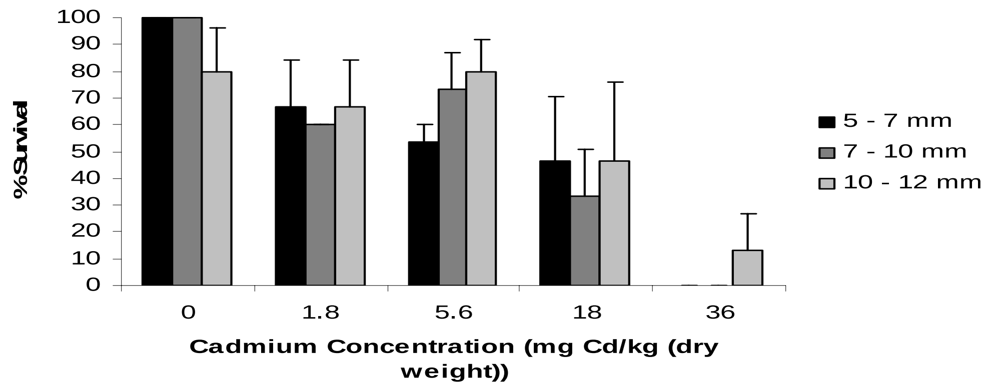


Figure 3.14 Average percentage survival (± 1 SE) of *Austrovenus stutchburyi* exposed to different concentrations of cadmium dosed sediments after 96 hours**.**

Table 3.11 Analysis of the effects of cadmium concentration on the survival after 96 hours in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.033	2	0.016	0.087	0.917
Concentration	7.528	4	1.882	9.965	< **0.001**
Size*Concentration	0.876	8	0.109	0.58	0.785
Error	5.099		0.189		

Burial responses to copper dosed sediments

The average burial rates for different lengths of *Austrovenus stutchburyi* exposed for two hours to different concentrations of copper dosed sediment ranged from 0 to 90% (Fig 3.15). As the copper concentration increased, burial success of cockles of 5 – 7 mm decreased (Fig 3.15). There was an increase in burial success of cockles of 7 – 10 mm from 0 to 5 mg Cu/kg (dry weight) and a steady burial rate between 5 and 10 mg Cu/kg (dry weight). The burial success increased to a peak at 25 mg Cu/kg (dry weight) and decreased dramatically at 50 mg Cu/kg (dry weight) (Fig 3.15). Cockles of 10 – 12 mm had no consistent burial pattern (Fig 3.15). There was a significant effect of length and copper concentration on the burial success of *Austrovenus stutchburyi* (Table 3.12). There was a significant interaction effect between length of the cockles and the concentration of copper (Table 3.12).

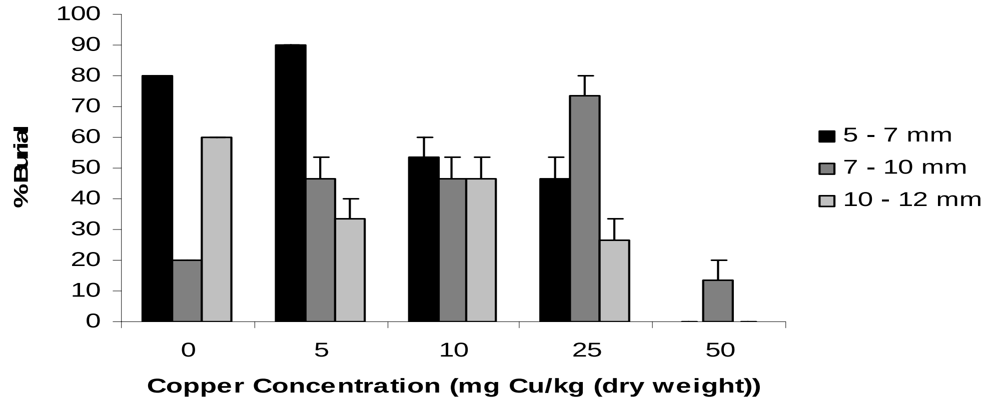


Figure 3.15 Average percentage burial (± 1 SE) of *Austrovenus stutchburyi* exposed to different concentrations of copper dosed sediments after 2 hours.

Table 3.12 Analysis of the effects of copper concentration on the burial after 2 hours in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.406	2	0.203	11.65	< **0.001**
Concentration	1.888	4	0.472	27.1	< **0.001**
Size*Concentration	1.301	8	0.163	9.338	< **0.001**
Error	0.47	27	0.017		

Burial responses to zinc dosed sediments

After two hours of exposure to different concentrations of zinc dosed sediments, *Austrovenus stutchburyi* average percentage burial was variable (Fig 3.16). Cockles in sediments with 160 mg Zn/kg (dry weight) had a lower burial rate than cockles in sediment with lower zinc concentrations (Fig 3.16). Successful burial ranged between 100% for cockles of 7 – 10 mm in a zinc concentration of 80 mg Zn/kg (dry weight), to 33.33% for cockles of 7 – 10 mm in a zinc concentration of 160 mg Zn/kg (dry weight) (Fig 3.16). Concentration of zinc in the sediment had a significant effect on the burial of *Austrovenus stutchburyi*; however, length had no significant effect (Table 3.13).

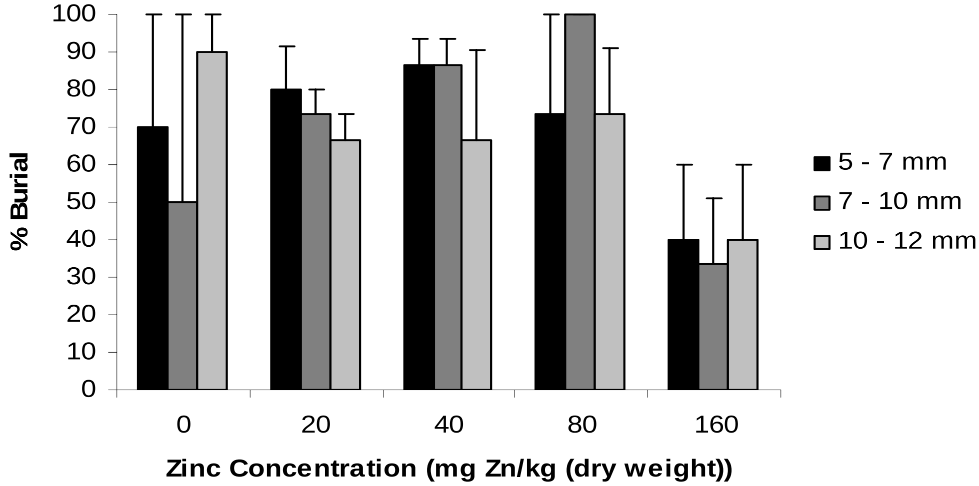


Figure 3.16 Average percentage burial (± 1 SE) of Austrovenus stutchburyi exposed to different concentrations of zinc dosed sediments after 2 hours.

Table 3.13 Analysis of the effects of zinc concentration on the burial after 2 hours in three length classes of *Austrovenus stutchburyi.* Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	0.064	2	0.032	0.126	0.883
Concentration	3.443	4	0.861	3.391	**0.023**
Size*Concentration	0.962	8	0.12	0.474	0.864
Error	6.852	27	0.254		

Burial responses to cadmium dosed sediments

A decrease in average burial percentage of *Austrovenus stutchburyi* exposed for two hours to cadmium dosed sediment occurred as the concentration of cadmium increased (Fig 3.17). There was a steady burial percentage of *Austrovenus stutchburyi* of 5 – 7 mm for each cadmium concentration, however at 1.8 mg Cd/kg (dry weight) there was a small decrease in burial percentage (Fig 3.17). There was a significant effect of both length and cadmium concentration on the burial rates of *Austrovenus stutchburyi* after 2 hours of exposure to the dosed sediments (Table 3.14). There was also a significant interaction effect of length and concentration on the burial of *Austrovenus stutchburyi* (Table 3.14).

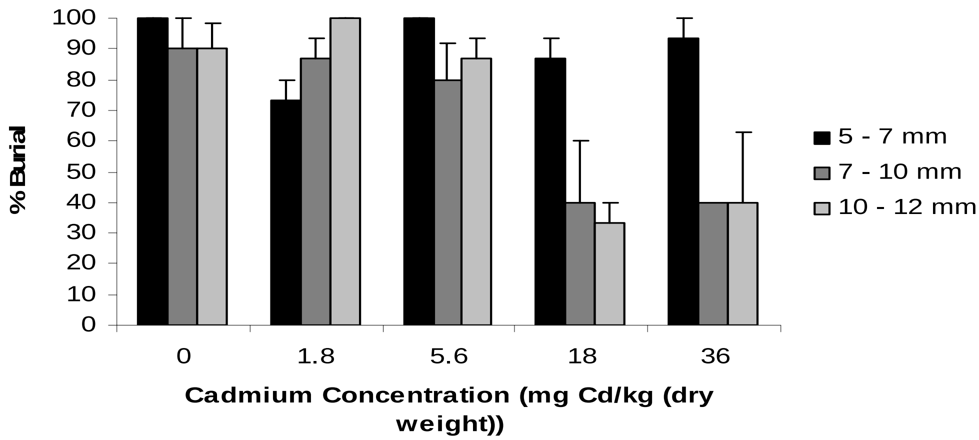


Figure 3.17 Average percentage burial (± 1 SE) of Austrovenus stutchburyi put in different concentrations of cadmium dosed sediments after 2 hours.

Table 3.14 Analysis of the effects of cadmium concentration on the burial after 2 hours in three length classes of *Austrovenus stutchburyi*. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Size	1.425	2	0.712	6.18	< **0.001**
Concentration	3.372	4	0.843	7.314	< **0.001**
Size*Concentration	2.751	8	0.344	2.984	**0.017**
Error	3.112	27	0.115		

3.4 Discussion

The results of the present study demonstrate that juvenile cockles, *Austrovenus stutchburyi*, have a clear behavioural and survival response when exposed to aqueous copper (0, 0.1, 0.05, 0.1 mg/L), zinc (0, 0.2, 0.4, 0.6 mg/L) and cadmium (0, 0.01, 0.05, 0.1 mg/L) and copper (0, 5, 10, 25, 50 mg/kg), zinc (0, 20, 40, 80, 160 mg/kg) and cadmium (0, 1.8, 5.6, 18, 36 mg/kg) dosed sediments. Concentrations of copper and zinc used in laboratory experiments were similar to those found in the natural environment; however concentrations of cadmium used were higher than those in the natural environment.

