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Cadmium and Zinc in Greenshell® mussels, *Perna canaliculus*, from Pelorus Sound, Marlborough, New Zealand

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Abstract

Pelorus Sound, Marlborough is the current centre of aquaculture in New Zealand. The main mariculture species of the area is the Greenshell® mussel, *Perna canaliculus*. To ensure that mussels grown in Marlborough can be exported to international food markets, the mussels’ trace metal levels have to be within limits set by food safety regulations. The levels of potentially toxic trace metals in mussels grown in Marlborough Sound were measured and found to be below the food safety limits set by the Australia and New Zealand Food Safety Council. Although all the levels tested showed some variability, those for cadmium (Cd) were the most variable. Detailed analysis of the variation of Cd and zinc (Zn) levels in mussels from Pelorus Sound was carried out, allowing a comparison of trends in the levels of these two metals.

Distance along Pelorus Sound and water depth explained most of the variation in the level of Cd in mussels. The distance from Pelorus River mouth was positively correlated with the level of Cd in mussels within inner Pelorus Sound. Zinc levels, though also variable between different farming locations, showed no relationship between Zn level and distance from Pelorus River mouth. The level of Zn was also dependent on the sex of the mussels, with the levels in female mussels being significantly higher; no such relationship was found for Cd.

Investigation of seasonal variability in Cd and Zn levels in mussels showed that both metal levels varied only slightly between months. Total metal content of the mussels, however, steadily increased as the tissue weight increased. Analysis of Cd and Zn levels within the tissue of mussels showed that the metals have a different distribution within tissue types. Highest Cd concentrations were in the gills and digestive tract, whereas Zn was distributed more evenly, with little differences between tissue types.

The comparison of Cd and Zn levels between a pure bred family line of mussels with mussels grown from wild-caught spat indicated a significant difference for both metals. Compared to *P. canaliculus*, significantly higher levels of Cd and Zn were found in *Mytilus galloprovincialis*. However, due to the presence of symbionts in *M. galloprovincialis* it was not possible to determine whether the difference in Cd and Zn levels was species related.

The level of Cd in Pelorus River water was determined, and the possible source(s) of Cd in the mussels from Pelorus Sound is discussed, along with suggestions for future research.
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Chapter 1
Introduction

Mussel aquaculture

The mussel *Perna canaliculus* (Gmelin 1791) is the most successfully farmed endemic species in New Zealand. Initially gathered from the wild, over-exploitation of the wild stock resulted in a collapse of the fishery (Stead 1971; Dias *et al.* 1986). Mussel landings from the wild reached a peak of 2000 tonnes in 1961 and then they dropped drastically throughout the 1970s and 80s to a final harvest of 15.4 tonnes in 1993 (New Zealand Seafood Industry 1994). To compensate for this declining catch, experimental raft farms were developed in Hauraki Gulf in 1963 (Greenway 1969), followed by Marlborough sounds in 1965 (Stead 1971). As technology and markets developed, farming of *P. canaliculus* became the fastest growing aquaculture industry in New Zealand.

The common name for *P. canaliculus* is the green-lipped mussel, however, the trade name Greenshell® mussel was established and registered allowing the development of exclusive marketing brands (New Zealand Fishing Industry Board and New Zealand Trade Development Board 1993). This branding prevents international markets of other green-lipped mussels (*Perna perna* and *P. viridis*) taking advantage of the New Zealand-based market development. Since the development of the Greenshell® name, significant funds have been used to develop a good brand image, using the “clean green” image of New Zealand to help promote the industry. In 2003 the export market was valued at NZ$ 133 million and the domestic market at NZ$ 30 million, with a harvest estimate of 78,000 tonnes (NZ Mussel Industry Council 2005).
The majority of New Zealand mussel farms are based on large long-line structures, a method originally developed in Japan and introduced in to New Zealand in 1974 (Jenkins et al. 1985). Increased mechanisation and adaptation of these structures to New Zealand’s weather conditions have allowed the development of the industry to become an efficient farming process (Jenkins et al. 1985). Generally, the mussel farms use up to ten long lines, each up to 110 m long. All long lines are anchored to the sea floor at both ends and supported on the surface by specially developed plastic buoys. The lines consist of two backbone ropes from which a continuous rope is looped (Figure 1.1).

![Diagram of mussel farm layout](image)

**Figure 1.1** Layout of a typical New Zealand longline mussel farm. Droppers loop every 0.5m with 30 - 40 surface buoys used to support the line depending on weight of mussel crop (Adapted from Jeffs et al. 1999).

Each loop, referred to as a *dropper*, is secured onto the backbone with a separate piece of rope. Depending on the depth of the site, the droppers are usually between 5 to 14 m long but can reach up to 30 m. Over 6000 m of continuous mussel line can be suspended on a single long line (Jenkins et al. 1985). Each line is seeded with mussel spat of approximately 40 mm length, at an average density of 180 mussels m\(^{-1}\) (Dick Jones pers. comm. 2004). The mussel spat is collected from three locations: Kaitaia and Golden Bay, each providing 40% of spat used in Marlborough, and locally-caught spat providing the remaining 20% (Dick Jones...
pers. comm. 2004). Small spat is grown to a suitable size on specially adapted spat farms with a seeding rate of up to 1000 spat m\(^{-1}\) (Patrick 2003). The spat from each location has different growth characteristics, which can enable a farmer to seed and grow out and harvest the mussels all year round (Patrick 2003).

Mussels are left for 12–18 months to grow until they reach harvestable size, of 80–110 mm (Jenkins et al. 1985). Once a suitable size is reached, the mussel’s flesh condition is tested regularly to enable harvesting at optimal weight. The closer mussels are to spawning, the heavier and therefore, more profitable the crop (Hickman et al. 1991). If the mussels have already spawned, and hence exhibit decreased condition, it is possible to leave them growing for another season. As the size and weight of mussels increase, the number of mussels that are lost, through dropping off the lines, dramatically increases. Although green mussels have the potential to grow much larger than average harvested size, there is only a small market for oversized mussels, and so extended growing periods are not economically viable (Jenkins et al. 1985).

The industry is located in four main regions: Marlborough/Nelson where over 75% of the industry is based, Coromandel, Stewart Island and to a small extent in Northland (Jeffs et al. 1999). Until recently mussel farms in New Zealand have been limited to locations with minimal exposure to violent wind and wave action and hence are often situated in sheltered bays close to the coast. Pelorus Sound in Marlborough, with many sheltered bays and inlets, has become the centre of mussel farming in New Zealand. This situation may change in the future with the proposed development of open ocean mussel farms offshore.

With the introduction of new legislation, the location of aquaculture farms is now dependent on regional councils setting aside suitable areas for marine farming. The Resource Management Amendment Act (2002) placed a moratorium on the development of new aquaculture farms for two years, to enable the government to properly legislate the industry under the Aquaculture Reform Bill (2004). The moratorium was put in place to allow regional councils time to determine the location of aquaculture management areas (AMAs), within which the industry is able to develop farms. The impact on the aquaculture industry
will be dependent on the individual regional council’s development of new Regional Coastal Plans, assigning suitable areas for AMAs through the Resource Management Act (1991).

In order for councils to assess the suitability of sites, many factors need to be determined, which include limiting environmental effects of aquaculture development and avoiding conflicts with other water users, while still providing water of suitable quality for aquaculture. Farms within an AMA are set terms of consent by the regional council, such as environmental impact monitoring. Existing farms have been temporally assigned AMA status until their permits expire. The allocation of new AMAs could potentially limit the development of new aquaculture ventures, especially if the water quality requirements of different aquaculture ventures are not considered individually.

**Trace metals in molluscs**

As little research has been carried out on trace metal uptake of *P. canaliculus*, it is important to give an overview of the variation in trace metal uptake in molluscs. It is widely accepted that molluscs bioaccumulate trace metals to high levels from sediment, water or food (Phillips 1977). Unfortunately the concentration of a trace metal in an animal might not relate directly to the concentration of that trace metal in the seawater. The cause of any discrepancies in the level of trace metal in seawater and a mollusc can be the result of changes in either environmental or physiological conditions (Phillips 1976; Graney *et al.* 1984). Anthropogenic inputs of trace metals into the marine environment can lead to some degree of elevated levels in seawater, but not to the levels typically observed in most marine invertebrates (Phillips 1977).

Most interspecies variation in trace metal levels can be explained by the different mechanisms of uptake, sequestering, storage, depuration of metals and in bivalves the clearance rate of water through the bivalve (Wang 2001). Intraspecies variation in trace metal levels can be almost as large as interspecies variation and is due to many different factors; some examples are age, condition (Lobel *et al.* 1991a), size (Ahn *et al.* 2001), season (Ouellette 1981) and sex (Latouche & Mix 1982). For example, because of relative rates of trace metal accumulation, the trace metal concentration in slow-growing animals can increase
with age, while in fast growing animals it can reduce with age, although in both cases total trace metal content increases (Phillips & Rainbow 1993). Increasing size can also reduce the surface-area-to-volume ratio, decreasing the effect of adsorbed trace metals to total body concentrations (Phillips & Rainbow 1993). Variation in trace metal levels of gametes, as well as sexual dimorphism, can explain some of the variation between sexes during certain stages of the reproductive cycle (Phillips & Rainbow 1993). Analysis of cadmium (Cd) variation between the sexes of *Mytilus edulis* shows that somatic tissue Cd concentration in females is significantly higher, but if the gonad and somatic tissues had not been separated, no difference would have been detected (Latouche & Mix 1982).

Seasonal changes relating to differences in environmental conditions, such as temperature and freshwater runoff, or physiological changes in the animal, such as reproductive cycle and dormancy, can have an effect on trace metal concentrations (Phillips & Rainbow 1993). Oysters have been found to exhibit much higher levels of seasonal variation than mussels (Phillips & Rainbow 1993). It has also been shown that a species may be affected by seasonal variations in one area and not in another (Phillips & Rainbow 1993). As a result, impacts of seasonal change on trace metal levels need to be analysed for each location and species (Fischer 1983).

Interaction and competition from other trace metals can enhance or reduce the level of trace metal uptake (Phillips & Rainbow 1993). Relationships between metals can be variable, changing with the concentration of trace metals, salinity, presence of chelating agents, pH and redox potential (Phillips & Rainbow 1993). For example, in a study of environmental variables that affect trace metal uptake in *Mytilus edulis*, Phillips (1976) found that low salinity increases net Cd uptake, especially at low temperatures, decreases the levels of lead (Pb), but has no affect on the uptake of zinc (Zn). In the same study, copper (Cu) levels were found to be so variable in the mussel, that it was suggested not to use the mussel in any Cu biomonitoring programme.

Even if all known sources of environmental and physiological variation of molluscs can be accounted for, there is still a level of "inherent variability" in the trace metal concentrations of molluscs (Phillips & Rainbow 1993). Variations in the uptake and
excretion of an element can result in variation between individuals of the same population, possibly indicating a genetic link to the inherent variability (Lobel et al. 1989). The solubility of elements is also linked to the level of inherent variability and thus alkali metals and non-metals, which are primarily stored in a soluble form, seem to have a low level of variability (Lobel et al. 1991b). Heavy metals, which are often found in insoluble granules within mollusc tissue, have the highest level of inherent variability (Lobel et al. 1991b).

Although most trace metal levels in molluscs have been investigated to some extent, the level of Cd in molluscs has been of particular interest. Although the mechanisms are not yet clearly understood, Cd accumulates in some molluscs to a high level. The accumulation of Cd in molluscs is particularly of concern in bivalves, where the level of Cd accumulated has become a concern for food safety reasons (Satarug et al. 2000).

Cadmiun in molluscs

The majority of the studies carried out on the Cd uptake have focused on pollution monitoring rather than food safety. As a result, extensive research has been carried out on a wide range of molluscs. Like most other trace metals, the concentration of Cd in a mollusc varies with changes in environmental and physiological parameters (Phillips 1976; Lobel et al. 1991b). Depending on the species of mollusc, the dissolved and particulate phases of Cd can have different effects on the level of Cd in the mollusc (Wang 2001).

Laboratory experiments indicate that Cd accumulated by Perna viridis correlated with the concentration of Cd in seawater after a period of 21 days (Chan 1988). The uptake of Cd by P. viridis from the dissolved phase has been found to be independent of the quality and quantity of seston (fine suspended matter), inferring that uptake from the dissolved and particulate phases is additive with no direct interaction between the two phases (Wang 2002). Yet the level of inorganic suspended matter has been reported to influence Cd uptake in the pearl oyster Pinctada carchariariam, from Shark Bay, Australia (McConchie & Lawrance 1991). Fine suspended hematite particles adsorbing Cd from high pH seawater, release Cd in the low pH of bivalve guts, explaining variations in Cd over an order of magnitude within a single bay (McConchie & Lawrance 1991). Laboratory experiments on the blue mussel M.
edulis show that the mussels accumulated the majority (82%) of Cd from suspended particles, but overall the concentration of Cd in mussels related proportionately to the total metal in both the dissolved and particulate phases (Wang et al. 1996).

Absorption efficiency, the rate at which trace metals can be absorbed from the environment, varies between species. For M. edulis the absorption efficiency for Cd from the dissolved phase was estimated at 0.31%, although salinity had an inverse effect on this rate (Wang et al. 1996). In the zebra mussel Dreissena polymorpha, absorption efficiency from dissolved phase for Cd was estimated at 1.02% (Ročiti & Fisher 1999). The mechanism by which Cd is absorbed by the molluscs is not fully understood. In freshwater bivalves dissolved Cd can be incorporated with the action of calcium (Ca) pumps (Markich & Jeffree 1994). To support molluscs’ high demand for Ca in shell formation, molluscs can use biological Ca pumps to increase levels of available Ca; the action of these pumps will incorporate Cd (Markich & Jeffree 1994). At low Ca concentrations, this route for Cd uptake can become predominant, especially at low salinities (Philips & Rainbow 1993).