3.4.1 Aqueous experiments –siphon extension

Failure of a behavioural response can lead to reduced fitness with adverse consequences for the population (Roper 1994); however behavioural effects of trace metal contamination have received relatively little attention (Roper 1995).

Behavioural effects shown by bivalves to the presence of trace metals include, the delayed burrowing of *Macomona baltica* and *Tellina tenuis* (Mohlenberg 1983) and the decreased reburrowing of the clam *Protothaca staminea* (Phelps 1985). In the present study siphon extension behaviour of juvenile cockles differed in increasing concentrations of aqueous copper, zinc and cadmium. Siphon extension of juvenile cockles in aqueous copper significantly decreased over 10 days in the first copper trials, however in the second trials, siphon extension decreased in increasing copper concentrations and over time. This may have been because the trials were not done at the same time and cockle health may have differed therefore a greater effect of copper concentration on siphon extension.

Larger juvenile cockles (10 – 12 mm) showed a decreased siphon extension percentage compared to smaller juvenile cockles in aqueous copper. Siphon extension of juvenile cockles exposed to increasing concentrations of zinc decreased over time; however, size or zinc concentration did not have an effect on siphon extension behaviour. Increasing aqueous cadmium concentrations did not affect siphon extension behaviour of juvenile cockles.

Roper *et al* (1995) found that both copper and zinc had a significant effect on the crawling behaviour of small (mean length 1.3 mm) *Macomona liliana*. *Macomona liliana* crawled away from dosed test sediments above 10 mg Cu kg $^{-1}$ and 40 mg Zn kg $^{-1}$, and stopped moving in response to higher levels of copper (50 mg kg $^{-1}$) and zinc (160 mg kg $^{-1}$). In the present experiment copper was the only trace metal to have a significant effect on the siphon extension behaviour of juvenile *Austrovenus stutchburyi*.

3.4.2 Aqueous experiments – survival

Aqueous concentrations of zinc (up to 0.6 mg Zn/L) and cadmium (up to 0.1 mg Cd/L) had no effect on the survival of juvenile cockles. However, both aqueous copper (0, 0.01, 0.05, 0.1 mg Cu/L) trials were conclusive that size had an effect on survival of juvenile cockles. In both copper experiments, none of the controls of 10 – 12 mm cockles survived by day ten, this, was an unusual result. The reason for low survival in the controls could have possibly been that the water in the experiments was contaminated.

Copper concentrations of 0.1 – 0.2 mg Cu/L have been found to be toxic to several species of bivalves such as *Mytilus edulis, Tellina tenuis* and *Macomona* *balthica* (Lewis 1982). The present study found that there was an effect of copper concentration between 0.01 to 0.1 mg Cu/L on the siphon extension of *Austrovenus stutchburyi,* however there was no effect of copper concentration on the survival of the juvenile cockles.

In an experiment, using the amphipod *Corophium volutator*, Bat, Raffaelli and Marr (1998) found that survival decreased in seawater with both sediment absent and present, although *Corophium* was less affected by trace metals when there was sediment present. Survival of *Corophium* also declined in increasing trace metal levels in sediment dosed with copper, cadmium and zinc (Bat 1998). There was less mortality in sediment containing only zinc compared to that containing only cadmium and mortality rates in cadmium decreased when zinc was present. Controls showed no mortality and copper was less toxic than cadmium and zinc (Bat 1998).

3.4.3 Sediment experiments – survival

Previous studies have focused mainly on the effects of pollutants in the surface water, however trace metals can be concentrated up to 1000 times in the sediments compared to that of the overlying water (Marsden 2001). Concentrations in the sediment experiments of the present study were much higher than those in the water experiments to simulate the reality of the field environment. Both cadmium and zinc dosed sediments decreased the survival of juvenile cockles in the sediment however copper dosed sediments had no effect on the survival of juvenile cockles.

For most species that are used routinely in toxicity tests, the relative importance of the different contaminant exposure pathways (overlying waters, pore waters, sediment and detritus) has not been evaluated (King 2005). King et al (2005) used radiotracer techniques to determine the rates of accumulation of copper and cadmium to the amphipod *Melita plumulosa* and the bivalve *Tellina deltoidalis* from water, sediment and algal sources. Cadmium influx increased linearly with increased metal concentration in *Macoma balthica* suggesting that uptake is not actively regulated (Griscom 2002). Cadmium may be more toxic to juvenile *Austrovenus stutchburyi* in the sediment than in water as there was no effect of cadmium on survival or siphon extension when the juveniles were exposed to cadmium dosed water. This may be because the cockles were ingesting sediment particles because of the disturbance caused from changing the water in the container each day. The deposit feeder *C. capitata* accumulates more cadmium from organic particles than it does from interstitial water (Trannum 2004) and in *Austrovenus stutchburyi* this mechanism could result in ingesting more cadmium via sediment particles that become suspended in the water.

Phelps *et al* (1985) found that sediment freshly dosed with copper caused abnormal burrowing in the bivalve *Protothaca staminea* (Steamer Clam), but after ageing the sediment for one day there was no effect. This could be due to rapid binding of the trace metal with the sediment and therefore making if unavailable to the animal. In the present study the trace metal dosed sediments were aged for 24 hours. Burrowing and survival of juvenile cockles was not affected by copper dosed sediments, however cadmium and zinc dosed sediments decreased burrowing and survival of juvenile cockles.

3.4.4 Sediment experiments - burial

Pollutants in estuaries can lead to a change in animal behaviour and avoidance of the pollutant, for example burrowing behaviour of *Macomona baltica* and *Tellina tenuis* became impaired when the bivalves were exposed to trace metals, oil and phenol (Mohlenberg 1983). Measuring changes in behaviour is essential to acknowledging the effects of trace metal contaminants in estuaries (McGreer 1979). In the present study concentrations of zinc, copper and cadmium in sediments had a detrimental effect on the burial of juvenile cockles into the sediment within the first two hours of being exposed. The size of the juvenile cockles also had an effect on burial in cadmium and copper experiments. The modification of behaviour is a sensitive indicator of environmental stress and can effect survival (Phelps 1983). Juvenile *Austrovenus stutchburyi* exposed to trace metals may cause modified burrowing behaviour with a prolonged amount of time on the sediment surface. This may cause increased predation pressure and make the cockles more vulnerable to current and tide action (Mohlenberg 1983). Roper *et al* (1995) found that burial behaviour of juvenile (1.3 mm long) *Macomona liliana* was influenced by sediment contamination. There was a significant reduction in the number of juvenile *Macomona liliana* burying after 10 minutes in 25 mg Cu kg^{-1}, which was not found in the present study. A decrease in burrowing depth or increase in time spent on the surface would increase their susceptibility to predation (Roper 1995).

Slow or modified burrowing behaviour may not be immediate but effects of trace metals may have a delayed response. Other studies have shown that trace metals cause delays in burrowing and settlement of bivalves (Furness 1990). For example McGreer (1979) found that burrowing behaviour in *Macoma balthica* was inhibited in sediments contaminated with copper (150 – 30 ppm dry weight), lead (74 – 18 ppm dry weight), chromium (90 – 40 ppm dry weight), silver (3.2 – 1.0 ppm dry weight), mercury (0.46 – 0.18 ppm dry weight) and cadmium (1.40 – 0.40 ppm dry weight) compared to the controls and that they had significant avoidance behaviour of sediment containing the highest trace metal levels.

In the present study, burial of juvenile *Austrovenus stutchburyi* was inhibited by copper, cadmium and zinc. Behaviour plays a role in preventing or promoting transport of juvenile bivalves (Hunt 2005), therefore if juvenile *Austrovenus stutchburyi* do not bury, they are likely to be swept away by the tide, waves and currents, causing mortality. If burying behaviour was influenced by trace metal contamination it could influence the susceptibility to predation of *Austrovenus stutchburyi* (Roper 1995).

3.4.5 Conclusions

Understanding the ecological effects of trace metal contaminants is fundamental in ensuring anthropogenic effects to the marine environment are minimized (Morrisey 1996). Estuarine species are known for their tolerance as they live in a wide range of variable environmental conditions, such as salinity, temperature, and sediment particle size; this however, may not reflect their ability to live in a contaminated environment (Marsden 2001). Microcosm experiments may compensate for severe drawbacks experienced in field studies such as inadequate reference sites, mixtures of different pollutants, and a high natural variability (Gyedu-Ababio 2006).

There is potential for contamination to still occur even though the source has ceased releasing chemicals into the estuary (Cundy 2003), therefore it may take years before estuaries will revert back to their ‘natural’ state. This may potentially impact the recruitment and settlement of juvenile *Austrovenus stutchburyi*. If they are unable to find a suitable habitat there will be greater mortalities, causing overall population declines. For example, drifting of *Macomona liliana* in response to sediments contaminated with copper and zinc, could result in a major distribution of juveniles and in the long term effecting the populations distribution (Roper 1995).

In California, USA the phoxocephalid amphipod *Rhepoxynius* spp. avoided sediments mixed with domestic sewage or concentrations of trace metals (zinc and cadmium), which are characteristic of marine sediments at waste-water discharge sites in this area (Oakden 1984). Oakden *et al* (1984) found that there was a >50% mortality of the amphipods when sediment contained zinc concentrations of 610 $\mu g\ g^{-1}$ and cadmium concentrations of 190 $\mu g\ g^{-1}$. The experiments in the present study show that burial and siphon extension behaviour and survival has been effected by the presence of copper, cadmium and zinc. In their natural environment a decrease in survival will be a cause for concern as it will eventually lead to population declines. A negative effect of burial and siphon extension behaviour will also be a cause for concern as the cockles ability to move away from contaminated habitats and avoid predators and water action such as tides and waves will be decreased. This will cause stress to the individual changing recruitment patterns, growth and fecundity.