**Trace metals in Perna canaliculus**

Most of the trace metal research carried out on mussels has used mussels of the genus *Mytilus*, especially in pollution monitoring programmes, such as Mussel Watch established in the 1970s. There appear to have been few published studies describing trace metal levels in *P. canaliculus* (Jeffs et al. 1999). Brooks and Rumsby (1965) analysed trace metal levels of molluscs in New Zealand, in all cases the level of Cd in mussels was below the limit of detection (10 ppm). Neilson and Nathan (1975), testing trace metal levels in molluscs from numerous locations around New Zealand, followed up this study, and found Cd levels in *P. canaliculus* ranged from < 0.1 – 1 ppm dry weight. A baseline study reported in 1985 of trace metal levels in shellfish from North Taranaki, found Cd levels in *P. canaliculus* of 0.9 – 1.1 ppm dry weight (Taranaki Catchment Commission 1985) and since then Cd and Zn levels have both dropped (Taranaki Catchment Commission 1988). A comparison of Cd wet weight levels in New Zealand finfish and shellfish showed that the level of Cd found in *P. canaliculus* from Nelson Bay (0.34 ppm) was closer to those of squid, *Notodarus* sp. (0.36 ppm), and paua, *Haliotus iris* (0.27 ppm), than to that of oysters, *Tiostrea lutaria* (4.75 ppm),
(Vlieg et al. 1991). More recently, a contaminant monitoring programme based in Auckland, which has monitored trace metal levels in oysters since 1987 and mussels since 1999, found Cd levels in most mussels to be below the limit of detection (0.37 ppm dry weight) with a maximum Cd level of 1.4 ppm dry weight (Kelly & McMurty 2004).

In a comparison of trace metal levels in *P. canaliculus* and *M. edulis*, by Mesa et al. (1999) examined the variation in trace metals of mussels from Spanish food markets. In this study the level of Cd in *P. canaliculus* was 0.46 ± 0.14 ppm dry weight, where the level of Cd in *M. edulis* was significantly lower and less variable at 0.38 ± 0.05 ppm dry weight. Of the other metals tested, only manganese (Mn) was notably different between the two species.

Anderlini (1992), in study of *P. canaliculus* trace metal levels in Wellington Harbour adjacent to a sewer outfall, found silver (Ag), Cu, Pb and Zn to have elevated levels due to the sewer input, but Cd levels were below the limit of detection (<1.0 ppm dry weight). In a similar study of trace metals in Auckland Harbour, Hickey (1992) investigated the seasonal difference in uptake of metals at polluted and unpolluted sites, using transplanted *P. canaliculus* as a biomonitor. In Hickey’s study, arsenic (As), aluminium (Al), iron (Fe), Mn, Cu, Zn and chromium (Cr) all exhibited some degree of seasonal variability, generally with winter maxima. Also, most of the metals showed a general trend of increasing concentration in industrialised areas, with decreased growth rates. Yet Hickey (1992) found the level of Cd in the mussels was below the limit of detection (not stated).

Only three studies, to date, have focused on factors that may affect the level of Cd in *P. canaliculus*. Kennedy (1986) examined the contribution of ingested sediment to the total body burden of molluscs in New Zealand. Some elements such as silicon (Si) and aluminium (Al) were found to originate totally from the gut contents, but sediment contribution for both Cd and Zn was low (<0.1 and <1.0% respectively) for both *P. canaliculus* and *M. galloprovincialis* (previously *M. edulis*). Neilsen (1974) examined the variation of Cd and Zn levels with depth in *P. canaliculus* grown on rafts in Kenepuru Sound, Marlborough, and off Waiheke Island, Hauraki Gulf. An increase in the Cd levels and decrease in the Zn levels with depth was found at the Kenepuru site, but no difference was detected at the Waiheke Island site. In Kenepuru Sound the mussel Cd levels ranged from 0.3 to 0.6 ppm wet weight
and Zn levels from 7 to 11 ppm. Finally, Arkless (2003) showed that *P. canaliculus* was able to bioaccumulate Cd directly from the dissolved phase.

**Cadmium health concerns**

Because of its toxicity, Cd is one of the main trace metals of concern in shellfish. Cd is one of the few elements that is not essential for biological systems and is toxic at low levels to most animals. Interfering with the uptake of essential minerals, Cd can cause irreversible renal damage and weakening of bones (Nath *et al.* 1984). A direct correlation between urinary Cd excretion over 2.5 μg g⁻¹ and an increased incidence of renal tubular dysfunction has been found (Buchet *et al.* 1990). Although the risk of kidney disorders from exposure to Cd is dependent on environment and genes (Bjorkman *et al.* 2000), it is especially high for women (Nishijo 2004). As a result, a provisional tolerable weekly intake of 7 μg Cd kg body weight⁻¹ has been accepted until more research can be conducted (JECFA 2003). Unfortunately this limit is already considered to be too high for 1% of the adult population (Satarug *et al.* 2000).

The main sources of Cd for humans are smoking and food, though only a small proportion of the total dietary Cd intake is absorbed and accumulated (Nath *et al.* 1984). The main source of dietary Cd depends on the individual. People especially susceptible to a high level of dietary Cd are those with a diet high in nuts, oils, seeds, molluscs or offal (especially liver and kidneys) (JECFA 2003). Regional differences in diet can lead to large variations in the risk of high Cd intake. In a study assessing an average diet of adult males in Catalonia, Spain, the weekly intake of Cd was calculated and the largest proportion of Cd found to be derived from shellfish (Llobet *et al.* 2003). In a contrasting study of typical Australian dietary intake and average Cd levels of food types, the main source of dietary Cd was found to be vegetables and cereals, including potatoes (Satarug *et al.* 2003). A high Cd intake can be due either to a high level of Cd in a food, or consumption of large volumes of food with a low level of Cd. Rice, wheat, starchy roots/tubers and molluscs have all been identified as major sources of Cd in diets, each with the potential to contribute 10% or more of weekly intake (JECFA 2003).
The present limit for Cd in Greenshell® mussels set by the Food Standards Australia New Zealand Authority is 2 ppm or 2 mg kg\(^{-1}\) wet weight (Commonwealth of Australia 2003). The CODEX on food additives and contaminants, an international food standards code developed by the Codex Alimentarius Commission, is currently considering a proposal to reduce the limit of Cd in mussels to 1.0 ppm wet weight (CODEX Alimentarius Commission 2005). Already the European Union has set a Cd limit of 1 ppm wet weight for bivalve molluscs (Commission of the European Communities 2001). The historical Cd content of \textit{P. canaliculus} sampled throughout New Zealand has been reported to range from 0.10 – 1.0 ppm wet weight (Nielsen & Nathan 1975). Reduction of the limit of Cd to 1.0 ppm could have an effect on the NZ mussel industry, particularly with respect to export markets such as the U.S.A. where the present recommended Cd limit is 3.7 ppm wet weight (Centre for Food Safety and Applied Nutrition 1993).

**Aims**

The aim of most trace metal biomonitoring studies is to determine the concentration of trace metal in an organism, isolating any variation in trace metal levels in the environment from other causative factors, to establish if the area is polluted or not. In contrast the main aims of this present study are to examine the level of trace metals in mussels to determine if variation in the uptake of trace metals and environmental levels of these metal could result in mussels exceeding food safety limits. The study’s aim specifically is to determine the level of Cd in \textit{P. canaliculus} within Pelorus Sound, analysing any spatial and temporal variation and identifying any factors that could cause variation in Cd levels, such as differences in size, condition and sex of mussels.

Together with Cd analysis, Zn levels in \textit{P. canaliculus} are also analysed. Determining the level of Zn in mussels will enable a comparison to be made between the two trace metals to establish if there is any correlation in their uptake. Unlike Cd, Zn is necessary for biological function, and any differences in the uptake of the two metals may highlight the different mechanisms for sequestering toxic metals in mussels.
By identifying factors that may be responsible for variation in Cd levels in mussels, it may be possible to identify sources of Cd pollution in the area. The identification of significant factors that affect the level of Cd in *P. canaliculus* may also allow the mussel industry to reduce or control levels of Cd in harvested mussels.

This study also compares trace metal content in the blue mussel *Mytilus galloprovincialis* with that of *P. canaliculus*. This research will determine if the uptake rate of the two mussel species is similar, allowing comparison with a wide range of trace metal research on *Mytilus* species.
Chapter 2

Historical Overview

Study Area

Marlborough Sounds is a remote and relatively unpopulated area of New Zealand. Excluding the main township Picton, there are approximately 2000 dwellings in Marlborough Sounds, 70% of which are holiday homes (Dias et al. 1986). Marlborough Sounds consists of three sounds, Queen Charlotte Sound, the location for the majority of the dwellings, Pelorus Sound and its side arm Kenepuru Sound, combined are currently the location of the over 50% of the mussel aquaculture industry in New Zealand (Figure 2.1). The main township of Pelorus Sound, Havelock (population 500), is situated at the head of Pelorus Sound near the mouth of Pelorus River. A port base in Havelock is the focus of most of the water use in Pelorus Sound, supporting a base of commercial and recreational fishers, with most of the recreational using the marina during the summer months (Sutherland 2000).

Pelorus sound is a drowned river valley, classified as a long residence time coastal inlet (Heath 1976). The total area high tide surface area of Pelorus Sound is $0.29 \times 10^3$ km² and has a residence time of 18 tidal periods (Heath 1982). Annual rainfall ranges from 1200 – 2000 mm, with the higher rainfalls generally being recorded over Mt Stokes (Figure 2.2) (Pascoe 1983).
Figure 2.1 Marlborough Sounds

Figure 2.2 Rainfall in Pelorus Sound (adapted from Pascoe 1983)
Historical Overview

Chapter 2

The average annual freshwater runoff into Pelorus Sound is 44 m³ s⁻¹ (Heath 1982). At times of high freshwater inflow a low salinity surface layer can form. This layer moves rapidly through the sound system mixing downward as it progresses (Heath 1982). Kenepuru Sound, a large side arm off the inner Pelorus Sound, can act to reduce the variability of the salinity. On the outgoing tide, Kenepuru Sound provides a large reservoir of seawater that mixes with lower salinity water from Havelock arm (Heath 1982).

The hydrology and nutrient fluxes in Pelorus Sound have been studied to determine if an adequate supply of nutrients, for phytoplankton growth, is available to sustain increased pressure for food from mussel farms (Bradford et al. 1987). High freshwater inflow from the Pelorus River provides a source of dissolved inorganic nitrogen and silica, and the open sea a source of nitrates (Bradford et al. 1987). The nitrogen source of the upper water column, when isolated from the benthic sediments during periods of stratification, is dominated by horizontal transport in Pelorus Sound during estuarine circulation of oceanic water (Gibbs et al. 2002).

Marlborough Sound topography is typically steep and consequently even small catchments generally drain directly into the sea (Laffan 1987). Therefore, changes in catchment land use and hydrology can have significant effects on the marine environment. Under heavy rainfall, substantial amounts of fine sediment can enter the marine ecosystem. From Pelorus River, suspended sediment input is estimated to be 319 x 10³ tonnes yr⁻¹, with another 246 x 10³ tonnes yr⁻¹ deposited from the Kaituna River and other sources (Griffiths & Glasby 1985). The high sediment output of the Pelorus and Kaituna Rivers has resulted in an extensive delta area of 18 km² in Pelorus Sound (Heath 1976 and 1982).

Soils up to 200 m above the water line are especially susceptible to land disturbances that expose the underlying subsoils, causing an increase in the production of fine sediments (Laffan et al. 1987). Soils below 200 m are “moderately to strongly acidic and moderately to strongly leached” (Laffan et al. 1987). Above 200 m the soils are less weathered and are not as susceptible to land disturbances. The NZ Genetic Soil Classification of Marlborough Sounds is southern yellow-brown earth (Gibbs 1980). This is a zonal soil type with the characteristics of the soil being dominated by the organic and climatic factors (Gibbs 1980).
Most of the soils in Marlborough Sounds have low nutrient status with low levels of phosphorus, molybdenum, and in some areas, sulphur (Laffan et al. 1987; Laffan 1987).

Geological research has shown there to be little differentiation of rock types in Pelorus Sound, with sandstone and siltstone dominating the area (Begg 2000). The majority of the rock in Pelorus Sound is part of the Caples terrane. The rock type changes to igneous and sedimentary rock in the northwest of Pelorus Sound where Crosilles melange enters into Tawhitinui Reach. Torelesse terrane on the south side of Queen Charlotte Sound, composed mainly of pelitic schist, is the only other terrane in Marlborough Sound (Figure 2.3). Torelesse terrane has high sulphide deposits, which have been shown to naturally concentrate Cd (Braithwaite & Rabone 1985).

Pelorus Sound has been extensively modified by settlement and farming practices. Over 70% of the land in Marlborough Sound had been cleared for new settlement by 1910 (cited in Sutherland 2000). High demands for land from new settlers and pastoral farming resulted in most of the original native bush being removed. Due to the poor nutrient status of the soils, to maintain suitable grass cover for livestock, pastoral farmers need to apply phosphate fertiliser without which the land quickly reverts to scrub (Laffan et al. 1987). With reductions in the profitability of pastoral farming due to access difficulties and high transportation, fertiliser and land costs, the majority of land has either been abandoned and has reverted to native bush, or it has been planted in pine (Pinus radiata) (Laffan 1987). The change to forestry has resulted in increased soil disturbances that could adversely affect marine life on near-shore sea-bed (Johnston et al. 1981).
Figure 2.3 Geological map of Marlborough Sound (after Begg 2000)
Historical Trace Metal Sampling

The Marlborough Shellfish Quality Programme (MSQP) monitors the quality of mussels and other shellfish as part of the IAIS 005 (Industry agreed implementation standards) NZ regulatory requirements for food safety. The primary responsibility of MSQP is to ensure that shellfish are only harvested from Marlborough Sounds at times when the shellfish will pose minimal health risk to the public. Two of the main concerns of MSQP are the level of microbiological contaminants and naturally occurring toxic shellfish poisons.

There are rigorous regulations imposed on the harvesting of shellfish to ensure that there is limited risk of exceeding safe levels of bacteria. The level of faecal coliform bacteria, used as an indicator of sewage contamination, is routinely tested to ensure food safety. As low levels of faecal bacteria in freshwater runoff from land can be quickly accumulated in shellfish, a rainfall maximum controls each mussel farming area. Once a harvest area has reached its freshwater limit the area is closed to harvest until the coliform count in the shellfish has diminished to below the regulatory limit of 14 / 100 ml of water or 300 / 100 g of shellfish (IAIS 005). Typically rainfall over a 24-hour period of 15 - 65 mm will result in closures of between two and seven days, and larger rainfall events would require longer closure periods (Jeremy Shearer pers. comm.).

A toxic algal bloom that produces biotoxins above the regulatory limit, risking toxic shellfish poisoning, closes an area to harvesting potentially for many months. Weekly shellfish and water samples are taken to test for biotoxins and the presence of any phytoplankton known to produce them. In contrast, the requirement for testing of trace metal levels in shellfish is once within every three years (IAIS 005).

In 1991 MSQP began an intensive programme to determine the level of trace metals throughout the Marlborough Sounds. Sampling at six monthly intervals, the variation in trace metal concentration between selected mussel farms over six years was investigated. From 1996, the trace metal levels of the mussels have been tested on a three-yearly basis. The data collected over the eleven years of sampling have been analysed in this present study.
Historical Methods

Pelorus Sound Variation of Trace Metals

The mussel trace metal testing programme was run throughout the sounds at 40 selected mussel farms. Samples were collected by MSQP from 1 m water depth during autumn or spring, with 227 samples collected over 11 years from 1991 to 2002. Although some farms were sampled only once, a selection of 16 locations were sampled regularly: Anakoha Bay, Hamilton Bay, West Forsyth, Wet Inlet, Whangakoko and Wairangi Bay on the outer coast, Brightlands, Four Fathom, Hallam Cove, Richmond Bay, Te Kopi Port Liglar in the outer Pelorus sound and Clova Bay East, Kenepuru Entrance, Waitaria Bay, West Beatrix, and Wilson Bay in the inner Pelorus Sound (Figure 2.4.).

Sampling started in August 1991, and between September 1992 and September 1996 samples were taken at six-monthly intervals. After this time, sampling was reduced to three-yearly as the trace metal levels were not found to increase enough with time to be a food safety concern.

Within-Bay Variation of Trace Metals

Testing was also carried out on the variation of trace metal levels within a bay. On 26 August 1993, five farms were sampled over an hour, all within 10 km of each other: Te Kopi Port Liglar, Cannon Hill, Danger Point, Horse Bay and Rat Point (Figure 2.5.).

Depth Variation of Trace Metal Levels

Three farms were also sampled to test for variation of trace metal levels with water depth: Hamilton Bay in Admiralty Bay on the open coast, Te Kopi Point in Port Liglar at the entrance to Pelorus Sound, and Kenepuru Entrance within inner Pelorus Sound. On 15 September 1992 each of these three farms was sampled at a depth of 10 m as well as 1 m. (Figure 2.5.).
Figure 2.4. Location of mussel farms sampled by MSQP for the analysis of temporal variation of trace metal levels in *Perna canaliculus* in Marlborough Sounds. NZ Map Grid 100,000.
Figure 2.5. Location of mussel farms sampled by MSQP for water depth and within-bay analysis of trace metal variation in *Perna canaliculus* in Marlborough Sounds, NZ Map Grid 100,000.
On each sampling occasion, up to 15 mussels were collected and sent to a commercial laboratory for trace metal analysis. In the laboratory, mussels were homogenised and a sample of the homogenate was used for chemical analysis. All results are presented as wet weight trace metal levels, as data on the dry weight of the homogenised samples was not available. An approximate conversion to dry weight is to multiply by a factor of five, calculated from the mean % solids (19.56%) reported by Hickman & Illingworth (1980) for *P. canaliculus* in New Zealand. Mussel samples were tested for total arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), nickel (Ni) and zinc (Zn). The analysis of the historical data was carried out as part of this current research.

Results

Trace Metal Variation in Pelorus Sound

Cr, Hg, Pb and Ni concentrations were frequently below the limit of detection, usually <0.03 ppm Hg, <0.5 ppm Cr and Pb, and <1.0 ppm Ni, although some results are reported lower than these limits. Therefore, it was not possible to consider these metals as part of the general analysis (Table 2.1). Mn levels were not tested in 1991.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mean (ppm)</th>
<th>Standard Deviation</th>
<th>Missing Data (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>0.016</td>
<td>0.007</td>
<td>48.7</td>
</tr>
<tr>
<td>Arsenic</td>
<td>2.08</td>
<td>0.91</td>
<td>0</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.64</td>
<td>0.44</td>
<td>0</td>
</tr>
<tr>
<td>Chromium</td>
<td>0.31</td>
<td>0.18</td>
<td>65.1</td>
</tr>
<tr>
<td>Copper</td>
<td>0.98</td>
<td>0.22</td>
<td>0</td>
</tr>
<tr>
<td>Lead</td>
<td>0.08</td>
<td>0.028</td>
<td>84.1</td>
</tr>
<tr>
<td>Manganese</td>
<td>3.01</td>
<td>1.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.39</td>
<td>0.18</td>
<td>60.8</td>
</tr>
<tr>
<td>Zinc</td>
<td>11.4</td>
<td>2.1</td>
<td>0</td>
</tr>
</tbody>
</table>
ANOVA (Analysis of variance) of the remaining trace metal concentrations showed that there was significant variation between the years for the levels of As, Cd, Mn and Zn, but only Cd and Mn levels differed significantly between the farms (Table 2.2). None of the trace metal levels were significantly different between the seasons sampled. Analysis of interactions between factors was not possible as too few samples were collected to determine this statistically.

Table 2.2 p values for ANOVA of trace metal levels in *Perna canaliculus* from Pelorus Sound from 1991 - 2002 (MSQP 2002).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Year</th>
<th>Season</th>
<th>Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>0.000</td>
<td>0.786</td>
<td>0.238</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.000</td>
<td>0.933</td>
<td>0.000</td>
</tr>
<tr>
<td>Copper</td>
<td>0.218</td>
<td>0.842</td>
<td>0.916</td>
</tr>
<tr>
<td>Manganese</td>
<td>0.002</td>
<td>0.876</td>
<td>0.047</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.000</td>
<td>0.119</td>
<td>0.104</td>
</tr>
</tbody>
</table>

Arsenic, Cd, Mn and Zn showed a reduction in concentrations over the 11-year sampling period. Tukey analysis of subset groupings showed only Zn levels had two significantly different subsets, with significantly higher Zn levels in 1991 and 1992 compared to the other years (Table 2.3). Cd showed the greatest variation among the years with four different year groups, but because of the limited number of samples and variation between farms, a significant difference was not evident as all year groups overlap to some extent.
Table 2.3 Tukey analysis of trace metals levels between years in *Perna canaliculus* from Pelorus Sound from 1991 – 2002. Each year is grouped into similar means and each group is ranked from lowest to highest (MSQP 2002)

<table>
<thead>
<tr>
<th>Year</th>
<th>Cadmium</th>
<th>Arsenic</th>
<th>Zinc</th>
<th>Manganese</th>
</tr>
</thead>
<tbody>
<tr>
<td>1991</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>1992</td>
<td>4</td>
<td></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>1993</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1994</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>1995</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>1996</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1999</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2002</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Both Cd and Mn showed significant difference between the farms (p<0.001 and p=0.047 respectively). Tukey analysis of the different Mn levels between the farms showed that this difference was only between Four Fathom and Whangakoko farms (Table 2.4). Variation of Cd levels between the farms also showed a significant difference. Tukey analysis of Cd showed seven different subsets between the farms, but all subsets overlap. Analysis of the different groupings for Cd showed that the level of Cd increased from the mouth of the Pelorus River to the open coast, with the exception of Wairangi Bay in Croisilles Harbour on the Nelson side of French Pass.
Table 2.4 Tukey analysis of trace metals levels between farms in *Perna canaliculus* from Pelorus Sound from 1991 – 2002. Farms are grouped with similar means, and groups are ranked for each metal from lowest to highest. Farms are listed in order from Pelorus River Mouth to the outer coast (MSQP 2002).

<table>
<thead>
<tr>
<th>Farm</th>
<th>Cadmium</th>
<th>Arsenic</th>
<th>Copper</th>
<th>Managanese</th>
<th>Zinc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenepuru Ent.</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Waitaria Bay</td>
<td>1 2</td>
<td>1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Four Fathom</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Wilson Bay</td>
<td>1 2 3</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Wet Inlet</td>
<td>2 3 4</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Clova Bay E.</td>
<td>2 3 4</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>West Beatrix</td>
<td>3 4 5 6</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Richmond Bay</td>
<td>3 4 5</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hallam Cove</td>
<td>4 5 6 7</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brightlands</td>
<td>6 7</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Te Kopi Pt Ligar</td>
<td>5 6 7</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>West Forsyth</td>
<td>4 5 6 7</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anakoha Bay</td>
<td>4 5 6</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Hamilton Bay</td>
<td>7 1</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Wairangi Bay</td>
<td>1</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Whangakoko</td>
<td>4 5 6</td>
<td>1 1 1</td>
<td>1 2</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Statistical analysis of the interaction of farm and year was also not possible due to the low numbers of samples taken. However, Cd levels in mussels showed significant variation between a number of farms and years. Graphical interpretation of the Cd results showed some degree of interaction as the difference in the Cd levels between the farms decreased over the sample years. This difference in Cd levels is illustrated in Figure 2.6, by selecting four farms representing each of the seven different groups isolated by the Tukey anlysis. Where initially there was a wide spread in Cd levels between the farms, this spread of results had completely disappeared in the 1999 samples.
Trace Metal Variation around Port Ligar

Analysis of the trace metal concentrations in mussels from five farms in and around Port Ligar showed that variation between the farms was low. The levels of Hg, Pb and Ni were all below the limit of detection. Standard deviations for the remaining metals for the five farms were all less than 16%, close to the error estimated for trace metal analysis (Table 2.5).
Table 2.5 Trace metal analysis of *Perna canaliculus* grown at five farms around Port Ligar on 26/08/1993, ppm wet weight (MSQP 2002).

<table>
<thead>
<tr>
<th></th>
<th>Hg</th>
<th>As</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Pb</th>
<th>Mn</th>
<th>Ni</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Te Kopi</td>
<td>&lt;0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.4</td>
<td>1.1</td>
<td>0.1</td>
<td>0.7</td>
<td>&lt;0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.1</td>
<td>&lt;0.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.9</td>
</tr>
<tr>
<td>Cannon Hill</td>
<td>&lt;0.01</td>
<td>1.4</td>
<td>1.2</td>
<td>0.1</td>
<td>0.7</td>
<td>&lt;0.5</td>
<td>1.2</td>
<td>&lt;0.5</td>
<td>9</td>
</tr>
<tr>
<td>Danger Point</td>
<td>&lt;0.01</td>
<td>1.6</td>
<td>0.8</td>
<td>0.1</td>
<td>0.9</td>
<td>&lt;0.5</td>
<td>1.4</td>
<td>&lt;0.5</td>
<td>9</td>
</tr>
<tr>
<td>Horse Bay</td>
<td>&lt;0.01</td>
<td>1.8</td>
<td>1.1</td>
<td>0.1</td>
<td>0.9</td>
<td>&lt;0.5</td>
<td>1.5</td>
<td>&lt;0.5</td>
<td>9.8</td>
</tr>
<tr>
<td>Rat Point</td>
<td>&lt;0.01</td>
<td>1.6</td>
<td>1.1</td>
<td>0.1</td>
<td>0.7</td>
<td>&lt;0.5</td>
<td>1.6</td>
<td>&lt;0.5</td>
<td>9.4</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td>&lt;0.01</td>
<td>1.56</td>
<td>1.06</td>
<td>0.1</td>
<td>0.78</td>
<td>&lt;0.5</td>
<td>1.36</td>
<td>&lt;0.5</td>
<td>9.22</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>0.17</td>
<td>0.15</td>
<td>&lt;0.01</td>
<td>0.11</td>
<td>0.21</td>
<td>0.38</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Lower limits of detection for Hg, Pb and Ni respectively

Trace Metal Variation with Depth

For all samples, Pb levels were below the limit of detection (<0.15 ppm wet weight). The difference between the 1 m and 10 m samples was greatest for Mn, with Kenepuru samples having 75% higher concentrations at 1 m than at 10 m. Both Mn and Cd had higher concentrations at 1 m at all farms, while Cr and Ni concentrations were lower at 1 m at all farms (Figure 2.7). The difference in Mn concentration with depth increased from the outer coast to the inner sound, whereas the opposite trend held for Zn, with mussel from Kenepuru entrance having the lowest concentration difference while the largest difference was in mussels from the outer coast.
Figure 2.7 Difference in trace metal concentrations in *Perna canaliculus* between 1 m and 10 m depth at three different locations in Marlborough Sounds September 1992 (ppm wet weight), (MSQP 2002). A positive difference indicates higher mussel trace metal concentrations at the surface.

Discussion

Marlborough Sounds could be considered to be a relatively pristine environment with low levels of anthropogenic inputs, yet variability of some trace metals levels in *P. canaliculus* was higher than expected. Some trace metal levels in mussels from Pelorus Sound are more variable than for populated locations of New Zealand such as Auckland (Hickey 1992) and Otago Peninsula (Linwood 1993). The level of Cd is particularly variable compared to other studies, even on an international level (Table 2.6). Some of the variation in trace metal levels can be explained by variation between sample sites and years. Although when the sample locations are grouped into farms from the inner Sounds, outer Sounds and outer coast, the variation of trace metal levels in mussels within each group is still large.
Table 2.6  A selection of reported concentrations of trace metals in *Mytilidae* (ppm wet weight). Dry weight results were converted to wet weight using a factor of 0.2.