Chapter 4: Field Studies

4.1 Introduction

Estuarine environments have been extensively modified and impermeable surfaces now replace vegetation, shorelines have been hardened and water flow patterns have been altered (Inglis 2000). In urbanized estuaries rapid accumulation of pollutants means their distribution is spatially heterogeneous with hot spots pollutants occurring around major discharge sources (Inglis 2000). For example, in a study of estuaries throughout the northern Gulf of Mexico contaminant variables ranged from non existent to biologically detrimental levels (Rakocinski 1997). Aquatic communities are extremely vulnerable to the impacts of trace metal pollutants (Gyedu-Ababio 2006), therefore understanding how organisms live in or consume trace metal contaminated sediments is essential to predict ecosystem effects (Griscom 2002). Rakocinski *et al* (1997) found that pollution effects on macrobenthic organisms are difficult to identify because of correlations between environmental variables. However, by using canonical correspondence analysis they found that trophic diversity in estuaries in the northern Gulf of Mexico decreased with sediment contamination and linked contaminant gradients with shifts in community function and structure (Rakocinski 1997).

Metal exposure may result in physiological and biochemical changes at the whole organism and population level, and selection pressure may act on recruitment and settlement (Wang 2005). It has been suggested that low recruitment may occur in sediments contaminated by pollution because cues that are important in settlement and recruitment have been changed (Watzin 1997). Therefore, trace metal contaminants in the sediment could result in mortality of those organisms that settle in undesirable habitats (Watzin 1997). It is important to measure the ecological effects of *Austrovenus stutchburyi* from contaminated sediments, as it is where they spend the majority of their life.

Macrobenthic communities are good indicators of biotic integrity (Rakocinski 1997). Toxicity, bioaccumulation and behavioural response experiments using choice and non-choice protocols are often carried out in the laboratory with macrobenthic species (Bat 1998). The majority of meiofaunal toxicity studies have occurred in the laboratory with lethal or sublethal (reduction in fecundity, increased developmental time) effects, which increase with increased metal concentrations (Sommerfield 1994). Oversimplification of field conditions is a disadvantage of laboratory testing as there is little ecological relevance because of the lack of multiple species and static environmental conditions (Gyedu-Ababio 2006). Manipulative field studies investigating the effects of trace metals on benthic faunal species are very rare in the marine environment probably due to the practical difficulties of introducing metals into the sediments (Morrisey 1996). Toxicity field studies have immense importance as they allow us to gain information on the effects of trace metals in ecologically relevant environmental conditions not seen in the laboratory.

Lindegarth and Underwood (1999) repeated an experiment similar to that of Morrisey *et al* (1996) to evaluate the method of increasing trace metal concentrations in the field using metal dosed plaster blocks. Lindegarth and Underwood (1999) increased the level of trace metals in each experimental unit and used copper, zinc and lead as experimental trace metals. They found that copper, zinc and lead levels increased in the sediment compared to controls although there was a lot of variation between sites. This variability was explained by physical factors, such as position on the shore, sediment characteristics, and flow regime which may affect the rate at which the metals disperse, the transport rate of the trace metals, and the binding properties of the trace metals to the sediment particles (Lindegarth 1999). They therefore concluded that this particular method of introducing trace metals into the sediment is very useful (Lindegarth 1999). Morrisey *et al* (1996) used cylindrical plaster blocks dosed with copper to examine the effect of copper on benthic macrofaunal assemblages in Botany Bay, New South Wales, Australia. This is the only field study to look at community effects of increased trace metals.

The objective of field experiments in this thesis is to offer an alternative approach to the laboratory experiments, which are trialed under static conditions. The present study is the first where sediments have been experimentally enhanced to find the effects on juvenile *Austrovenus stutchburyi*. *Austrovenus stutchburyi* is a common bivalve in estuaries around Canterbury and therefore is easily collected and transferred to new locations. Survival of juvenile cockles is extremely important to the future of the population and if trace metals effect their survival in the field, it will have negative consequences. This study will also show if contaminants may have played a role in the current distribution patterns of *Austrovenus stutchburyi*.

4.2 Aim

The aim of these transfer experiments is to determine if increased levels of copper, cadmium and zinc in the sediment have an effect on the survival of juvenile *Austrovenus stutchburyi* in the field.

4.3 Methods

Trace metals were introduced into sediments in the field by impregnating small plaster blocks with copper, cadmium and zinc with methods modified from Morrisey *et al* (1996).

Collection of Austrovenus stutchburyi *and sediments*

Sand for the experiments was collected from Shag Rock, Sumner and sieved through a 0.5 mm mesh to remove any unwanted macrofauna and debris. Juvenile *Austrovenus stutchburyi* of 5 – 10 mm were collected from Beachville Road on the south side of Avon-Heathcote Estuary on the same day as the translocations were to take place. Cockles were kept in cool seawater during transfer to their new location.

Preparation of plaster blocks

Cylindrical blocks (10 cm diameter, 3 cm deep) were made using 180 g building plaster; 120 g distilled water; and 0.071 g of $CuSO_4$ (100 mg/kg of copper) or 0.12 g of $ZnCl_2$ (320 mg/kg of zinc) or 0.026 g of $CdCl_2$ (72 mg/kg of cadmium). These were well mixed and set into plastic pipe lined with cling film for easy removal of the blocks. These concentrations were determined by the amount of trace metals in the laboratory sediment experiments and levels that Morrisey *et al* (1996) used. Trials of copper chloride mixed with building plaster to make blocks were undertaken as copper chloride was used in the water and sediment experiments in the laboratory. A reaction occurred between the plaster and copper chloride, and the mixture turned bright green and frothed. Therefore, copper sulphate was used as there was no reaction with the building plaster. Zinc chloride and cadmium chloride did not react with the building plaster.

Experimental chambers were two litre ice cream containers that had holes of 6 mm diameter punched into the sides and bottom to allow a small amount of flow through of water. Mesh of 0.5 mm was attached with a hot glue gun to the inside of the containers so the cockles could not escape through the holes. The lid of the container was cut out and a mesh of 0.5 mm attached to it with a hot glue gun. One trace metal dosed block was placed in the bottom of each of the containers. Sieved sand from Shag Rock was poured into the container to cover the plaster block. Containers and *Austrovenus stutchburyi* were then transported to field sites.

Experimental design

There were five transfer sites within three estuaries around Canterbury. These were: McCormacks Bay, Water Treatment Ponds and Tern Street site 1, within the Avon-Heathcote Estuary, Saltwater Creek and Takamatua Bay (Fig 4.1, 4.2 and 4.3). Experimental treatment units consisted of six containers. Four replicate containers containing plaster blocks dosed with trace metals and two controls, with plaster blocks that were not dosed with trace metals. All three experimental trace metal experiments were run at the same time to minimise temporal variation. Around three hours before high tide the containers were embedded into the sediment so the top sat level with the top of the sediment. Containers were fixed in place by packing sand firmly around them. Five cockles were put onto the sediment surface in each of the containers. Containers containing trace metals blocks were placed 10 meters away from control containers and containers containing different trace metals were 50 meters away from each other at the same site. Three hours after the same high tide the containers were checked to ensure all the cockles had successfully burrowed into the sand. The containers were left at each site for four days and were then removed before the next high tide on that day. The containers were returned to the laboratory and the cockles were sieved out of the sand, placed in clean seawater. Those cockles that showed movement, extended their siphons, or foot were recorded alive within one hour and those that showed no activity were recorded as dead.

On removal from the habitat, a sample of sand from each of the containers was collected and sent to Hill Laboratories to be analysed for copper, cadmium and zinc levels. These samples were analysed by Nitric/hydrochloric acid digestion, ICP-MS

(Low level). US EPA 200.2. Cadmium was analysed to 0.01 mg/kg dry weight, copper was analysed to 0.2 mg/kg dry weight and zinc was analysed to 0.4 mg/kg dry weight.

Experiments exposing cockles to trace metals in three sites in the Avon-Heathcote Estuary (Fig 4.1) were done in May (Autumn) 2007. Experiments exposing cockles to trace metals at Tern Street site 1 (Fig 4.1), Takamatua (Fig 4.3) and Saltwater Creek (Fig 4.2) was repeated twice in May (Autumn) and July (Winter) 2007.

Figure 4.1 Image of the Avon-Heathcote Estuary showing the *Austrovenus stutchburyi* collecting site (BR – Beachville Road) and experimental sites (OX- Water Treatment Ponds, TS1 – Tern Street 1 site, and MB – McCormacks Bay).

Figure 4.2 Image of Saltwater Creek Estuary showing the experimental field site (SC – Saltwater Creek).

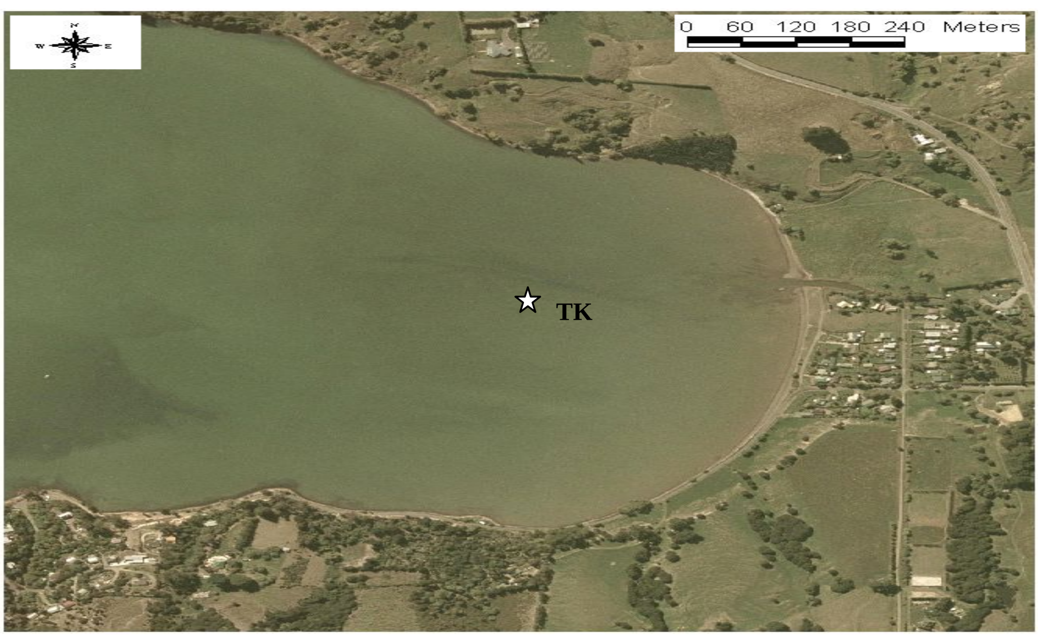


Figure 4.3 Image of Takamatua Bay showing the experimental field site (TK – Takamatua).

Statistical analyses

A factorial ANOVA was used to test the survival differences between sites and trace metal type in the transfer field experiments and a one way ANOVA was used to test the trace metal levels from the experimental and control sediments. Data were ArcSin transformed as they violated the assumptions of ANOVA.

4.4 Results

Exposure of cockles to trace metals within the Avon-Heathcote Estuary

Survival of *Austrovenus stutchburyi,* exposed to plaster blocks impregnated with copper, cadmium and zinc ranged from 20% to 80% (Fig 4.4) and there was a significant effect of trace metals on survival (Table 4.1). *Austrovenus stutchburyi* survival was greatest in control experiments compared to those experiments where copper, cadmium and zinc had been added; however, there was no difference in survival of cockles between the three trace metal types. Cockle survival was similar (mean = 60 to 100%) in all the control experiments. There was a significant effect of site on survival of juvenile cockles (Table 4.1). Survival was similar at McCormacks Bay (mean = 75%) and Tern Street (mean = 60%), however, cockles from the Water Treatment Ponds (mean = 41%) had significantly lower survival as shown by a Tukey test.

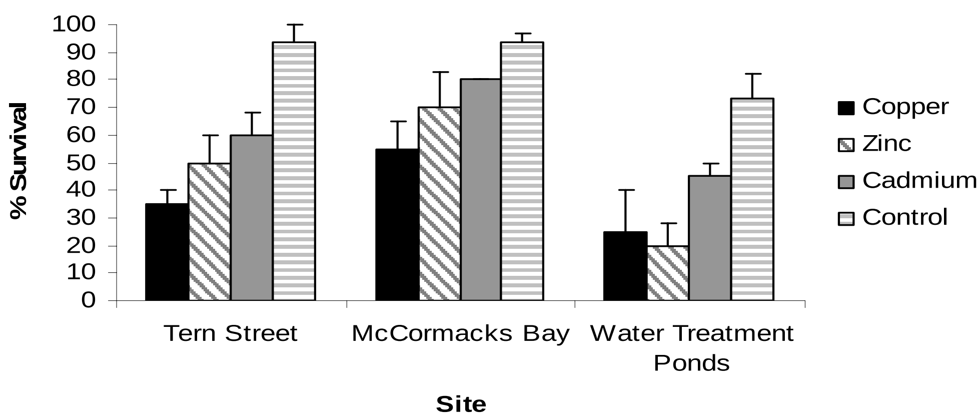


Figure 4.4 Survival of *Austrovenus stutchburyi*, (+1SE) exposed to added copper, cadmium and zinc at Tern Street, McCormacks Bay and the Water Treatment Ponds, in May 2007.

Table 4.1 Analysis of the survival of *Austrovenus stutchburyi* exposed to copper, cadmium, zinc and control at Tern Street, Water Treatment Ponds, and McCormacks Bay, Avon-Heathcote Estuary. Univariate analysis of variance. May 2007. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Site	2.15	2	1.075	9.842	**< 0.001**
Metal	5.753	3	1.918	17.66	**< 0.001**
Site x Metal	0.298	6	0.497	0.455	0.837
Error	4.587	42	0.109		

Exposure of cockles to trace metals between estuaries (May - Autumn)

Survival of *Austrovenus stutchburyi*, exposed to added levels of copper, cadmium and zinc ranged from 25 % to 80 % (Fig 4.5). There was no significant difference in survival (mean = 70 to 90%) of juvenile *Austrovenus stutchburyi* in the control experiments. There was a significant effect of metal on the survival of juvenile *Austrovenus stutchburyi* (Table 4.2). Survival of the cockles was greatest in the control experiments compared to the experiments with added copper, cadmium and zinc. However, survival of the juvenile cockles exposed to copper, cadmium and zinc were similar. Site had no significant difference on survival of juvenile *Austrovenus stutchburyi* as shown by univariate analysis of variance (Table 4.2).

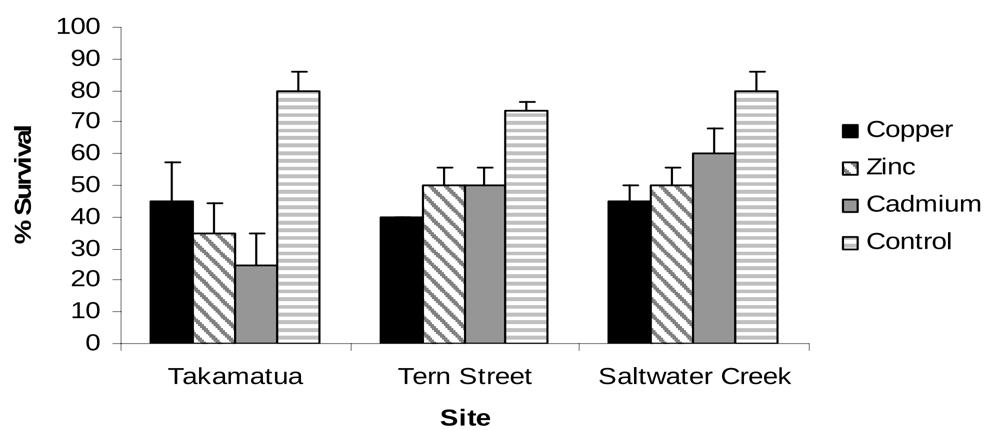


Figure 4.5 Survival of *Austrovenus stutchburyi*, (+1SE) exposed to added copper, cadmium and zinc at Takamatua, Tern Street, and Saltwater Creek, in May 2007.

Table 4.2 Analysis of the survival of *Austrovenus stutchburyi* exposed to copper, cadmium, zinc and controls at Tern Street, Takamatua, and Saltwater Creek. Univariate analysis of variance. May 2007. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Site	0.165	2	0.083	1.804	0.177
Metal	2.608	3	0.869	18.96	**< 0.001**
Site x Metal	0.368	6	0.061	1.34	0.261
Error	1.926	42	0.046		

Exposure of cockles to trace metals between estuaries (July - Winter)

Survival of *Austrovenus stutchburyi* transferred from Beachville Road to Tern Street, Takamatua and Saltwater Creek ranged from 0% to 80% (Fig 4.6). There was a significant difference in survival of juvenile *Austrovenus stutchburyi* in the control experiments (Tukey test: $p = 0.005$). Survival of cockles in Saltwater Creek and Tern Street control experiments was similar and the survival of cockles in the Tern Street and Takamatua control experiments was similar. A reason for the difference in control survival in these experiments is that at Saltwater Creek, one of the control containers was tipped upside down, possibly because of human interference, and the cockles had escaped. Data were analysed with this control container removed, however, the analysis did not change so the control was left in the analysis. There was a significant effect of site on the overall survival of juvenile cockles exposed to copper, cadmium and zinc (Table 4.3). Takamatua (Mean = 39%) and Tern Street (Mean = 38%) had a similar percentage of overall survival of juvenile cockles however, Saltwater Creek (Mean = 17%) had significantly lower survival. Treatment had a significant effect on the survival of juvenile *Austrovenus stutchburyi* exposed to copper, cadmium and zinc (Table 4.3). Greatest survival of juvenile cockles occurred in the control experiments compared to the copper, cadmium and zinc experiments; however, survival was similar in the three trace metals tested.

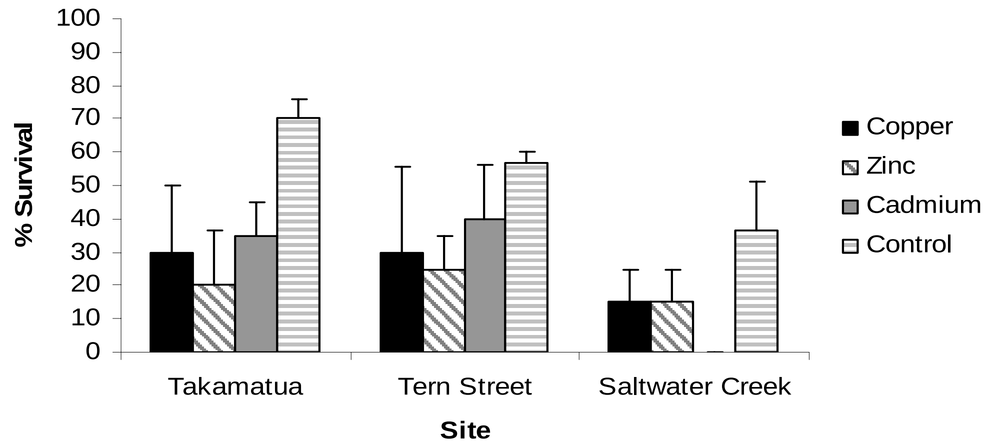


Figure 4.6 Survival of *Austrovenus stutchburyi,* (+1SE) exposed to elevated copper, cadmium and zinc at Takamatua, Tern Street, and Saltwater Creek, in July 2007.

Table 4.3 Analysis of the survival of Austrovenus stutchburyi exposed to copper, cadmium, zinc and controls at Tern Street, Takamatua, and Saltwater Creek. Univariate analysis of variance. July 2007. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Site	0.639	2	0.32	11.69	< **0.001**
Metal	1.512	3	0.504	18.43	< **0.001**
Site x Metal	0.264	6	0.044	1.607	0.169
Error	1.149	42	0.027		

Juvenile *Austrovenus stutchburyi* were transferred from Beachville Road to Tern Street, Takamatua and Saltwater Creek during May 2007 and July 2007. There was a significant effect of season on the survival of juvenile cockles exposed to copper, cadmium and zinc (Table 4.4). Overall survival of juvenile *Austrovenus stutchburyi* was greater in May than in July. There was also a significant effect of treatment on the survival of juvenile cockles between May and July (Table 4.4). Cockles in control experiments had a significantly higher percentage of survival than those cockles in copper, cadmium and zinc, however there was no difference in survival of cockles between the three trace metals.