<table>
<thead>
<tr>
<th>Location</th>
<th>Species</th>
<th>Cd</th>
<th>Cr</th>
<th>Cu</th>
<th>Mn</th>
<th>Zn</th>
<th>Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marlborough MSQP data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inner Sounds</td>
<td><em>P. canaliculosis</em></td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>0.7</td>
<td>0.6</td>
<td>2.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Outer Sounds</td>
<td><em>P. canaliculosis</em></td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>0.7</td>
<td>0.5</td>
<td>1.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Outer Coast</td>
<td><em>P. canaliculosis</em></td>
<td>0.1</td>
<td>&lt;0.1</td>
<td>0.8</td>
<td>0.6</td>
<td>1.5</td>
<td>0.7</td>
</tr>
<tr>
<td>New Zealand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banks Peninsula¹</td>
<td><em>Mytilus sp.</em></td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.4</td>
<td>0.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Otago Peninsula¹</td>
<td><em>Mytilus sp.</em></td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>0.5</td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Doubtful Sound¹</td>
<td><em>Mytilus sp.</em></td>
<td>0.4</td>
<td>1.0</td>
<td>0.1</td>
<td>0.4</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Auckland²</td>
<td><em>P. canaliculosis</em></td>
<td>0.3</td>
<td>0.6</td>
<td>1.3</td>
<td>2.3</td>
<td>3.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Tasman³</td>
<td><em>P. canaliculosis</em></td>
<td>0.2</td>
<td>0.3</td>
<td>0.2</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kenepuru Sound³</td>
<td><em>P. canaliculosis</em></td>
<td>0.3</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marlborough³</td>
<td><em>P. canaliculosis</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wellington³</td>
<td><em>P. canaliculosis</em></td>
<td>0.1</td>
<td>1.0</td>
<td></td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stewart Island³</td>
<td><em>Mytilus sp.</em></td>
<td>0.6</td>
<td>1.6</td>
<td></td>
<td>2.0</td>
<td>17.0</td>
<td></td>
</tr>
<tr>
<td>International</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Netherlands⁵</td>
<td><em>Mytilus sp.</em></td>
<td>0.1</td>
<td>0.6</td>
<td>0.3</td>
<td>0.4</td>
<td>2.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Hong Kong⁶</td>
<td><em>P. viridis</em></td>
<td>&lt;0.1</td>
<td>0.3</td>
<td></td>
<td>1.7</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>Mediterranean⁷</td>
<td><em>Mytilus sp.</em></td>
<td>0.1</td>
<td>1.2</td>
<td>0.1</td>
<td>5.8</td>
<td>3.5</td>
<td>30.8</td>
</tr>
<tr>
<td>Canada⁸</td>
<td><em>Mytilus sp.</em></td>
<td>0.2</td>
<td></td>
<td></td>
<td>1.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia⁹</td>
<td><em>Mytilus sp.</em></td>
<td>0.2</td>
<td>0.1</td>
<td></td>
<td>3.4</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Northern Ireland¹⁰</td>
<td><em>Mytilus sp.</em></td>
<td>0.1</td>
<td>1.4</td>
<td>0.3</td>
<td>89</td>
<td>3.9</td>
<td>3.0</td>
</tr>
<tr>
<td>England¹¹</td>
<td><em>Mytilus sp.</em></td>
<td>0.2</td>
<td>0.6</td>
<td>0.1</td>
<td>1.1</td>
<td>3.0</td>
<td>8.2</td>
</tr>
</tbody>
</table>

¹ Linwood (1993)
² Hickey (1992)
³ Neilsen and Nathan (1975)
⁴ Neilsen (1974)
⁵ Stronkhorst (1992)
⁶ Phillips (1985)
⁷ Fowler & Oregioni (1976)
⁸ Lobel et al. (1990)
⁹ Richardson et al. (1994)
¹⁰ Gault et al. (1983)
¹¹ Giusti et al. (1999)
Most research into trace metal levels in bivalves, so far, has focused on the impact of pollution. A study of trace metal levels in mussels in Wellington Harbour showed that sewage resulted in a 50% increase in levels of Ag, Cu and Zn in mussels, whereas Pb showed a 600% difference in levels (Anderlini 1992). Variation of Cd levels in mussels from Pelorus Sound (almost ten fold difference) would indicate a source of pollution, yet none is apparent that would account for such high variation.

With such a high degree of variation in trace metal levels and no replication of sampling, the power of any analysis of these data is low. Therefore, there is a high risk of a Type II error (accepting no difference between populations when one exists). Only very large differences between populations can be detected. However, a significant difference between the growing areas for Cd and Mn has been shown. It is also possible to detect a significant difference between years for the levels of Cd, Mn, As, and Zn, with the level of all but Mn decreasing during the sampling period. The reduction in trace metal levels over the sampling period and the variation in trace metal levels between the sample sites cannot, as yet, be explained by simple known causes of variation in trace metal levels, such as pollution input or weathering of geological deposits.

Analysis of homogenised samples reduces the variability of trace metal levels to some extent, giving a mean trace metal level for all the mussels processed. A homogenised sample size of 10 – 15 mussels was shown to give a representative mean of trace metal levels in a mussel population (Bayne et al. 1981; Lobel et al. 1991a). Analysis of trace metals levels in mussels from around Port Ligar illustrates the concept behind homogenised samples. The variation of trace metal levels between farms around Port Ligar ( < 16 %), was lower than what would be expected between individual bivalves at the same location (approximately 30%), (Daskalakis 1996). The low level of variation between the farms in Port Ligar indicates that variation in Cd levels throughout Pelorus Sound is related, to some extent, to large distances of over 10 km.

The mussel samples taken from 1 m and 10m show that there is a change in the relationship between some of the metals and the growing region with water depth, although too few samples were taken to analyse this difference statistically. A simple description of
the findings shows that Cd and Mn are at a consistently higher concentration in mussels from 1 m, conflicting with the Kenepuru Sound findings of Nielsen (1974). Ni and Cr show the opposite trend, with higher concentrations in the deeper mussel samples. The changes in the relationship between metal concentration and depth at the three different sites indicate the complexity of the relationship between trace metals in water and physiological mechanisms of mussels for processing different metals.

The most important implication for food safety concerns is the variation of Cd between the different growing areas. The results clearly indicate the need for monitoring of Cd levels on a wide scale throughout the Marlborough Sounds area. They also raise the question as to the source of Cd for the mussels. If the source is anthropogenic or sedimentary arising from riverine input, one would expect the level of Cd to be higher within the Sounds and decrease towards the open ocean (Cook Strait). However, in fact higher Cd levels are found in the outer Pelorus Sound and on the outer coastline within Cook Strait, which implies an oceanic source of Cd.

The level of trace metals found in mussels from Pelorus Sound were below the maximum level of metal contaminants in food, set by Australia New Zealand Food Standards, but some locations exceed the current European and proposed CODEX limit of 1 ppm for Cd (Table 2.7).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Arsenic (ppm)</th>
<th>Cadmium (ppm)</th>
<th>Lead (ppm)</th>
<th>Mercury (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia and New Zealand</td>
<td>1*</td>
<td>2b</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>European Community</td>
<td></td>
<td>1</td>
<td>1.5†</td>
<td>0.5</td>
</tr>
<tr>
<td>America</td>
<td>86</td>
<td>4</td>
<td>1.5</td>
<td>1.0</td>
</tr>
<tr>
<td>Proposed CODEX</td>
<td></td>
<td>1c</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

*Commonwealth of Australia (2003),
†Commission of the European Communities (2001)
‡Commission of the European Communities (2005)
†FDA (2001)
§CODEX Alimentarius Commission (2005)
*Arsenic limit is inorganic arsenic only
bExcluding Bluff oysters and scallops
cExcluding oysters and scallops
As only a small number of farms exceed these limits, the impact to the mussel industry currently would only extend to restricting exports to Europe from certain farms. The proposed CODEX limit for molluscs has just been sent to step five of an eight-step process (CODEX Alimentarius Commission 2005). Although the proposed maximum Cd level for molluscs has received much attention and discussion, most opposition to the proposed limit was due to the inclusion of oysters. As oysters have now been removed from the proposed limit, it is possible that there will be little objection to its introduction. If the CODEX maximum limit for Cd of 1 ppm is introduced, then the few farms that exceed current European Cd limits within Marlborough Sounds could become unfeasible.

Conclusion

The historical data collected by MSQP between 1991 and 2002 show that there is some variation in trace metal levels between the years and growing areas. Because the data set for individual farms is small, the analysis is only able to detect large differences. Even so, it is possible to detect a significant difference in the concentrations of As, Cd, Mn and Zn.

No information is available on the variation of trace metals of individual mussels in each sample, as the analysis was carried out only on homogenised samples. Without establishing the level of variation between individual mussels, the results do not give sufficient information about the ability of different farm areas to grow mussels within the food safety limits specified for mussels. The historical mean Cd level in mussels from Pelorus Sound, 0.64 ± 0.44 ppm wet weight (Table 2.1), is well below the current NZ food safety limits of 2 ppm. However, there is still a possibility that certain growing areas may be excluded from some export markets, especially to Europe where the limit is 1 ppm wet weight.

The decline in the level of Cd, As and Zn over the period of testing also raises the question as to why this has occurred and whether the decline will continue. The only apparent change in anthropogenic inputs into Pelorus Sound during that period has been a reduction in the proportion of land used for pastoral farming (Sutherland 2000), and an increase in the number of mussel farms. If this decline in the trace metal content of the mussels grown in
Pelorus Sound continues, then the risk of Cd levels exceeding the food safety limits may be minimal.
Chapter 3
General Methods

Selection of Sample Sites

The historical MSQP data show a trend of decreasing Cd levels over the eleven-year sampling period. During earlier sampling from 1991 to 1996, a clear trend of increasing Cd levels in P. canaliculus along the inner reaches of Pelorus Sound was apparent. The lowest concentrations of Cd were in mussels grown at Kenepuru entrance, with Cd levels increasing out to the open ocean (Chapter 2). By 1999, Cd concentrations in mussels had declined such making any trend harder to distinguish. The reasons for the decline in Cd levels in mussels over sampling years and the increasing level of Cd towards the ocean was not understood. In order to examine these trends in-depth, a more intense sampling programme was required. Rather than sample the whole of the Pelorus Sound, which would spread the sampling over a large area, the present study focuses on the variation within the inner Pelorus Sound for analysis of spatial variation. Port Ligur was selected for analysis of temporal variation as it exhibits the largest standard deviation in trace metal levels between the years of historical sampling within Pelorus Sound (see Figure 2.5).

The final selection of the farms was made in the field, with the selection depending in part on the size of the mussels. Only farm lines with mussels of harvestable size were selected, ensuring any mussel samples would be similar to the size of mussels marketed commercially. Within the farm, the line that was selected for sampling had mussels that were expected to be close to harvestable condition, although this did not always prove to be the case. This process of selection ensured that size and condition of the sampled mussels were as similar as possible throughout the sampling, limiting the independent variation of size and condition between sample sites. Each selected bay within inner Pelorus Sound was sampled twice, once in April and once in October 2004, except for Little Nikau Bay and Clova Bay, which were sampled only in October, and Beatrix Bay for which two separate farms were sampled in April and only one farm in October.
In Port Ligur the mussels were not in harvestable condition all year round and so it was not possible to select for harvest size or condition for the analysis of temporal variation. Samples from Port Ligur were collected monthly by MSQP staff and frozen before dispatch to Dunedin for analysis. Some sampling periods for Port Ligur were delayed due to weather restricting access to the site resulting and in no sampling for some months, but two samples were collected in the following month.

Sample Size

The sampling procedure used by MSQP for the historical data did not allow direct comparison of the variation of trace metal levels within the samples of *P. canaliculus*, as the data were limited to homogenised groups of mussels. A Cd concentration of 0.19 ± 0.04 ppm (95% confidence interval, n=14), with a standard deviation of 36%, has been observed for individual mussels sampled from Kaikai Beach, Otago (Arkless 2003). Similar levels of variation were found for Cd and Zn in the oyster, *Crassotrea virginica* (29 and 38 % respectively) (Daskalakis 1996). The number of samples required to detect a significant difference between the sample sites depends on the difference between the sites and the number of samples tested, as shown in Table 3.1.

Table 3.1 Required sample size to detect a two-sided significant difference between populations with a standard error of 36% and a significance level of 0.05 (UCLA 2004).

<table>
<thead>
<tr>
<th>Difference (%)</th>
<th>Sample size</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Power =0.90</td>
<td>Power =0.80</td>
</tr>
<tr>
<td>10</td>
<td>131</td>
<td>99</td>
</tr>
<tr>
<td>20</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>30</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>40</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

To keep the statistical power high and the number of samples low, the difference between the populations must be high. The Pelorus Sound data from MSQP (2002) exhibited differences in Cd levels between the farms ranging from 15 to greater than 100%. A sample size of 10 to 15 mussels has been recommended as a suitable sample size for homogenised samples (Bayne *et al.* 1981; Lobel *et al.* 1991a). Assuming little variation with trace metal
levels with depth, a sample size of 15 at each farm would enable the detection of 30% differences between the farms. To ensure that sufficient mussels were collected to allow the detection of an optimum range of differences between the depths and sites, a field sample size of 10 was chosen for each depth. Once back in the laboratory, a reduced number of the mussels were randomly selected from each sample. This allowed the calculation of optimal sample size post-collection of five at each depth and a minimum of 15 from each farm.

**Mussel Sampling Procedure**

A mussel farm boat was used to collect mussels from the selected sites. To undertake this task, a mussel dropper line was raised by winch above the water and samples were collected. Surface, mid dropper and bottom of dropper were sampled at each farm during initial sampling. On subsequent sampling, the sample was taken from mid dropper except for Grants Bay where the sample was taken from all three depths. Not all of the farms grow mussels in water of the same depth; as a result the length of the droppers varies between some farms. As the length of the dropper varies, so does the depth at which the mussels were sampled in each farm. Although this selection method is not ideal, it has enabled a representative sample to be collected for the range of growing depths. Once sampled, mussels were washed to remove most of the epibiota and attached sediments. Barnacles and tubeworms were too difficult to remove in the field and as a result, a small percentage of epibiota remained on the shells. Once washed, samples were placed into clean polythene bags and kept cool until their return to port in Havelock.

Mussels sampled on 6th April 2004 were then weighed to determine a live whole weight and individually bagged before being frozen at -25°C. During the weighing of the mussels it was noted that some had already started to gape and thus losing internal cavity fluid. As it was not possible to reduce the time between sampling and weighing, whole live weight was not used in the subsequent analysis. Mussels sampled on 26 October 2004 were frozen directly on return to port and no live whole weight was recorded. All mussels were frozen within 12 hours of collection. Mussels sampled outside these dates, were collected by MSQP staff from mid-water and then frozen.
Depuration, a process of enabling live mussels in clean water to purge gut contents, is recommended in many biomonitoring studies, as the contribution of some trace metals from the sediment and food in the gut can be significant (Phillips & Rainbow 1993). For example Cr, Mn, and Pb levels have been found to correlate with the level of trace metal in the ingested sediment, while other metal levels, such as Cd and Zn, do not (Flegal & Martin 1977; Bertine & Goldberg 1972). Kennedy (1986) showed in New Zealand that ingested sediment in *P. canaliculus* from unpolluted sites contributed undetectable quantities of Cd and less than 1% of total Zn. The depuration process could potentially introduce a higher level of contamination than the contribution of Cd and Zn from sediment in the gut. Mussels harvested from Pelorus Sound are currently not depurated. Therefore, as the contribution of Cd and Zn from ingested sediment is probably low, and to provide a representative sample of harvested mussels, mussels sampled in the present study were not depurated.