Table 4.4 Univariate analysis of varianceof survival of *Austrovenus stutchburyi* exposed to copper, cadmium and zinc at Tern Street, Takamatua Bay and Saltwater Creek during two seasons. May and July 2007. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Site	0.093	2	0.047	1.275	0.285
Metal	4.026	3	1.342	36.66	**< 0.001**
Season	1.75	1	1.75	47.8	**< 0.001**
Site x Metal	0.372	6	0.062	1.695	0.132
Site x Season	0.712	2	0.356	9.721	**< 0.001**
Metal x Season	0.094	3	0.031	0.856	0.467
Site x Metal x Season	0.26	6	0.043	1.184	0.323
Error	3.075	84	0.037		

Metal concentrations of sediments

Levels of cadmium ranged from 0.02 mg/kg dry weight in the controls to 0.2 mg/kg dry weight in cadmium containers at Takamatua. Levels of copper ranged from 2.5 mg/kg dry weight in the controls to 3.3 mg/kg dry weight at Takamatua. Levels of zinc ranged from 29.9 mg/kg dry weight in zinc experiments at Saltwater Creek to 32.9 mg/kg dry weight at in the controls (Table 4.5). Levels of copper, cadmium and zinc in replicate experimental containers were similar, which shows that the release of metals was the same in each of the containers. There was a significant increase in copper and cadmium levels in the sediment in metal containers compared to controls; however, there was no significant increase in zinc levels in the sediment (Table 4.6).

Table 4.5 Levels of copper cadmium and zinc in sediments from the experimental containers in July (winter) 2007. Single samples.

Site	Metal in plaster	Copper (mg/kg dry wt)	Cadmium (mg/kg dry wt)	Zinc (mg/kg dry wt)
Takamatua	Control	3.0	0.02	32.1
Takamatua	Control	2.6	0.02	29.6
Takamatua	Copper	3.3	0.03	33.3
Takamatua	Copper	3.2	0.02	31.4
Takamatua	Cadmium	3.7	0.2	34.6
Takamatua	Cadmium	3.1	0.04	32.1
Takamatua	Zinc	2.6	0.02	31.4
Takamatua	Zinc	2.6	0.02	30.7
Tern Street	Control	2.8	0.02	32.9
Tern Street	Control	2.9	0.02	32.4
Tern Street	Copper	3.0	0.02	32.1
Tern Street	Copper	3.2	0.02	33.1
Tern Street	Cadmium	2.9	0.06	34.1
Tern Street	Cadmium	2.8	0.03	33.4
Tern Street	Zinc	2.7	0.02	32.8
Tern Street	Zinc	2.7	0.02	32.3
Saltwater Creek	Control	2.5	0.02	29.3
Saltwater Creek	Control	2.9	0.02	31.2
Saltwater Creek	Copper	2.8	0.02	28.6
Saltwater Creek	Copper	2.8	0.02	30.9
Saltwater Creek	Cadmium	2.7	0.04	33.4
Saltwater Creek	Cadmium	2.5	0.01	32.6
Saltwater Creek	Zinc	2.6	0.02	29.9
Saltwater Creek	Zinc	2.9	0.02	32.2

Table 4.6 Analysis of zinc, copper and cadmium in the sediment of control and metal dosed containers at Tern Street, Takamatua Bay and Saltwater Creek. Values in bold indicate significance where $p < 0.05$.

Source of Variation	SS	*df*	MS	*F*	*p*
Zinc	0.27	1	0.27	0.156	0.701
Error	17.31	10	1.73		
Copper	0.213	1	0.213	5.039	**0.049**
Error	0.423	10	0.042		
Cadmium	0.001	1	0.001	20.46	**0.002**
Error	< 0.001	8	< 0.001		

4.5 Discussion

The results of the present study conclude that juvenile *Austrovenus stutchburyi* show decreased survival when exposed to copper, zinc and cadmium in the field.

Experiments in the laboratory allow for identification of toxic effects of contaminants in controlled conditions; this unfortunately does not allow us to factor in the wide range of environmental fluctuations in the field (Morrisey 1996). Therefore, effects seen in the laboratory may not reflect what is happening in the field because of static laboratory conditions. One advantage of transplanting bivalves is that it provides a combination of the experimental control of laboratory experiments with the environmental realism of field monitoring (De Luca - Abbott 2001). The idea behind transplanting individuals from a clean environment to a contaminated one is that the individuals have not had the opportunity to regulate their physiology to the contaminant stress which enables the evaluation of the initial stress response (De Luca - Abbott 2001). De Luca – Abbott (2001) transferred *Austrovenus stutchburyi* from a clean site to a range of sites with higher contaminants to establish whether cockles suffer sublethal stress with increased exposure to contaminated sediments. She found that the energetics, such as the reproductive potential and growth, of the cockle are affected by environmental sublethal stress. In the Whitford Estuary of the Hauraki Gulf, Norkko *et al* (2006) transferred two species of adult bivalves, the cockle *Austrovenus stutchburyi*, and the clam *Paphies australis*, to estuarine sites with different concentrations and types of suspended sediment to determine the long term response of RNA of the bivalves to changes in sedimentation. *Austrovenus stutchburyi* of 20 – 35 mm and *Paphies australis* of 45 – 65 mm were transplanted to five sites with different suspended sediment concentrations and left for 11 weeks. They found that mortality of caged bivalves was highest at muddy sites and RNA of both species differed significantly between sites. The relative increase in RNA at the two most exposed sites was larger in *Austrovenus stutchburyi* than in *Paphies australis* (Norkko 2006).

4.5.1 Movement of cockles from unfavourable sediment

The benthic macrofaunal community is often regarded as relatively sedentary and cannot avoid deteriorating conditions (Dauer 1995). However, in a preliminary field trial experiment the juvenile cockles escaped out of the holes in the containers with trace metal dosed plaster blocks. Some cockles escaped out of the control containers; however, more cockles remained in the control containers than in the trace metal treated containers. It is known that adult *Austrovenus stutchburyi* crawl across the surface leaving tracks which are readily visible and can easily move 30 cm over a single tide (Hewitt 1996). Juvenile *Macomona liliana* have been found to move away from sandy sediments treated with copper in the laboratory (Roper 1994). The above behaviour of *Austrovenus stutchburyi* suggests that juveniles also have an ability to move away from unfavourable conditions. Trace metal contamination may disrupt post-settlement dispersal because the unfavourable habitats cause individuals to move to find more suitable habitats by actively emigrating. If active emergence away from unsuitable sediments results in increased transport rates, juvenile cockle sediment choice can influence adult distributions (Lundquist 2004). The present study showed that the presence of a copper, cadmium and zinc caused a higher percentage of mortality of juvenile *Austrovenus stutchburyi* than did control experiments in both May and July field experiments. This is consistent with the distribution of juvenile cockles found from population surveys in the present study which showed that there is a negative correlation between juveniles and copper and zinc in the sediment.

4.5.2 Seasonal effects

Trace metals and temperature are common stressors in estuaries and their effects can vary seasonally because trace metals can have increased adverse effects when temperatures are elevated (Cherkasov 2006). Transplants in the present study, were undertaken at two different times of the year to investigate whether season had any influence on survival of *Austrovenus stutchburyi* exposed to copper, cadmium and zinc. Survival of juvenile *Austrovenus stutchburyi* was higher in the May (autumn) field transfer experiments than it was in the July (winter) field transfer experiments. The air temperatures during the July experiments was colder (Average air temperature: 5.9 ^{o}C) (Salinger 2007a) than in the May (Average air temperature: 11.4 ^{o}C) (Salinger 2007b) experiments which possibly accounts for the difference in survival rates, especially the decrease in control survival. Temperature plays a key role in the effect of trace metals because of its role in physiological processes (Lannig 2006), however, multiple stressors such as temperature and trace metal effects are not well understood because they can have additive or non-additive effects (Cherkasov 2006). The juvenile cockles may not cope with a change in environment conditions and therefore the trace metals may cause a lower percentage of survival.

Environmental stress sometimes results in a reduction of the net energy balance of organisms because of a reduction in assimilation of energy or its conservation and an energy deficit can have adverse effects on survival of organisms and their populations (Cherkasov 2006). Biological responses can vary seasonally because of seasonal fluctuations in food supply, energy pools, and reproductive cycles which may mean organisms are more vulnerable to additional stress at certain times of the year (Adams 1990). Cockles retain low tissue body-weight over winter and then weight increases in the spring due to somatic growth and gamete development (Marsden 2004a), therefore a difference in condition between season may cause a decrease or increase in survival when juveniles are exposed to copper, zinc and cadmium. Cherkasov *et al* (2006) found that increased temperatures and cadmium exposure in the oyster *Crassostrea virginica* results in energy deficiency because of elevated energy demand and reduced mitochondrial capacity possibly reducing survival in higher temperatures. In the present study the survival of juvenile cockles may be because of trace metal effects, temperature effects or a combination of the two. Temperature was possibly the main factor in decreasing survival in juvenile cockles in July. Cherkasov *et al* (2006) found that elevated temperatures and trace metals had an effect on oysters, not decreased temperatures, although this is beyond the scope of the present study.

4.5.3 Sediment testing

Sediments from the experimental containers were tested for an increase in trace metal content to see if the trace metals had leached out of the plaster blocks. In the present study both the copper and cadmium levels were significantly higher in the sediment compared to the controls, however, zinc levels were not, possibly because they were already high. Morrisey *et al* (1996) impregnated building plaster with copper sulphate and found that copper levels in the sediment ranged from 140 – 1200 μg/g compared with 29 – 40 μg/g prior to the experiment beginning. Copper was introduced into the sediment by burying plaster impregnated with copper sulphate. Treatment units consisted of a one metre diameter circle with six copper blocks (10 cm diameter, 10 cm deep) placed evenly around. Cores were taken within the treatment circle at 0, 30, 60, 120, and 180 days so macrofaunal counts could be made, however there was some inconsistency among replicate units within treatments. Decreases in animals in the copper treatment compared to the controls may have been because of mortality or emigration. Apart from bivalves, other abundant taxa in the sediment is unlikely to leave any identifiable remains. Bivalve shells are already naturally present in the sediment and therefore an increase due to the experiment was unlikely to be detected (Morrisey 1996).

4.5.4 Conclusions

Introduction of trace metals in plaster blocks is a technique that is useful in soft-sediment habitats to enhance the metal concentration of the sediment (Morrisey 1996, Lindegarth 1999). The present study showed that both copper and cadmium were increased in the sediment by introducing plaster blocks with the trace metals set into them and that there was an effect of increased trace metals on the survival of juvenile *Austrovenus stutchburyi*. Trace metal levels may be higher when immediately placed in the sediment in the experimental containers and slowly decrease over time which may have an immediate effect on the juvenile cockles rather than a long term effect. Therefore if sediment samples were taken within 12 hours of the trace metal blocks being placed in the sediment it would tell us if trace metals leach into the sediment immediately. Removal of cockles from the experiment within a smaller amount of time may also show any immediate behavioural effects on individuals.