**Condition Index**

One of the most variable factors in the analysis of mussels is their nutritive status (Hickman & Illingworth 1980). The Condition Index (CI), a comparison between the amount of soft mussel tissue and the internal shell capacity, is commonly used as a simple method to gauge the health of the mussel (Crosby & Gale 1990). As a mussel approaches spawning, the CI increases as the mussel reaches its highest percentage flesh content, post-spawning the CI drops (Hickman & Illingworth 1980). There are many established methods to determine the condition of a bivalve mollusc (Crosby & Gale 1990). The main distinction between the methods is usually the technique used to estimate the internal shell capacity, which, due to the irregular shape of a shell, is not easy to measure accurately. A displacement method can be used to establish volume (Baird 1958), but is considered to be an inefficient and inaccurate method for a large number of shellfish (Hickman & Illingworth 1980; Lobel et al. 1991).
Hickman & Illingworth (1980), in a study of the condition cycle of *P. canaliculus* around New Zealand, compared seven different methods of analysis. From this and other research, the “precise index” recommended for biological studies is:

\[
\text{Condition Index} = \frac{100 \times \text{dry flesh weight}}{\text{Whole (live) weight} - \text{Shell weight}}
\]

(Hickman & Illingworth 1980; Lawrence & Scott 1982).

This method uses a simple premise that the internal cavity of a live bivalve will be completely full with soft tissue and cavity fluid. Assuming a density of all the contents to be 1 g cm\(^{-3}\), the whole weight minus the shell weight gives an accurate estimate of the internal shell cavity, accounting for any variations in the shell size as well as any epibiota (Abbe & Albright 2003). For this method to work, the mussels must be weighed before they start to gape and lose cavity fluid. Unfortunately, the time between collection and processing prior to freezing in the present study did not allow this measurement to be made accurately, as some of the mussels had started to gape in the intervening 6 hours. Mussels could not be kept in water or returned to water after sampling, as contamination risks were too high for successful subsequent trace metal analysis. As a result, this preferred method of determining CI had to be rejected in the present study.

Measurement of the size of a bivalve can be used to estimate the volume, provided the shell shape is consistent. Shell length and whole live weight are directly correlated in *P. canaliculus*, \( r^2 = 0.98 \) (Hickman 1979), but length alone does not account for all the variations in shell size. The use of the three basic dimensions of a shell provides a rapid and consistent representation of the volume of the mussel (Lobel *et al.* 1991\(^a\)). Mussels were measured in the present study along the length, width and height of their shells to the nearest 0.1 mm using digital callipers (Figure 3.1). The cultivated mussels are fast growing with similar shell shape and thickness, and so this method gives a representative value for the volume of the shell, thus providing a simple method of establishing the CI of the mussel.
To obtain an accurate measurement for the soft tissue content, the dry weight is preferred, as the wet tissue is susceptible to rapid water loss (Hickman & Illingworth 1980). Wet weight of the soft tissue can fluctuate dramatically over short periods of time, as bivalves can increase water uptake to compensate for poor condition (Lucas & Beninger 1984).

From all the above considerations, the following CI from Lobel et al. (1991) was used in the present study:

\[
\text{Condition Index} = \frac{1000 \times \text{Soft Tissue Dry Weight (g)}}{\text{Length} \times \text{Width} \times \text{Height of Shell (mm)}}
\]

**Sexing the mussels**

On opening the shell of a mussel it is possible to determine the sex of a mature mussel by colour. A mature female mussel develops an orange gonad and intermantle while the male mussel tissue is a creamy white colour (Jenkins et al. 1985). While this colour differentiation is simple and quick, it does not always identify females that have yet to reach maturity. To
determine the sex of all the mussels accurately would require a microscopic examination of
the gonad tissue (Buchanan 2001). As the mussels collected for most of the present research
were expected to be mature and close to peak condition, this method was considered to be
unnecessary.

Spat Origin

A significant genetic difference exists between North and South Island populations of
mussels (Smith 1988). Two genetically isolated populations of mussels have been identified,
a southern population and a northern population which includes the top of the South Island
north of 42° S (Star et al. 2003). The mussel spat used in Marlborough Sounds is collected
from three main areas: Kaitai, Golden Bay and locally caught spat. Although all the mussels
used in Marlborough Sounds would originate from the northern population, Patrick (2003)
identified differences in the condition index and growth rate between the different stock types.
Mussels originating from Kaitai and Golden Bay have different shell forms (Dick Jones pers.
comm. 2004). Kaitai spat are clearly identifiable by the dark banding that runs along the
length of the shell, and generally the shell is a rich emerald green with little yellow
colouration. Golden Bay spat have no dark banding and have a yellow-brown central shell
colour graduating to a bright green at the edge. Locally caught spat have a combination of
both characteristics, some with obvious Kaitai traits and others dominated by the Golden Bay
shell colour trait. The colouration of each shell was assessed and placed into one of four
groups: Kaitai spat (K), Golden Bay spat (GB), Kaitai-like local spat (LK), and Golden Bay-
like local spat (LGB) (Figure 3.2).
Figure 3.2 Local mussel types from Malborough Sound, a) Golden Bay-like b) Kaitaia-like

Chemical Analysis of the Mussel Soft Tissue

Sample preparation for analysis of the Cd and Zn content of the mussel soft tissue was adapted from the protocol for Analysis of Fish and Seafood: Dry Ashing Procedure (Perkin Elmer 1994). Many methods of trace metal analysis are in use, because no single method has consistently proved to be better than any other (Galloway et al. 1983). The most important consideration is to incorporate some inter-laboratory calibration of the analysis (Galloway et al. 1983). All the methods used in this present study were selected based on access to equipment and to minimise contamination.

The frozen mussels were defrosted and shucked, using acid-cleaned plastic knives. At this stage the sex of the mussel and presence of internal symbionts could be determined. The mussel flesh was then placed in an acid-cleaned 50 ml conical flask and the wet weight recorded. Mussel samples were then oven-dried at 80°C for 48 hours, and the dry weight was recorded on cooling. The low drying temperature was selected to reduce the likelihood of
trace metal loss through uncontrolled boiling. Mo and Neilson (1994) recommended 60 hours at 80°C for oysters, or until constant weight. Testing of this method showed mussels reached a constant dry weight after 48 hours of drying, with a variation of less than 0.015 g between 46 and 48 hours drying (Appendix 1). Samples were transferred to a muffle furnace and ashed for 40 hours at 430 – 450°C to oxidise any organic material, forming gaseous CO₂. The relatively low temperature for ashing was selected to reduce loss of Cd and Zn through volatilisation (Perkin Elmer 1994) and to avoid melting glassware. Once cool, the ash weight of the mussel sample was recorded.

In order to determine the concentration of trace metal in a sample all the organic matter has to be removed to release any trace metal that is bound to the organic matrix (Smith & Williamson 1986). To remove any remaining organic matter and dissolve the inorganic material, the sample was acid digested using quartz-distilled nitric acid. Approximately 10 ml of acid was slowly added to the sample and gently heated in a fume cupboard until the cessation of brown nitrate fumes. Loose acid-clean lids were then placed on the samples and heat was slowly increased until just below boiling point. The sample was then left to digest at this temperature for up to 10 hours until the solution had cleared. Once the sample was clear, the lid was removed to allow the acid to evaporate until approximately 1 ml of sample remained. The remaining acid ensured acidic conditions in order that all trace metals remained in solution (Smith & Williamson 1986). The samples were then made up to approximately 30 ml with Milli-Q® water. The final volume was calculated by weight and density corrections and the sample was sealed in trace metal-clean polyethylene containers until analysis.

All steps in this chemical analysis procedure for trace metals require that all apparatus be trace metal free to avoid any contamination. All glassware and plastic ware (including plastic knives) were initially soap washed and rinsed in distilled water. Then each piece was left in a solution of 10% nitric acid for 1 week for acid cleaning to dissolve any metals in particulate form. Each piece was then rinsed at least three times in Milli-Q® water, dried and stored in trace metal clean plastic bags until use. All samples were cooled in a desiccator to prevent any uptake of moisture in the cooling process. The ashing process limited the number of samples analysed to a maximum of 15 in each of two furnaces to ensure sufficient room to
allow complete heating and ashing of samples. All weights were recorded using a Mettler PM2500 balance (0.001 ± 0.002 g).

The dissolved mussel samples were analysed using Flame Atomic Absorption Spectrometry technique (FAAS) using Perkin Elmer AAnalyst 100 with background correction. Samples were aspirated using a high-level precision impact bead nebuliser and vaporised in an air-acetylene flame. During this process any molecular ions present in the digest are dissociated into atoms and these quantitatively absorb light at a wavelength characteristic of a particular element as provided by a specific cathode lamp (see Dulsí 1999). By measuring the amount of wavelength absorbed one can determine, with the use of standards, what concentration of a specific atom is present in the solution. Each element requires different flame conditions and different concentrations of standards to take advantage of the optimal linear absorbance (Table 3.2).

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelength (nm)</th>
<th>Slit (nm)</th>
<th>Gas</th>
<th>Flame type</th>
<th>Linear range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>228.8</td>
<td>0.7</td>
<td>Dry air and acetylene</td>
<td>Lean blue</td>
<td>0.028 - 2</td>
</tr>
<tr>
<td>Zinc</td>
<td>213.9</td>
<td>0.7</td>
<td>Dry air and acetylene</td>
<td>Lean blue</td>
<td>0.018 – 1.0</td>
</tr>
</tbody>
</table>

Each sample was analysed five times by FAAS and the mean concentration of these analyses reported. Due to the variable nature of FAAS analysis, a maximum limit of 5% was set for the relative standard deviation (RSD) of the concentration as calculated by Perkin Elmer AAnalyst 100. Low Level Precision (LLP) and High Level Precision (HLP) reference samples, produced by ChemSearch, were used to test the extremes of the calibrations. To accept the calibration, the LLP and HLP reference samples had to be within 10% of the known concentration. The use of precision samples allowed the accuracy and consistency of the FAAS calibration between each analysis to be tested.

Any sample above the concentration of the highest standard was diluted to bring the concentration within range. Any sample in the lower ranges of the calibration with a relative
standard deviation above 5% is reported as being below the detection limit of the element. All the mussel digests had Zn levels that exceeded the concentration limits of the FAAS analysis. Rather than dilute each sample, the head of the burner was rotated to reduce the sensitivity of FAAS until a linear absorbance relationship for a calibration standard of 10 mg L\(^{-1}\) was achieved. This method allowed a rapid assessment of the Zn concentration in solution, but reduced the accuracy of the Zn analysis by a factor of ten.

**Controls**

As a limited number of samples could be processed at once, samples were analysed in batches of up to 24 mussels. To ensure the reliability and consistency of treatment, numerous checks were put in place to determine the accuracy of each batch. Laboratory blanks and standard reference material were put through at a ratio of 1:10 and 1:20 to test for contamination. The laboratory blanks allow correction for any unidentified sources of contamination such as insufficient cleaning and atmospheric contamination introducing dust particles. Standard reference material is used to test the efficiency of the trace metal extraction; both a certified reference material and a laboratory made mussel reference material were used.

The certified standard reference material used was the internationally-recognised dogfish liver certified reference material for trace metals (DOLT-3) obtained from the National Research Council in Canada (NRC). As it was not possible to acquire any certified mussel reference material, a secondary reference material was produced from a random sample of 20 mussels (GSM). The mussels for GSM were homogenised and freeze-dried before being ground into a fine power. The analysis of the trace metal content of DOLT-3 was compared to the certified values supplied by NRC, ensuring some inter-laboratory comparison of methods. Using GSM as a reference ensures that any matrix interference specifically from the mussel tissue itself can be tested to see it is consistent throughout the whole analysis procedure.
The level of Cd and Zn found in the DOLT 3 reference material, although more variable than the certified values, was within the recommended limits of analysis, with all results being within 10% of the certified values (Table 3.3). GSM analysis proved to be more variable with results ranging just below 30% for both Cd and Zn. The low level of Cd and Zn in GSM often resulted in concentrations in the sample being near the limit of detection, explaining some of the variation in results. If these samples had not been diluted to 30 ml, the accuracy of the results would have significantly improved. The laboratory blanks consistently show a level of contamination below the limits of detection for the analysis, and were corrected for within the calibration of the results.

<table>
<thead>
<tr>
<th></th>
<th>DOLT 3 Reference</th>
<th>DOLT 3 Results n=26</th>
<th>GSM n=36</th>
<th>Range of Blank n=50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td>19.4 ± 0.6</td>
<td>18.6 ± 1.01</td>
<td>0.68 ± 0.19</td>
<td>-0.010 - 0.011</td>
</tr>
<tr>
<td>Zinc</td>
<td>86.6 ± 2.4</td>
<td>85.12 ± 6.23</td>
<td>44.64 ± 12.38</td>
<td>-0.1 - 0.09</td>
</tr>
</tbody>
</table>

Standard deviation expressed as error term.

**Matrix Effect**

Matrix effect is the interaction between the elements in solution. Sometimes a solution is such that the interaction of the elements reduces or enhances the concentration determined by FAAS (Cullen 2004). In order to determine any matrix effect from the mussel tissue itself, a spiked analysis of known amounts of trace metals was undertaken. Five mussels were randomly selected and homogenised with 10 ml of Milli-Q® water and the resulting sample was split into 15 flasks each containing approximately 6 g of mussel homogenate. The 15 flasks were split into five groups of three; three groups were treated with a spiked solution of trace metals, and the other two groups were used as mussel tissue controls after the addition of a similar volume of Milli-Q® water. The concentration of trace metals in the spiked samples
was estimated to be one, two, and ten times the concentration of that in the tissue. Corrections for weight were made for each spike addition to ensure the precise amount of added trace metals was known for each sample. The samples then subjected to the same analytical process described above. The retrieval of each trace metal was calculated using a mean of the mussel tissue control measurements. According to the European Communities for trace metal analysis requirements (Commission of the European Communities 2001), the level of recovery should be 80-120%. For both Cd and Zn, the retrieval of spiked metal solutions in the present study was within these recommended limits for trace metal analysis (Table 3.4).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spike added (µg)</th>
<th>Metal Recovered (%)</th>
<th>Standard Deviation (% recovery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cadmium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spike 1</td>
<td>0.48</td>
<td>101.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Spike 2</td>
<td>0.95</td>
<td>101.4</td>
<td>1.8</td>
</tr>
<tr>
<td>Spike 3</td>
<td>4.97</td>
<td>100.3</td>
<td>1.9</td>
</tr>
<tr>
<td>Zinc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spike 1</td>
<td>6.02</td>
<td>101.4</td>
<td>3.9</td>
</tr>
<tr>
<td>Spike 2</td>
<td>11.83</td>
<td>97.2</td>
<td>9.1</td>
</tr>
<tr>
<td>Spike 3</td>
<td>61.94</td>
<td>92.8</td>
<td>8.8</td>
</tr>
</tbody>
</table>

1 Concentration of metals in control samples: Cd 0.12 ± 0.01 ppm and Zn 8.2 ± 0.5 ppm

Statistical analysis

All the statistical analyses used the commercial software package SPSS Version 11.5 for Windows. If the distribution of the results was not normal, a natural log transformation was used. A full factorial ANOVA analysis with interaction terms followed by Tukey
analysis was used to test the significance of any difference between factors. A Pearson's correlation index or Spearman's Rho correlation for nonparametric data was used to analyse any correlations. All maps were drawn using the LINZ Topo 4 Vector Coastline data set.

The statistical analysis of the data uses multiple comparisons to determine the significant factors for each variable. However, this method of analysis increases the risk of making a type I error (rejecting null hypothesis when it is true). Rather than reducing the power of the analysis using a Bonferroni correction, the false discovery rate (FDR) was tested (Garcia 2003). Analysing all the p-values reported in this study gives a proportion of significant tests of 83%; determined by using QVALUE through RGui software (Storey 2002). The analysis produces a q-value for each reported p-value to determine the positive FDR (Storey & Tibshirani 2003). If an acceptable positive FDR is set at 1% then all q-values must be less than 0.01 to be within this rate. As a result, in this present study any analysis reported with a p-value over 0.033 may have a high probability of making a type I error.
Chapter 4

Spatial Variation in Cadmium and Zinc Levels

Introduction

The MSQP data of trace metal levels in mussels from Marlborough Sounds 1991 - 2002 showed a difference in the level of Cd and no difference in Zn between farms (Chapter 2). Cd levels in mussels increased from Kenepuru Sound to the outer coast. The reasons for the changes in Cd levels are not clearly identified, as many factors could influence the level of Cd in mussels. Physiological factors such as size, age, sex, growth rate and reproductive condition have been shown to affect the concentration of some trace metals in molluscs (Latouche & Mix 1982; Lobel et al. 1991a; Ahn et al. 2001). Environmental factors such as season, temperature, salinity and habitat have also been shown to directly affect the bioaccumulation of trace metals in molluscs (Phillips 1976; Graney et al. 1984; Blackmore & Wang 2003). Even using all these factors to explain the variability between mussel samples, there is still a problem with the unexplained variability of trace metals in biological tissue (Boyden & Philips 1981; Lobel et al. 1982). It is at least in part because of this variability, that many pollution-monitoring programmes use homogenised samples of 5 - 10 organisms, as suggested by Lobel et al. (1991a), in order to reduce the problem of high variability in trace metal levels between individual animals.

Variation in the concentration of Cd in mussels from Pelorus Sound could be due to environmental differences in the availability of Cd or to variations in physiological factors between farms. In order to ascertain whether the cause is at least in part environmental, it is important to determine whether any of the physiological factors differ significantly between sample sites. Using homogenised samples it is not possible to separate all the likely physiological causative factors. By analysing individual mussels, although more time consuming, it is possible to better determine the significance of physiological factors, as well as isolate the level of residual variability.
Hickman et al. (1991), in a study of mussels grown in Pelorus Sound over a period of two years, recognised three patterns of condition index, referred to as high, medium and low. Mussels from farms within Kenepuru Sound had a high condition index along with those from Clova Bay and Crail Bay. Most other farms tested by Hickman et al. were classified as being in the medium condition index group, with two farms in outer Pelorus Sound classified as low. No individual environmental factor correlated with the changes in condition index. A combination of changes in salinity and food availability best explained changes in mussels condition index, with periods of high salinity most frequently explaining low condition index. Previous studies have shown that condition index is correlated with the concentration of trace metals in mussels (Lobel et al. 1991a). As the condition index of a mussel increases, the proportion of soft tissue increases, effectively diluting the concentration of trace metals in the soft tissues. The historical data for Cd levels (Chapter 2) exhibited a similar pattern to the groups of condition index identified in Hickman et al.'s study. If the pattern of Cd levels in mussels is directly correlated with changes in condition index, then the difference in the Cd levels between growing areas may be due to differences in growing conditions rather than differences in Cd exposure.

The analysis of historical data outlined in Chapter 2 identified two trends for trace metal levels in mussels within Pelorus Sound that can be investigated in this research. Mussels from farms closer to the Pelorus River mouth had noticeably lower Cd levels than mussels from farms further out towards the open ocean (Cook Strait). There was also a trend of reducing Cd and Zn concentration over the eleven years of sampling.

Methods

Variation with distance along Pelorus Sound

To analyse whether the variation with distance along Pelorus Sound was significant required a sampling design to take account of variation both within the farms and between individual mussels. Nine bays were selected for analysis in order to maximise comparability with historical data (sites 1 - 9), as well as produce a representative range along the length of
the inner Pelorus Sound (Figure 4.1). The distance of each sample site from the Pelorus River mouth was calculated to the nearest 0.5 km using the central channel (Table 4.1.). Wilsons Bay was sampled using two neighbouring farms, as the original farm did not have mussels in harvestable condition for the October sampling period. As the location of the two farms is very similar, these samples have been grouped and analysed as sample site 4.

Table 4.1. Mussel farms sampled during April and October 2004 for analysis of spatial variation of trace metal levels in *Perna canaliculus* in inner Pelorus Sound.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Farm Area</th>
<th>Farm ID*</th>
<th>Water Depth (m)</th>
<th>Dropper Depth (m)</th>
<th>Distance from Pelorus River Mouth (km)</th>
<th>Sample Dates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Kenepuru Entrance</td>
<td>91</td>
<td>24.3</td>
<td>12</td>
<td>12.5</td>
<td>06/04 and 26/10</td>
</tr>
<tr>
<td>2</td>
<td>Little Nikau</td>
<td>179</td>
<td>8.9</td>
<td>8</td>
<td>18</td>
<td>26/10</td>
</tr>
<tr>
<td>3</td>
<td>Yencya Bay</td>
<td>29</td>
<td>20.8</td>
<td>12</td>
<td>25</td>
<td>06/04 and 26/10</td>
</tr>
<tr>
<td>4</td>
<td>Wilsons Bay</td>
<td>27</td>
<td>24</td>
<td>12</td>
<td>32</td>
<td>06/04</td>
</tr>
<tr>
<td>4</td>
<td>Wilsons Bay</td>
<td>112</td>
<td>24</td>
<td>12</td>
<td>32</td>
<td>26/10</td>
</tr>
<tr>
<td>5</td>
<td>Old Homeward Bay</td>
<td>96</td>
<td>27.4</td>
<td>12</td>
<td>34</td>
<td>06/04 and 26/10</td>
</tr>
<tr>
<td>6</td>
<td>Grants Bay</td>
<td>395</td>
<td>30.2</td>
<td>16</td>
<td>38</td>
<td>06/04 and 26/10</td>
</tr>
<tr>
<td>7</td>
<td>Clova Bay</td>
<td>163</td>
<td>27.7</td>
<td>12</td>
<td>39</td>
<td>26/10</td>
</tr>
<tr>
<td>8</td>
<td>West Beatrix Bay</td>
<td>160</td>
<td>30.1</td>
<td>14</td>
<td>40</td>
<td>06/04 and 26/10</td>
</tr>
<tr>
<td>9</td>
<td>Central Beatrix Bay</td>
<td>265</td>
<td>30.2</td>
<td>14</td>
<td>41</td>
<td>06/04</td>
</tr>
</tbody>
</table>

*Farm identification number refers to either the permit number or the farm licence number that is use to identify individual farms.
Figure 4.1 Location of mussel farms sampled during April and October 2004 for analysis of spatial variation of trace metal levels in Perma canaliculus in Pelorus Sound, NZ Map Grid 100,000.
Variation with Water Depth

Water depth has previously been found to be a significant factor in Cd concentration variation in Kenepuru Sound (Neilson 1974). Analysis of the historical data showed some variation of trace metal levels with depth that disagreed with Neilson’s findings. In order to determine whether depth is a factor in the variation of Cd and Zn levels in mussels, each sample site was sampled on one occasion at three depths: top, middle, and bottom. The mussel dropper line was raised by winch above the water and samples were collected from the top of the dropper, half way, and at the bottom of the dropper where, as mussels hang down from the line, a change in growth direction was observed (see Figure 1.1). Sampling by this method ensured that the mussels collected would represent the range of depths at each sample site. Not all farms grow mussels to the same depth as the length of the droppers varies depending on the water depth at each site. Mussels were sampled at depths of approximately 1 m, 6 m, and 12 m, except for sample sites in Beatrix Bay (1 m, 7 m and 14 m), Grants Bay (1 m, 8 m and 16 m), and Little Nikau (1 m, 4 m and 8 m). This sampling method gave representative samples over the range of growing depths, with a minimum numbers of samples to allow time for processing.

Variation within Farms

Four farms, sample sites 1, 4, 5, and 8 (Kenepuru Entrance, Wilsons Bay, Old Homeward Bay and West Beatrix Bay) were each sampled from two locations during the April collection to determine if there was any variation in trace metals within the farms themselves. These four farms were selected to be representative of the farms sampled in inner Pelorus Sound. Where possible, two separate farm lines were sampled to enable a larger range to be analysed, but this was limited by the age and condition of the mussels (see Chapter 3).
Results

Variation with Distance along Pelorus Sound

Cadmium

Cadmium levels in mussels sampled from Pelorus Sound vary considerably, ranging from 0.17 to 5.29 ppm dry weight. Almost all sample sites have larger within-site variability than expected, with all sample sites, except site 2, exceeding 30% standard deviation. The range of Cd levels found within some of the sample sites was much greater than expected. For example, mussels from Grants Bay, sample site 6, had the highest level of Cd, and it also had the largest range at almost twice the mean for the site (Table 4.2).

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.45</td>
<td>0.39</td>
<td>0.64</td>
<td>0.79</td>
<td>1.15</td>
<td>2.35</td>
<td>1.78</td>
<td>2.01</td>
<td>2.36</td>
</tr>
<tr>
<td>Minimum</td>
<td>0.17</td>
<td>0.29</td>
<td>0.37</td>
<td>0.43</td>
<td>0.69</td>
<td>0.78</td>
<td>0.76</td>
<td>0.83</td>
<td>1.46</td>
</tr>
<tr>
<td>Maximum</td>
<td>0.77</td>
<td>0.51</td>
<td>1.12</td>
<td>1.80</td>
<td>2.77</td>
<td>5.29</td>
<td>3.71</td>
<td>3.48</td>
<td>3.93</td>
</tr>
<tr>
<td>Range</td>
<td>0.61</td>
<td>0.22</td>
<td>0.76</td>
<td>1.37</td>
<td>2.08</td>
<td>4.51</td>
<td>2.94</td>
<td>2.65</td>
<td>2.47</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>35.6</td>
<td>20.5</td>
<td>31.2</td>
<td>31.6</td>
<td>37.4</td>
<td>47.0</td>
<td>42.7</td>
<td>34.3</td>
<td>33.9</td>
</tr>
</tbody>
</table>

Even with the large range, an increase in Cd concentration, observed in the historical data from Pelorus River to the open ocean, is still apparent. Initially, there is only a gradual increase in Cd in mussels along Pelorus Sound until sample site 6, where a rapid increase in the Cd levels of mussels occurred (Figure 4.2).
Figure 4.2 Mean cadmium concentration for *Perna canaliculus* grown in Pelorus Sound sampled in April and October 2004. Error bars show standard deviation, n=235.

The distribution of the dry weight results for Cd levels is left-skewed. Since ANOVA requires a normal distribution of the data, Cd levels were transformed by natural log. A Q-Q plot of the residuals after ANOVA showed this transformation to be adequate. ANOVA and Tukey analysis of Cd levels from all the inner Pelorus Sound sample sites for both harvest dates showed that the sample sites could be split into four significantly different groups (p<0.001): sample sites 1 and 2, < site 3, < site 4 and 5, < sites 6, 7, 8 and 9. Partial eta-squared estimates of the variance explained by each variable showed that over 80% of the variance in Cd levels was explained by distance alone.

ANOVA showed there was a significant difference in Cd levels with the depth of sample (p<0.001), with bottom>middle>top. Partial eta-squared estimates showed that over 14% of variance in Cd levels were explained by depth of sample. However, Cd levels were not significantly affected by harvest date (p=0.448) or by sex of mussel (p=0.722). The different spat types also showed no significant difference between Cd levels (p=0.438). Although there was a significant interaction between distance and spat (p=0.047), with a
partial eta-squared estimate of 3%, not all spat types were present in all samples. Analysis of interaction between sample site and depth showed significant difference in Cd levels (p=0.013), with a partial eta-squared estimate of 21%. At all sample sites, except sample site 6, mussels from the bottom of the dropper had higher Cd levels. The mid-dropper sample from sample site 6, Grants Bay, was the deepest of all mid-line samples at 8 m, and was the only location where mussels from the mid-line sample showed Cd levels higher to those found in the bottom line sample.