A difference in survival at differing times of the year is important to surviving seasonally or globally elevated temperatures. A decrease in survival of juvenile *Austrovenus stutchburyi* because of pollution caused by trace metals will ultimately have an effect on the cockle population as a whole. However the present study has also shown that juvenile *Austrovenus stutchburyi* have the ability to move away from sediments containing high levels of trace metals which may have a long term effect on the distribution of cockle populations.

Chapter 5: General Discussion

5.1 Summary of Major Findings

This thesis investigated the relationship between *Austrovenus stutchburyi* populations and environmental variables in estuaries around the Canterbury region. The relationship between cockle densities and densities of cockles < 10 mm was tested against the environmental variables; trace metal levels (copper, cadmium and zinc), sediment particle size, nitrogen and phosphorus levels, pore water and organic matter in the sediment and the density of flora and fauna.

Positive correlations occurred between the average density of cockles and fine sand (125 μm), phosphorus levels and percentage cover of *Ulva* sp. However, cockle density was negatively correlated with cadmium and zinc concentration, and percentage of mud present. The density of small cockles < 10 mm was negatively correlated with copper and cadmium concentration in the sediment and positively correlated with *Diloma subrostrata* numbers, *Zostera muelleri* percentage cover, *Ulva* sp. percentage cover, and sediment particles sizes of <63 μm (mud), 63 μm (very fine sand), and 125 μm (fine sand).

Laboratory experiments were conducted to determine behavioural (siphon extension and burial) and survival responses of cockles between 5 – 7 mm, 7 – 10 mm and 10 – 12 mm shell length exposed to trace metal dosed aqueous solution and sediments. These experiments indicated that the smallest cockles were most susceptible to increasing trace metal concentrations.

Field experiments to investigate the survival effects of trace metals were conducted on juvenile cockles transferred from a clean environment to artificially contaminated environments dosed with copper, cadmium and zinc. When juvenile *Austrovenus stutchburyi* were transferred within three sites in the Avon Heathcote Estuary, site and exposure to copper, cadmium and zinc reduced cockle survival. Transfer of cockles between estuaries showed similar effects. In a second transfer of cockles between estuaries, site and treatment had an effect of survival on juvenile cockles.

5.2 Monitoring/Indicators

The present study has shown that benthic invertebrates such as *Austrovenus stutchburyi* can be useful indicators of estuarine health. Environmental influence on the density and population structure of *Austrovenus stutchburyi* is extremely complex and is unlikely to be related solely to one variable. Species-environment correlations are important tools for ecology and conservation, but there is generally lack of knowledge of how spatial extent, habitat and environmental heterogeneity interact to affect relationships (Rossi 2007). It is important to choose a monitoring species that is ecologically or commercially important in the receiving environment (Lam 2001). Only a small number of estuaries were sampled in the present research, and it is recommended that by extending the number of estuaries surveyed, an improved insight into the correlations between environmental variables and *Austrovenus stutchburyi* populations can be developed. The use of *Austrovenus stutchburyi* as a bioindicator will allow us to assess the risk of various activities to the ecosystem and human health (Roach 2001). Monitoring of estuaries at regular intervals over a number of years will help to gain an understanding of the natural temporal and spatial variations in the cockle population.

5.3 Recruitment into Trace Metal Elevated Sediments

It has been suggested that diminished recruitment may occur in sediments containing trace metals because cues important in settlement have been changed or eliminated (Watzin 1997). Trace metal influence on the survival and behaviour of *Austrovenus stutchburyi* is highly complex. However, the present research has shown there to be decreased survival and a change in behaviour (measured as siphon extension and burial) in the presence of copper, cadmium and zinc. Regular population monitoring may help to determine the extent to which trace metals are having an effect on the settlement and recruitment of juvenile cockles.

Investigations of behavioural and survival changes of juvenile cockles in the presence of multiple trace metals might highlight interactions between trace metals that would be greater than the effect of individual trace metals. These studies might also give a more accurate representation of the effects of trace metals in the field. It would also be useful to undertake recruitment experiments in the field using multiple trace metals to determine if they prevent or minimise cockle recruitment and settlement.

5.4 Multiple Stressors – Temperature and Trace Metals

Temperature and trace metals are common stressors in estuaries and global climate change is causing them to become increasingly important (Cherkasov 2006). The potential effects of increased temperatures in our estuaries and coastal bays and combined effects of trace metals on the behaviour and survival of *Austrovenus stutchburyi* are unknown. Metabolic regulation helps maintain an optimal energy balance in some organisms and matches energy demand with sufficient energy supply. However, the energy balance may be reduced by environmental stresses and this may have adverse effects on the survival of organisms and populations (Cherkasov 2006). To understand the role of environmental stressors, such as temperature, and the additional stress of trace metals an analysis of effects on *Austrovenus stutchburyi* is required. Experiments examining the affects on survival of juvenile cockles of trace metal exposure combined with elevated temperatures are urgently needed. Other variables such as reduced oxygen, PCB's, other trace metals (for example lead), and different contaminants (for example sewage) could be investigated to determine the effects on survival, recruitment and settlement of juvenile cockles.

5.5 Conservation

Effective management of ecological, ethical and economic values of *Austrovenus stutchburyi* is critical to help stop their decline. Cockle populations are declining in some areas due to habitat change and over harvesting (Cummings 2007). This is a serious problem throughout New Zealand as populations are unlikely to restore themselves without the intervention of human assistance. Closing areas for recreational harvesting is one way of reducing over harvesting, as is currently implemented at Port Levy, Banks Peninsula. Although this will reduce numbers being taken, it may not increase recruitment into the population; therefore, it is important to help re-establish the population. In Port Levy some reasons for the population not increasing may be because there are insufficient juveniles, a lack of effective settlement success or the environmental conditions have changed and the habitat is no longer suitable for juvenile cockles.

Restoration of natural conditions within the estuary or coastal bays is important to help with the re-establishment of populations. However, it is not feasible to remove pollutants like trace metals that are already present within the sediment, but better management systems may help to minimise future trace metal contamination. Better management of land use and interactions between land and drainage systems, would allow more areas of suitable cockle habitat, especially in areas that have not already had large impacts from urban and industrial use, for example, Saltwater Creek Estuary. Water systems such as runoff and drainage systems can be managed more efficiently to decrease the amount of pollutants entering our coastal water bodies.

Large numbers of people walking in areas suitable for cockle habitat can cause a reduction in their survival. Rossi *et al* (2007) showed that trampling by humans modified the abundance and population dynamics of the clam *Macoma balthica* and the cockle *Cerastoderma edule*. They found that there was a negative impact on both species, probably because footsteps directly killed or buried the animals, provoking asphyxia (Rossi 2007). Conservation and management of mudflats by closing areas for human use over a period of time removes the source of disturbance and may allow the cockle population to re-establish (Rossi 2007).

Transfer of individuals may be another good way to help re-establish diminished cockle populations around not only Canterbury but also, New Zealand. The population survey conducted in the present study has given good insight into the habitat preferences for cockles and in particular juvenile cockles < 10 mm. This information can be used to select sites that are suitable for cockle enhancement. Cummings *et al* (2007), conducted experimental transfers of cockles 20 – 32 mm in length, in the Whangarei Harbour to determine the optimal density for transferring cockles. They have shown that irrespective of density around 30% of adults transferred to 30 x 30 cm plots remained in the plots 12 months later and survival was high within the first 29 weeks of transfer (July 2004 to January 2005) (Cummings 2007). Stewart (2005) found that survival of transplanted cockles was clearly dependent on habitat quality which decreased with increased pollution. Other factors that may be important for selecting sites for the transfer of juvenile cockles are sediment particle size, food availability, time of year, quality and size of stock, predator refuge, for example sea grass, salinity and nutrients. Using information about the best transfer density and habitat preference from the present study, a successful transfer program to enhance cockle populations by transferring cockles could be implemented.

5.6 Aquaculture

The cockle is a popular bivalve collected by many people for their own personal consumption and as fishing bait. However, because of reduced habitat and declining cockle numbers, there is a possibility that the freedom of collection for personal use may be removed in the future to help conserve the cockle population. Currently 150 cockles/day/person are allowed to be collected for recreational use, which is very high compared to other species of shellfish such as mussels (50/person/day) and oysters (50/person/day). Restoration of shellfish populations is becoming an increasing practice worldwide as natural populations are exposed to habitat loss or degradation and over harvesting (Gaffney 2006).

Stewart (2005) suggests that cockle populations are maintained by low level recruitment and there is therefore a potential for populations to collapse following several years of poor recruitment. Additional stresses such as, harvesting and trace metal contaminants will have detrimental effects on the *Austrovenus stutchburyi* population.

There may be a potential to cultivate *Austrovenus stutchburyi* within the lab for commercial and conservational reasons. It has already been shown that cockles may be reared under laboratory conditions (Stephenson 1979). The possibility of aquaculture of the cockle in New Zealand needs to be researched further. If cockles can be reared in a laboratory environment to a size where they will cope with transfer of environments, they may later be released into suitable habitats in the natural environment where, if successful, could be used for commercial and/or recreational harvest. It is recommended that an extensive trial be conducted to determine the optimal size, ecologically and economically, so that the cockles should be transferred into the natural environment. If there was a long-term aim to have sustainable or harvestable *Austrovenus stutchburyi* populations, management practices would need to include the whole catchment of the estuary. This may also require the whole ecosystem to be restored as all environmental factors and species are linked in a chain.

Hatchery propagation of stocks for restoration of populations may cause some genetic impact on the recipient population if the planted stocks survive and reproduce (Gaffney 2006). There are three primary genetic concerns of population restoration. These are; identification and use of the correct genetic material for producing hatchery lines; maintenance of genetic variability in hatchery stocks; and maintaining population size in the wild population (Gaffney 2006). Enhancement of degraded wild populations with improved genetic strains may help reverse negative fisheries selection if coupled with appropriate harvesting (Gaffney 2006).