![Graph showing Cd levels in mussels across different sample sites and depths.](image)

**Figure 4.3** Mean dry weight cadmium concentration (ppm dry weight) in *Perna canaliculus* collected from three depths from Pelorus Sound in April and October 2004. Error bars show standard deviation.
Zinc

The level of variation in Zn levels in mussels within the sample sites was much lower than the variation found for Cd levels. However, the range of Zn levels found within sample sites was greater than variation between most sample sites (Table 4.3).

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>60.7</td>
<td>62.1</td>
<td>49.2</td>
<td>46.5</td>
<td>47.1</td>
<td>41.7</td>
<td>35.4</td>
<td>46.1</td>
<td>54.5</td>
</tr>
<tr>
<td>Minimum</td>
<td>43.2</td>
<td>45.2</td>
<td>32.1</td>
<td>35.6</td>
<td>32.3</td>
<td>29.9</td>
<td>27.8</td>
<td>22.3</td>
<td>38.3</td>
</tr>
<tr>
<td>Maximum</td>
<td>76.7</td>
<td>73.9</td>
<td>64.5</td>
<td>67.7</td>
<td>68.8</td>
<td>67.7</td>
<td>56.9</td>
<td>67.7</td>
<td>72.2</td>
</tr>
<tr>
<td>Range</td>
<td>33.5</td>
<td>28.7</td>
<td>32.4</td>
<td>32.1</td>
<td>36.6</td>
<td>37.8</td>
<td>29.1</td>
<td>45.4</td>
<td>33.9</td>
</tr>
<tr>
<td>Standard Deviation (%)</td>
<td>15.2</td>
<td>13.5</td>
<td>15.6</td>
<td>17.9</td>
<td>16.6</td>
<td>24.0</td>
<td>20.3</td>
<td>22.3</td>
<td>16.5</td>
</tr>
</tbody>
</table>

Table 4.3 Range of zinc concentrations (ppm dry weight) in *Perna canaliculus* grown in Pelorus Sound and sampled in April and October 2004.

![Graph](image.png)  

*Figure 4.4* Mean zinc concentration (ppm dry weight) in *Perna canaliculus* grown in Pelorus Sound and sampled in April and October 2004. Bars show Standard deviation, n=235.
The distribution of the dry weight results for Zn levels were also left-skewed and transformed by natural log. ANOVA of Zn levels showed the sample sites to be significantly different (p<0.001). Tukey analysis showed that only sample site 7, Clova Bay, Zn levels differed significantly from all other sample sites. The remaining sample sites were classified into four overlapping groups showing some difference between the sites, with a pattern of increasing Zn levels away from Clova Bay: sample site 7, < sample sites 4, 5, 6 and 8, ≤ sample site 3, ≤ sample site 9, ≤ sample sites 1 and 2. Partial eta-squared estimate showed that 56% of variance in Zn levels was explained by distance.

ANOVA showed the Zn concentration was also significantly affected by depth (p=0.001), sex of mussel (p<0.001), and harvest date (p=0.027). Depth and sex each accounted for just over 10% of the variability in Zn levels, whilst harvest date accounted for only 4%. Similar to Cd levels, the Zn levels in mussels were highest in the bottom samples: bottom > middle > top. However unlike Cd, female mussels had significantly higher levels of Zn. Spat types showed no significant difference between Zn levels (p=0.536). There were more significant interactions between the different factors for Zn levels compared to Cd, although the interaction between depth and sample site was not significant (p=0.96) (Figure 4.5). There was a significant interaction between sample site and sex (p=0.041), with female mussels having higher Zn levels in all samples, apart from sample sites 3 and 7 (Yncyca Bay and Grants Bay). The interaction between distance and harvest (p=0.024) showed Zn levels in April were lower than those for sample sites 1 and 4 (Kenepuru Entrance and Wilsons Bay), but higher for all other sample sites. There was also some significant interaction between sex and spat (p=0.010), but in all cases female mussels had higher levels of Zn than males.
Figure 4.5 Mean dry weight zinc concentration (ppm dry weight) in *Perna canaliculus* collected from three depths from Pelorus Sound in April and October 2004. Error bars show standard deviation.

**Condition Index**

ANOVA for the condition index showed that there was significant variation between the sample sites and depth (both $p=0.001$). Very little variation in condition of the mussels was explained by these factors alone, with partial eta-squared estimates of 19%, and 11% respectively. Interactions of mussel condition between depth and site, sex and spat, and sex and harvest were all significant ($p=0.002$ $p=0.013$ and $p=0.036$ respectively). Sample sites 4 and 6 had the highest condition index but Tukey analysis of the difference in condition index between the sample sites showed all groups of sites overlapped to some extent. Variation of
condition index with depth showed mussels from the surface samples had a higher condition index, top > middle > bottom, though interactions between sites changed this relationship. The interaction between site and depth explained the largest degree of the variation in condition of the mussels, but it was still only able to account for 24% of the variation. Analysis of the interaction of condition index between the sample sites and depth showed that sample sites 2 and 7 (Little Nikau Bay and Grants Bay) had a significantly lower condition index for the bottom samples. The droppers from the mussel farm in Little Nikau, as a shallow water site, extended to within 1 m of the bottom, and Grants Bay droppers were the deepest sampled extending down to 16 m, sample sites 2 and 7. These two extremely low condition indices may have influenced the significance of condition index with depth (Figure 4.6).

Figure 4.6 Mean condition index for *Perna canaliculus* collected from three depths from Pelorus Sound in April and October 2004. Error bars show standard deviation.
Shell Volume

ANOVA of the variation of the volume of the mussels sampled showed that there was a significant variation between the sample sites, depth and spat type (p=0.002, p=0.004 and p=0.033 respectively). Very little variation in volume of the mussels was explained by these factors alone, with partial eta-squared estimates of 18%, 9% and 7% respectively. Interactions of mussel shell volume between sex and depth, and sex, site and depth were significant (p=0.038, and p<0.001 respectively). Sample sites 5, 7 and 8 had significantly larger mussels. Also, mussels were significantly larger in top and mid-dropper samples, top>middle>bottom, although this relationship changed with the interaction effect from sample site and depth (Figure 4.7).

**Figure 4.7** Mean shell volume (cm$^3$) for *Perna canaliculus* collected from three depths from Pelorus Sound in April and October 2004. Error bars show standard deviation.
Dry Weight

ANOVA of the variation of the dry weight of the mussels sampled showed that there was a significant variation between the sample sites, depth and spat type (p<0.001, p<0.001 and p=0.006 respectively). Compared to volume, more variation in dry weight of the mussels was explained by these factors, with partial eta-squared values of 23%, 23% and 10% respectively. Interactions of mussel length between sex and depth, sex and harvest, and site, sex and depth, were significant (p=0.006, p=0.023, and p=0.008 respectively). Similar to the shell volume analysis, heavier mussels were collected from sample sites 4, 5, 7 and 8. The significant difference with depth again showed mussels were significantly heavier in top dropper samples, top>middle>bottom (Figure 4.8).

![Graph showing dry weight at three depths in *Perna canaliculus* from Pelorus Sound in April and October 2004. Error bars show standard deviation.](image)

**Figure 4.8** Mean dry weight at three depths in *Perna canaliculus* from Pelorus Sound in April and October 2004. Error bars show standard deviation.
Analysis of covariance of Cd and Zn levels with mussel size, weight and condition

ANCOVA of Cd levels in mussels with dry weight, condition index and shell volume of the mussels showed that none of the three variables was a significant covariate (p=0.470, p=0.331 and p=0.919 respectively). Similarly, ANCOVA of Zn levels in mussels with dry weight, condition index and volume of the mussels found all covariates were insignificant (p=0.325, p=0.681 and p=0.130 respectively). Although shell length, condition index, dry weight, Cd and Zn levels from mussels in Pelorus Sound all showed significant difference between the sample sites, a comparison of the Tukey analysis from the five variables showed that none of the groupings were consistent (Table 4.4).
Table 4.4 Tukey analysis of cadmium and zinc concentration, dry weight, shell volume and condition index in *Perna canaliculus* from Pelorus Sound, 2004. Each sample site is classified into groups with similar mean results, and each group is ranked from lowest to highest.

<table>
<thead>
<tr>
<th>Sample site</th>
<th>Cadmium Concentration (Ln dry weight)</th>
<th>Zinc Concentration (Ln dry weight)</th>
<th>Shell Volume (cm$^3$)</th>
<th>Condition Index (g cm$^{-3}$)</th>
<th>Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>v</td>
<td>A B</td>
<td>a b c</td>
<td>w x y</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>v</td>
<td>A</td>
<td>a</td>
<td>w</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>iii iv</td>
<td>A</td>
<td>b c</td>
<td>w x</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>ii iii</td>
<td>A B</td>
<td>d</td>
<td>x y z</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>ii iii</td>
<td>B C</td>
<td>c d</td>
<td>y z</td>
</tr>
<tr>
<td>6</td>
<td>4</td>
<td>ii</td>
<td>A B C</td>
<td>c d</td>
<td>x y</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>i</td>
<td>C</td>
<td>b c d</td>
<td>z</td>
</tr>
<tr>
<td>8</td>
<td>4</td>
<td>ii iii</td>
<td>C a b</td>
<td>x y z</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>iv v</td>
<td>A a b</td>
<td>w</td>
<td></td>
</tr>
</tbody>
</table>
Analysis of correlations between physiological factors and mussel Cd and Zn levels

Non-parametric analysis of the correlation between Cd and Zn levels in the mussels with the mussel dry weight, shell volume and condition index showed that Zn levels in the mussels were negatively correlated to all three of these factors. Cd levels in the mussels were also negatively correlated to condition index. However, analysis of the total trace metal content (total trace metal = dry weight x trace metal concentration), showed that the total Cd and Zn content in the mussels were positively correlated with both dry weight and shell volume, but only total Zn content was slightly correlated with condition index (Table 4.5).

<table>
<thead>
<tr>
<th></th>
<th>Dry Weight (g)</th>
<th>Shell Volume (cm³)</th>
<th>Condition Index (g cm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cd] (µg/g)</td>
<td>-0.037(ns)</td>
<td>0.113(ns)</td>
<td>0.153(*)</td>
</tr>
<tr>
<td>Total Cd (µg)</td>
<td>0.320(**)</td>
<td>0.373(**)</td>
<td>0.007(ns)</td>
</tr>
<tr>
<td>[Zn] (µg/g)</td>
<td>-0.411(**)</td>
<td>-0.180(**)</td>
<td>-0.366(**)</td>
</tr>
<tr>
<td>Total Zn (µg)</td>
<td>0.657(**)</td>
<td>0.601(**)</td>
<td>0.144(*)</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).
ns Correlation is not significant
n=235

The physical parameters of tissue weight, shell volume and condition index of the mussels were all highly correlated. Dry weight was positively correlated with both shell volume and condition index. However, the correlation between shell volume and mussel condition index was negative (Table 4.6).
Table 4.6 Pearson’s correlations between mussel dry weight, shell volume and condition of *Perna canaliculus*, sampled Inner Pelorus Sound, 2004

<table>
<thead>
<tr>
<th></th>
<th>Dry Weight (g)</th>
<th>Shell Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shell Volume (mm)</td>
<td>0.718(**)</td>
<td>-</td>
</tr>
<tr>
<td>Condition Index (g cm⁻³)</td>
<td>0.486(**)</td>
<td>-0.232(**)</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).

n=235

Analysis of any correlations between Cd and Zn in the mussels showed that there was a negative correlation between Cd and Zn concentrations, but total Cd and Zn content did not correlate. However, Cd concentration and total Cd content were positively correlated and also, to a lesser extent, Zn concentration and total Zn content correlated (Table 4.7).

Table 4.7 Spearman’s Rho correlations between trace metals in soft tissue of *Perna canaliculus*, sampled Inner Pelorus Sound, 2004

<table>
<thead>
<tr>
<th></th>
<th>[Cd] (µg/g)</th>
<th>Total Cd (µg)</th>
<th>[Zn] (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Cd (µg)</td>
<td>0.894(**)</td>
<td>-0.184(**)</td>
<td>-0.334(**)</td>
</tr>
<tr>
<td>[Zn] (µg/g)</td>
<td>-0.231(**)</td>
<td>0.001(ns)</td>
<td>0.368(**)</td>
</tr>
</tbody>
</table>

** Correlation is significant at the 0.01 level (2-tailed).

ns Correlation is not significant

n=235

There was a strong linear relationship between Ln Cd concentration and Ln total Cd, (Figure 4.9). However a linear relationship was not evident between Ln Zn concentration and Ln total Zn (Figure 4.10), as reflected in the presence of a relationship between total Zn content and dry weight (Figure 4.11).
Figure 4.9 Linear relationship between the cadmium concentration (dry weight ppm) and total cadmium (μg) of soft tissues of *Perna canaliculus* sampled from Pelorus Sound in April and October 2004 ($R^2=0.83$).

Figure 4.10 Linear relation between the zinc concentration (dry weight ppm) and total zinc (μg) of soft tissues of *Perna canaliculus* sampled from Pelorus Sound in April and October 2004 ($R^2=0.11$).
Variation between Harvest Dates

Samples were harvested in April and October, but only six sites were harvested on both dates due to difficulties in finding farms with mussels in harvestable condition. To ensure analysis of all the data was consistent, a comparison between the six sites sampled from mid dropper between harvest dates was carried out. ANOVA analysis of natural log transformed data showed both Cd and Zn levels were not significantly different between sample dates (p=0.383 and p=0.521 respectively), and all interaction terms with harvest date were not significant. Grants Bay, sample site 7, was the only site to be sampled at all depths for both harvest dates. This site when analysed separately still showed no significant difference between harvest dates for Cd levels (p=0.075), but was significantly different for Zn levels with these dropping from April to October (p>0.001).

Variation of trace metal levels in mussels within farms

Analysis of the variation of Cd and Zn concentration within farms showed a significant difference between the duplicate sites for Cd levels, but not for Zn levels (p=0.011, p=0.212 respectively). There is no significant interaction between sample site and duplicate number.
for Zn levels (p=0.101). However, there is a significant interaction with the sample site and depth for Cd levels, with only sample site 1, Kenepuru Entrance, being significantly different between duplicates (p=0.023). A plot of the duplicate mean Cd levels in mussels from the four sample sites showed the difference between the duplicates samples is small (Figure 4.12). The difference between the two mean Cd levels for sample site 1 was 0.35 ± 0.07 and 0.55 ± 0.08 ppm dry weight (95% CI), a difference very close to the limit of detection. Consequently, the significant difference between the two duplicate sites could be due to experimental error in the chemical analysis of the mussels.