Current management methods of daily bag limits are insufficient and do not take into account additional stresses such as pollution (Hartill 2005). The use of aquaculture to restock cockle populations needs the support of the local people, fishermen and Māori. In a giant clam restocking project in the Philippines, one of the major problems was illegal fishing and poaching. Marine protected areas were established and groups of people used to protect the restocked areas (Gomez 2006).

5.7 Conclusions

Estuarine ecosystems are characterized by spatial and temporal variations such as temperature, sediment particle size and water depth and quality (Thrush 1997). Populations are distributed along gradients that reflect scales of influence of environmental factors (Edgar 2002), and physical factors such as substrate and habitat characteristics have been suggested to be important (Hewitt 1997). Effects of pollution may be expected to be greatest during recruitment of individuals to sedimentary bottoms (Olsgard 1999). Human activities threaten to decrease the diversity and abundance of marine benthic organisms and interfere with vital ecological processes (Lindegarth 1999). Behavioural modification from trace metal pollution can reduce fitness of individuals and cause detrimental impacts on whole populations (Roper 1994). Identification of spatial patterns in marine benthic organisms is fundamental to their management and can be identified by sampling patterns of natural scales of variation (Cole 2000).

Cockle populations in the present research have shown a strong correlation with environmental variables and this can be used for management and conservation. Laboratory experiments demonstrated changes in burial and siphon extension in relation to copper, cadmium and zinc and showed decreases in survival at some concentrations of these metals. Field experiments such as those carried out in the present research have not been done previously and they have shown that copper, cadmium and zinc reduce the survival of juvenile *Austrovenus stutchburyi*. Manipulative experiments such as the ones in the present sudy are essential in identifying environmental impacts (Lindegarth 1999).

The mortality of juvenile *Austrovenus stutchburyi* due to increased levels of trace metals may have ongoing implications. For example, the cockles are more likely to be picked off by predators because they are not able to burrow quickly once disturbed. Settlement of juveniles in trace metal contaminated areas may decrease the ability of cockles to move and settle in non-contaminated areas. The research in this thesis is a start to understanding the effects and implications of contaminants on survival, behaviour and recruitment of juvenile cockles. Future research needs to include more field experiments and be extended to include a greater range of contaminants and environmental variables throughout estuaries and bays of the New Zealand coastline. The present and future research will benefit management strategies for increasing population numbers of *Austrovenus stutchburyi*.

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Appendix 1: Calculations for Stock Solutions

STOCK SOLUTION: COPPER

The amount of trace metal salt $CuCl_2.2H_2O$ to be added to form the stock solution was calculated as follows:

Molecular mass of salt: 170.48 g $CuCl_2.2H_2O$

Molecular mass of metal in salt: 63.55 g Cu

Grams of salt added per container = (Volume of container)(Desired concentration of metal)(Molecular mass of salt/Molecular mass of metal in salt) (Frantz Smith pers. comm.)

=(1 L container) (1 g Cu/L) (170.48 g $CuCl_2.2H_2O$/63.55 g Cu)

=2.68 g $CuCl_2.2H_2O$/ 1 L of dH_2O

= 1 g Cu/L solution

STOCK SOLUTION: ZINC

The amount of trace metal salt $ZnCl_2$ to be added to form the stock solution was calculated as follows:

Molecular mass of salt: 136.3 g $ZnCl_2$

Molecular mass of metal in salt: 65.41 g Zn

Grams of salt added per container = (Volume of container)(Desired concentration of metal)(Molecular mass of salt/Molecular mass of metal in salt) (Frantz Smith pers. comm.)

=(1 L container) (1 g Zn/L) (136.3 g $ZnCl_2$/65.41 g Zn)

=2.08 g $ZnCl_2$/ 1 L of dH_2O

= 1 g Zn/L solution

STOCK SOLUTION: CADMIUM

The amount of trace metal salt $CdCl_2$ to be added to form the stock solution was calculated as follows:

Molecular mass of salt: 228.34 g $CdCl_2$

Molecular mass of metal in salt: 112.411 g Cd

Grams of salt added per container = (Volume of container)(Desired concentration of metal)(Molecular mass of salt/Molecular mass of metal in salt) (Frantz Smith pers. comm.)

=(1 L container) (1 g Cd/L) (228.34 g $CdCl_2$/112.411 g Cd)

=2.03g $CdCl_2$/ 1 L of dH_2O

= 1 g Cd/L solution

Appendix 2: Surface Flora/Fauna Recorded during the General Survey

Site	*Amphibola crenata*	*Diloma subrostrata*	*Cominella glandiformis*	*Zeacumantus subcarinatus*	*% Ulva*	*% Seagrass*
BR1	0	0	0	0	0	0
BR2	0	0	0	0	0	0
BR3	0	3	0	0	0	0
BR4	0	8	0	0	40	0
BR5	0	0	0	0	0	0
BR6	0	16	0	0	0	0
BR7	0	4	0	0	0	0
BR8	0	5	0	0	0	0
BR9	0	3	0	0	0	0
BR10	0	6	0	0	0	0
TS1-1	0	5	0	0	1	0
TS1-2	0	5	0	0	0	0
TS1-3	2	2	0	0	0	0
TS1-4	0	6	0	0	0.5	0
TS1-5	2	1	0	0	0	0
TS1-6	0	3	0	0	5	0
TS1-7	2	11	0	0	4	0
TS1-8	4	3	0	0	1	0
TS1-9	0	4	0	0	0	0
TS1-10	3	2	0	0	0	0
TS2-1	1	2	0	0	5	80
TS2-2	1	12	0	0	0	90
TS2-3	0	0	0	0	3	90
TS2-4	0	15	0	0	3	80
TS2-5	0	15	0	0	25	72
TS2-6	2	5	0	0	7	80
TS2-7	4	16	0	0	5	70
TS2-8	1	7	0	0	5	80
TS2-9	1	8	0	0	0	90
TS2-10	1	8	0	0	0	90

Site	*Amphibola crenata*	*Diloma subrostrata*	*Cominella glandiformis*	*Zeacumantus subcarinatus*	% *Ulva*	% *Seagrass*
PPJ 1	1	0	0	0	0	0
PPJ 2	4	0	0	0	0	0
PPJ 3	3	0	0	0	0	0
PPJ 4	2	0	0	0	0	0
PPJ 5	2	0	0	0	0	0
PPJ 6	3	0	0	0	0	0
PPJ 7	4	0	0	0	0	0
PPJ 8	2	0	0	0	0	0
PPJ 9	3	0	0	0	0	0
PPJ 10	3	0	0	0	0	0
HS 1	0	0	0	0	0	0
HS 2	0	1	0	0	0	0
HS 3	0	1	0	0	0	0
HS 4	0	0	0	0	0	0
HS 5	0	1	0	0	0	0
HS 6	0	3	0	0	0	0
HS 7	0	1	0	0	0	0
HS 8	0	3	0	0	0	0
HS 9	0	0	0	0	0	0
HS 10	0	0	0	0	0	0
HD 1	2	0	0	0	0	0
HD 2	2	0	0	0	0	0
HD 3	0	0	0	0	0	0
HD 4	2	0	0	0	5	0
HD 5	2	0	0	0	1	0
HD 6	0	0	0	0	0	0
HD 7	0	0	0	0	0	0
HD 8	2	0	0	0	0	0
HD 9	0	0	0	0	0	0
HD 10	4	0	0	0	0	0
PPY 1	2	0	0	0	0	0
PPY 2	1	0	0	0	0	0
PPY 3	1	0	0	0	0	0
PPY 4	0	0	0	0	0	0
PPY 5	3	0	0	0	0	0
PPY 6	1	0	0	0	0	0
PPY 7	1	0	0	0	0	0
PPY 8	2	0	0	0	0	0
PPY 9	1	0	0	0	0	0
PPY 10	0	1	0	0	0	0

Site	*Amphibola crenata*	*Diloma subrostrata*	*Cominella glandiformis*	*Zeacumantus subcarinatus*	*% Ulva*	*% Seagrass*
HC 1	1	1	0	0	0	0
HC 2	0	0	0	0	0	0
HC 3	2	1	0	0	25	0
HC 4	2	1	0	0	0	0
HC 5	2	0	0	0	0	0
HC 6	1	0	0	0	0	0
HC 7	0	0	2	0	0	0
HC 8	0	0	0	0	0	0
HC 9	0	0	0	0	0	0
HC 10	0	0	0	0	0	0
TK 1	0	1	0	0	0	0
TK 2	0	0	8	0	0	0
TK 3	0	0	1	0	0	0
TK 4	0	0	4	0	0	0
TK 5	0	0	4	0	0	0
TK 6	0	0	3	0	0	0
TK 7	0	0	0	0	0	0
TK 8	0	0	3	0	0	0
TK 9	0	0	0	0	0	0
TK 10	0	0	3	0	0	0
SP 1	0	2	0	0	40	0
SP 2	0	2	0	0	5	0
SP 3	0	4	0	0	0	0
SP 4	0	1	0	0	5	0
SP 5	0	1	0	0	2	0
SP 6	0	0	0	0	5	0
SP 7	0	1	0	0	0	0
SP 8	0	1	0	0	1	0
SP 9	0		0	0	2	0
SP 10	0	2	0	0	0	0
OX 1	0	0	0	0	0	0
OX 2	0	0	0	0	0	0
OX 3	0	0	0	0	0	0
OX 4	0	0	0	0	0	0
OX 5	0	0	0	0	0	0
OX 6	0	0	0	0	0	0
OX 7	0	0	0	0	0	0
OX 8	0	0	0	0	0	0
OX 9	0	0	0	0	0	0
OX 10	0	0	0	0	0	0

Site	*Amphibola crenata*	*Diloma subrostrata*	*Cominella glandiformis*	*Zeacumantus subcarinatus*	% *Ulva*	% *Seagrass*
MB 1	0	0	0	50	0	0
MB 2	0	0	0	4	0	0
MB 3	2	0	0	50	0	0
MB 4	0	0	0	48	0	0
MB 5	0	0	0	38	0	0
MB 6	1	0	0	8	0	0
MB 7	0	0	0	3	0	0
MB 8	0	0	0	0	0	0
MB 9	1	0	0	0	0	0
MB 10	1	0	0	12	0	0
SC1-1	9	0	0	0	0	0
SC1-2	8	0	0	0	0	0
SC1-3	5	0	0	0	0	0
SC1-4	4	0	0	0	0	0
SC1-5	5	0	0	0	0	0
SC1-6	8	0	0	0	0	0
SC1-7	3	0	0	0	0	0
SC1-8	6	0	0	0	0	0
SC1-9	5	0	0	0	0	0
SC1-10	5	0	0	0	0	0
SC2-1	4	0	0	0	0	0
SC2-2	5	0	0	0	0	0
SC2-3	4	0	0	0	0	0
SC2-4	4	0	0	0	0	0
SC2-5	4	0	0	0	0	0
SC2-6	7	0	0	0	0	0
SC2-7	4	0	0	0	0	0
SC2-8	6	0	0	0	0	0
SC2-9	4	0	0	0	0	0
SC2-10	3	0	0	0	0	0
PL 1	0	0	0	0	0	0
PL 2	0	0	0	0	0	0
PL 3	0	0	0	0	0	0
PL 4	0	0	0	0	0	0
PL 5	0	0	0	0	0	0
PL 6	0	0	0	0	0	0
PL 7	0	0	0	0	0	0
PL 8	0	0	0	0	0	0
PL 9	0	0	0	0	0	0
PL 10	0	0	0	0	0	0

Appendix 3: Environmental Variables Recorded during the General Survey

Site	Total Nitrogen g/100g	Total Phosphorus mg/kg	Cadmium mg/kg	Copper mg/kg	Zinc mg/kg	% pore water	% organic content
TS1-1	0.06	348	0.03	4	38.9	22.2392829	1.382217953
TS1-2	0.08	363	0.04	5	44.1	27.92941043	2.251791198
TS1-3	0.09	434	0.05	5	43.7	24.64880996	1.98019802
TS2-1	0.07	340	0.04	4.6	42.4	31.68807936	2.547315796
TS2-2	0.09	388	0.05	5.6	47.9	33.080466	3.137651822
TS2-3	0.1	412	0.05	6	48.5	31.51591249	2.954657641
MB1	0.087	472	0.05	8.7	72	19.03818444	2.68446422
MB2	0.086	503	0.05	8.9	69	41.40027456	5.220883534
MB3	0.098	524	0.05	9.5	69.5	34.75343661	4.748766826
SP1	0.07	460	0.12	5.5	59.9	24.82424975	1.63901458
SP2	0.06	411	0.13	5.9	62.7	26.43342088	1.859211856
SP3	0.08	482	0.14	7.3	67.3	27.85185185	2.163391024
PL1	0.05	975	0.05	9.5	55.4	23.25581395	1.623611385
PL2	0.05	693	0.05	9.4	59.4	19.12245461	1.543781046
PL3	0.05	747	0.06	10	66	21.09801308	1.763277373
HS1	0.05	284	0.06	3.2	35.6	21.32810058	0.759314851
HS2	0.05	276	0.06	3.3	35.1	28.55855856	0.707275618
HS3	0.05	286	0.07	3.6	36.3	21.32523759	0.731494531
HD1	0.05	369	0.1	5.5	59.4	22.23318585	1.109201048
HD2	0.05	380	0.1	5.6	60.3	20.62044653	1.077688299
HD3	0.05	368	0.1	5.5	58.4	23.96482372	1.192884187
HC1	0.05	398	0.16	5.9	75.7	21.0592686	1.441795691
HC2	0.05	425	0.17	6.4	80.1	23.16087565	1.457617293
HC3	0.05	413	0.16	6.3	76.2	21.26720706	1.437709412
PPJ1	0.06	536	0.12	8.5	66.1	22.82227186	1.359173127
PPJ2	0.08	609	0.13	10.6	74.3	21.97536378	1.211485262
PPJ3	0.06	574	0.1	8.7	63.9	23.71458118	1.805660991
PPY1	0.11	729	0.19	15.3	91.9	30.05164146	3.011126931
PPY2	0.1	702	0.17	13.8	87.9	27.19375997	2.442983524
PPY3	0.11	762	0.2	15.4	99	26.55014159	2.335371798

Site	Total Nitrogen g/100g	Total Phosphorus mg/kg	Cadmium mg/kg	Copper mg/kg	Zinc mg/kg	% pore water	% organic content
BR1	0.05	409	0.05	4.9	40	28.4	2.045413588
BR2	0.05	380	0.04	4.5	37	23.23948372	1.542080502
BR3	0.05	383	0.04	4.3	37.9	23.79984147	1.415585003
OX1	0.08	397	0.17	8	74.3	23.87070227	1.891778272
OX2	0.07	358	0.15	6.8	66.6	24.60170973	2.093667461
OX3	0.08	413	0.16	8.2	74.2	27.27456694	2.164839446
TK1	0.11	1040	0.06	7.9	49.1	28.14814815	4.824341908
TK2	0.09	1190	0.06	9.8	59.6	24.69599672	4.218452327
TK3	0.08	908	0.06	7.6	49.2	27.11295063	4.921405646
SC1-1	0.11	503	0.03	8.3	49.8	19.91918751	1.077321696
SC1-2	0.09	587	0.03	8.5	51.8	19.87765434	1.053093462
SC1-3	0.15	606	0.04	9.2	52.5	21.65403624	1.261670452
SC2-1	Missing	Missing	Missing	Missing	Missing	23.39079962	1.990274794
SC2-2	Missing	Missing	Missing	Missing	Missing	25.73574409	2.172447374
SC2-3	Missing	Missing	Missing	Missing	Missing	24.11580148	1.691411389

Appendix 4: Average Number of Cockles/25 cm^2, Average Size and Total Number of <10 mm Cockles/25 cm^2 Recorded during the General Survey

SITE	**BR**	**BR**	**BR**	**BR**	**BR**	**BR**	**BR**	**BR**	**BR**	**BR**
TOTAL	73	58	53	67	64	25	20	14	3	21
SIZE	30.80	32.59	26.81	30.73	31.70	32.16	33.30	26.86	11.33	34.33
<10 mm	5	7	7	6	3	0	1	1	2	0

SITE	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**	**TS1**
TOTAL	30	46	41	81	56	55	41	71	100	62
SIZE	23.19	21.84	24.12	25.28	24.13	23.42	24.22	24.78	27.40	26.68
<10 mm	3	4	2	3	1	7	1	6	5	2

SITE	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**	**TS2**
TOTAL	31	36	37	38	46	88	40	17	62	36
SIZE	15.63	18.62	14.02	14.18	16.47	16.50	11.57	14.68	14.76	12.81
<10 mm	10	4	12	12	10	21	22	6	24	17

SITE	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**	**PPJ**
TOTAL	3	6	2	2	7	5	2	1	2	8
SIZE	17.12	17.92	23.93	18.74	21.02	4.06	23.08	22.57	32.06	24.10
<10 mm	1	1	0	0	2	5	0	0	0	1

SITE	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**	**HS**
TOTAL	1	9	7	8	6	8	5	2	10	4
SIZE	15.02	18.98	14.98	25.00	21.43	15.12	10.74	18.84	15.17	9.82
<10 mm	0	1	2	0	1	3	2	0	2	2

SITE	**HD**	**HD**	**HD**	**HD**	**HD**	**HD**	**HD**	**HD**	**HD**	**HD**
TOTAL	2	2	4	4	3	3	6	5	7	9
SIZE	19.13	9.97	8.91	13.74	24.82	14.19	20.00	3.27	7.45	11.31
<10 mm	1	1	2	1	0	1	0	5	4	4

SITE	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**	**PPY**
TOTAL	0	1	0	0	0	0	0	0	0	0
SIZE	0	20.21	0	0	0	0	0	0	0	0
<10 mm	0	0	0	0	0	0	0	0	0	0

SITE	**HC**	**HC**	**HC**	**HC**	**HC**	**HC**	**HC**	**HC**	**HC**	**HC**
TOTAL	12	16	16	11	13	7	11	11	11	4
SIZE	10.27	11.83	11.16	16.26	14.81	11.40	12.08	17.03	17.32	10.74
<10 mm	6	5	7	4	2	2	5	3	1	1

SITE	**TK**	**TK**	**TK**	**TK**	**TK**	**TK**	**TK**	**TK**	**TK**	**TK**
TOTAL	52	41	57	26	38	70	41	86	43	53
SIZE	20.24	22.75	21.12	18.26	20.11	16.17	22.31	20.50	19.58	22.14
<10 mm	4	0	4	5	4	12	1	7	5	2

SITE	OX	OX	OX	OX	OX	OX	OX	OX	OX	OX
TOTAL	6	5	11	12	6	5	12	5	11	6
SIZE	29.25	21.73	28.82	29.45	26.29	20.18	29.88	30.61	30.65	29.78
<10 mm	1	1	1	0	0	0	0	0	0	0

SITE	SP	SP	SP	SP	SP	SP	SP	SP	SP	SP
TOTAL	41	22	35	14	30	37	16	39	42	17
SIZE	24.32	25.59	23.89	23.85	21.88	25.99	22.16	21.49	23.93	26.92
<10 mm	1	0	0	1	3	1	1	5	0	0

SITE	MB	MB	MB	MB	MB	MB	MB	MB	MB	MB
TOTAL	9	11	3	14	5	2	3	0	6	4
SIZE	26.69	26.89	21.82	18.95	13.52	18.68	20.96	0.00	12.27	15.93
<10 mm	1	0	1	4	0	1	0	0	2	1

SITE	PL	PL	PL	PL	PL	PL	PL	PL	PL	PL
TOTAL	7	13	10	2	12	5	9	4	2	3
SIZE	22.43	26.48	25.47	24.43	21.94	22.11	22.32	19.77	15.47	24.26
<10 mm	0	0	1	0	0	0	0	0	1	0

SITE	SC1	SC1	SC1	SC1	SC1	SC1	SC1	SC1	SC1	SC1
TOTAL	23	17	11	8	24	11	17	30	14	17
SIZE	16.95	14.84	14.39	20.59	17.05	18.22	18.20	18.96	16.66	20.15
<10 mm	2	4	1	0	3	0	1	1	0	1

SITE	SC2	SC2	SC2	SC2	SC2	SC2	SC2	SC2	SC2	SC2
TOTAL	11	27	5	10	13	5	12	8	9	15
SIZE	26.45	28.72	24.71	26.74	21.41	7.31	23.05	17.50	16.85	22.47
<10 mm	1	0	0	0	2	3	1	4	3	3