![Figure 4.12 Mean cadmium concentration (ppm dry weight) of duplicates within sample sites in mussels sampled from Pelorus Sound in April 2004. Error bars are standard deviation](image)

**Discussion**

Analysis of the Cd and Zn levels in mussels from inner Pelorus Sound has shown that the pattern of accumulation for the two metals is not similar. The variation of Cd concentration within Pelorus Sound, with the lowest levels at sample sites closest to Pelorus River, was similar to that found by the earlier sampling of MSQP in 1996 (Chapter 2).
However, Zn concentration in mussels was almost the opposite to that of Cd, with the higher Zn concentrations found in sample sites closest to Pelorus River.

Although the variation of Cd levels in mussels within the sample sites was much higher than expected, it was still possible to isolate some significant factors that may have influenced the uptake of Cd. Location of sample site was the main controlling factor, explaining over 80% of the variation in Cd levels in the mussels, followed by depth, explaining a further 14% of the variation. The increase in Cd levels with depth agrees with the findings of Neilsen (1974), where a significant increase in Cd levels of mussels with depth was observed in Kenepuru Sound. Neilsen proposed that in Kenepuru Sound, as a shallow water site, close association of mussels with sediment might account for the increase in Cd levels in mussels. During this present study, only sample site 2, Little Nikau, was collected from a shallow water site, yet an increase in mussel Cd levels with depth was found at all sample sites. The Cd concentration in the mussels also showed a low level of significant interaction between spat type and sample site, but as not all spat types occurred in all samples, it is difficult to assess the importance of this interaction.

The Zn levels in mussels from Pelorus Sound were less variable than the Cd levels, but again the location of sample sites was the main controlling factor, explaining 56% of the variation in Zn levels. Depth, harvest date and sex of mussel were also significant factors in Zn levels. Depth and sex accounting for just over 10% of the variability in Zn levels and harvest date only 4%. Neilsen (1974) found that Zn levels in mussels from Kenepuru Sound decreased with depth, while in this present study Zn levels in the mussels was found to increase with depth. Also, in contrast to this present study, Phillips (1976) found a surface winter maximum for both Cd and Zn in *M. edulis* that correlated with a trace metal-rich hyposaline surface water layer that formed during winter. However, higher Zn levels in female mussels, found at most sample sites in this present study, does concur with previous research into variation of trace metal levels with sex of molluscs. For example the black mussel, *Choromytilus meridionalis*, was found to have a significantly higher concentration of Zn in the female mussels during winter (Orren et al. 1980) and higher Zn levels were found only in the gonad tissue of *M. edulis* (Latouche & Mix 1982; Lobel et al 1991a; Sokolowalski et al. 2004). The significant variation of Zn levels in mussels between harvests when
analysed separately was found to relate only to sample site 7, Grants Bay, where there is a significant drop in Zn levels between the mussels harvested in April and October. As a significant difference between harvest date and Zn levels was found, the interpretation of the Zn levels with distance along Pelorus Sound must proceed with caution.

Analysis of the variation of Cd and Zn in mussels from Pelorus Sound is complicated by the significant variation of physiological factors in the mussels. Condition index, dry weight and shell volume were significantly different between sample sites and depth, yet none of the three variables were significant co-variables to Cd or Zn levels in the mussels. Analysis of the variation of condition index, dry weight and shell volume with mussels from inner Pelorus sounds showed only a small amount of variation can be explained by sample site, depth, sex and spat type. Again, like Cd and Zn, sample site was the major factor in explaining variation of condition index, dry weight and shell volume. However, opposite to Cd and Zn, the top-dropper samples had significantly higher condition index, dry weight and shell volume. Previous research (Lobel et al. 1991) has shown that condition index and trace metal levels are negatively correlated, as the increase in tissue dilutes the trace metals. Various studies have reported contrasting results with respect to the affect of size on trace metal level in molluscs. For example, Boyden (1977) studied the effect of size on the trace metal content in *M. edulis* and found it was negatively correlated for Zn and independent of size for Cd. However, Riget et al. (1996) found Zn levels to be independent of size, and Cd levels to be positively correlated with size in *M. edulis*. In this present study, the mussel Zn levels negatively correlated with both condition index and shell volume, agreeing with Boyden (1977), and mussel Cd levels had a slight positive correlation with condition index that was not expected.

The small number of samples at each site resulted in incomplete sampling of spat types at all locations and depths. This uneven experimental design has confounded the ability to detect differences in spat type. Yet even with such low numbers of different spat types, both Cd and Zn levels in mussels showed some significant interactions between the spat types. A laboratory analysis of different spat types limiting the variation in factors such as location, depth and sex may show a difference in trace metal levels to be significant.
Analysis of the Cd total body loading and Cd concentration in mussels tested showed a direct correlation, but this was not the case for Zn. If the size of mussels was a significant factor in the trace metal content, then the relationship between total trace metal content and trace metal concentration would be expected to be curved and not linear as observed for Cd (Figure 4.9). The strong positive correlation between the dry weight and total Zn content in the mussels (Figure 4.11), and the lack of any relationship between total Zn content and Zn concentration (Figure 4.10), shows the Zn levels found in mussels from Pelorus are relatively consistent when compared to Cd levels.

**Conclusion**

Results showed that the level of Cd and Zn in *P. canaliculus* grown in inner Pelorus Sound is variable. The main factor that significantly influenced the variation of Cd and Zn levels in *P. canaliculus* in Pelorus Sound was the location of the mussel farm, with farms close to the Pelorus River having mussels with significantly lower levels of Cd and almost the opposite being true for Zn levels. The other major factor in the Cd levels of mussels was the depth at which the mussels are grown, with mussels grown near the surface exhibiting lower levels of both Cd and Zn. Sex and harvest date also influence the Zn level in *P. canaliculus* with female mussels having significantly higher levels of Zn.

Analysis of the variation of volume and condition of mussels, limited by the design of the experiment, showed little or no correlation with Cd levels and negative correlations with Zn levels.

Results showed that the level of Cd at the mussel farms tested did not exceed the present NZ and Australian Food Safety limit of 2 ppm wet weight. Some of the mussels tested were above 5 ppm Cd dry weight, equivalent to over 1 ppm wet weight, and so exceeding the present European Food Safety limit of 1 ppm wet weight.
Chapter 5

Seasonal variation of Cadmium and Zinc in *Perna canaliculus*

Introduction

Mussels have been used in pollution monitoring because of their potential to accumulate trace metals to measurable levels and in equilibrium with their environment (Goldberg 1975). Large fluctuations in the levels of trace metals in the marine environment may not be detected by “snap shot” water sampling. The concentration of trace metals in mussels fluctuates over a much longer time period incorporating the natural variability of trace metals in the water (Bryan *et al.* 1985). An ideal bio-monitor should be able to reach equilibrium with the trace metal in their environment with no other physical or biological factors affecting the equilibrium. In this situation it would be possible to conclude that any variation in trace metal levels in the bio-monitor, indicates that the level of these trace metals in their environment is variable too.

Unfortunately, changes in environmental or physiological conditions can alter the equilibrium of a trace metal with a bivalve (for example Philips 1976; Lobel *et al.* 1991a; Blackmore & Wang 2003). Changes in trace metal concentrations in mussels over a season could indicate that either the level of trace metal in the water is changing, or that the bivalve’s ability to reach equilibrium with the surrounding water is changing. Factors such as temperature and food availability may change the clearance rate of bivalves (Boyden 1977, Philips 1980), while changes in freshwater input and water circulation may alter the level or availability of trace metals in the water column (Philips 1977; Engel *et al.* 1981). Seasonal variation may be location dependent. For example, in Devon, England, the trace metal levels in *Mytilus edulis* have been shown not to vary with season (Boalch *et al.* 1981), while in Newport, Oregon, the trace metal levels in *M. edulis* were reported to fluctuate with season (Latouche & Mix 1981). Whether this difference in the ability to detect seasonal fluctuations in this mussel species is due to experimental design or a genuine dependence on location has not been determined.
Seasonal variation in trace metal levels was not apparent in MSQP's historical data from Pelorus Sound. However, significant variation between years was identified, with a decline in most metal levels tested, especially for Cd and Zn (Chapter 2). Sampling only once during each season has limited the ability to detect a difference between seasons. As a result, variation of trace metal levels between seasons may have been masked by variation between years. The aim of the present study was to determine if the level of trace metals in mussels varied between seasons, and, if so, to determine whether this variation was due to changes in environmental levels of the trace metal, and/or physiological changes in mussels.

**Mussel Sampling**

The logistics of the present study did not allow an in-depth analysis of temporal variation at all sites. Instead, just a single farm in Port Ligar was selected based on MSQP's historical data set, which indicated that the variation in the level of Cd in mussels grown in this location was the highest for any location within Pelorus Sound, at over 45% standard deviation.

MSQP staff sampled ten mussels each month for the 11-month period (April 2004 to February 2005) between 5 – 7 m depths from Cannon Hill in Port Ligar, (Figure 5.1). Mussels were collected from the same farm line during the whole sampling period. Soft tissue Cd and Zn concentrations were determined in five individual animals randomly selected from each of these monthly samples using the methods described in Chapter 3.
Results

Cadmium

Except for samples collected in December 2004, Cd levels in mussels collected from Port Ligar were highly variable throughout the sampling period. During this time the mean Cd concentration was $1.64 \pm 0.13$ ppm dry weight (95% confidence interval). The standard deviation for Cd levels in mussels for the whole sampling period was 0.50 ppm or 30% of mean concentration. The variability between all months (15 – 24 %) was lower than the variability between individual samples within a sample date (10 – 44 %). High variability of Cd levels between individual mussels makes it difficult to determine if there is any difference between sample dates. ANOVA of natural log transformed Cd concentration values with date showed a small but significant difference between dates ($\text{Ln Cd dry weight} p=0.043$). Tukey post-hoc analysis showed only one group with no significant difference for concentration of Cd in mussels between sample dates. However, analysis of total Cd content in mussels showed there was a significant difference between sample dates ($p=0.001$). Tukey analysis of total Cd content produced three overlapping subgroups, with a peak in September and a smaller peak in February (Table 5.1).
Table 5.1 Tukey analysis of natural log transformed data for sample dates for total cadmium content in *Perna canaliculus* soft tissues collected from Cannon Hill, Port Ligur, 2004-2005. Each sample date is classified into groups of similar means, and each group is ranked from lowest to highest.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Ln Cadmium Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-APR-04</td>
<td>1 2 3</td>
</tr>
<tr>
<td>09-JUN-04</td>
<td>1 2 3</td>
</tr>
<tr>
<td>29-JUN-04</td>
<td>1</td>
</tr>
<tr>
<td>13-JUL-04</td>
<td>1</td>
</tr>
<tr>
<td>03-AUG-04</td>
<td>1 2 3</td>
</tr>
<tr>
<td>14-SEP-04</td>
<td>1</td>
</tr>
<tr>
<td>05-OCT-04</td>
<td>1 2 3</td>
</tr>
<tr>
<td>03-NOV-04</td>
<td>1 2</td>
</tr>
<tr>
<td>07-DEC-04</td>
<td>1 2 3</td>
</tr>
<tr>
<td>05-JAN-05</td>
<td>1 2 3</td>
</tr>
<tr>
<td>01-FEB-05</td>
<td>2 3</td>
</tr>
</tbody>
</table>

A peak in total Cd in mussels sampled during September, although not independently significant from the other sampling dates, showed total Cd in mussels did change over the year (Figure 5.2). The minimum uptake of Cd by the mussels, not accounting for efflux, for the ninety-day period from 3/11/2004 to 1/02/2005, where the total Cd in the mussels increased gradually, is calculated to be at least 29 ng Cd day$^{-1}$ (uptake = difference in total content/time period).
Zinc

In contrast to Cd, the concentration of Zn in the soft tissue of the mussels differed significantly between sample dates (p<0.001). Post-hoc Tukey analysis indicated five overlapping subgroups, with a drop in concentration in September. ANOVA of total Zn content also showed a significant difference between sample dates (p<0.001). Tukey analysis showed three overlapping subgroups, with February samples being significantly higher than all sample dates expect for January (Table 5.2). Compared to total Cd, the total Zn content showed a more consistent increase over the sampling period (Figure 5.3). The minimum uptake of Zn by the mussels, not accounting for efflux, from 3/11/2004 to 1/02/2005, is calculated to be at least 1.4 μg Zn day$^{-1}$, almost fifty times the uptake of Cd.
Table 5.2 Tukey analysis of natural log-transformed data for sample dates for zinc concentration and total content in *Perma canaliculus* soft tissues collected from Cannon Hill, Port Ligar, 2004-2005. Each sample date is classified into groups of similar means, and each group is ranked from lowest to highest.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Ln Zinc Concentration</th>
<th>Ln Zinc Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-APR-04</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>09-JUN-04</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>29-JUN-04</td>
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<tr>
<td>13-JUL-04</td>
<td>4</td>
<td>5</td>
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<td>03-AUG-04</td>
<td>3</td>
<td>5</td>
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<td>14-SEP-04</td>
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<td>05-OCT-04</td>
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<td>03-NOV-04</td>
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<td>1 2</td>
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<tr>
<td>07-DEC-04</td>
<td>1 2</td>
<td>1 2</td>
</tr>
<tr>
<td>05-JAN-05</td>
<td>1 2</td>
<td>2 3</td>
</tr>
<tr>
<td>01-FEB-05</td>
<td>1 2</td>
<td>3 3</td>
</tr>
</tbody>
</table>

Figure 5.3 Zinc in soft tissue of *Perma canaliculus* sampled from Cannon Hill, Port Ligar, 2004 – 2005. • dry weight concentration of Zn (ppm), ■ total Zn content (µg). Error bars indicate standard deviation.
The soft tissue dry weight of the mussel and volume of their shell were significantly different between sample dates (p<0.001). Tukey analysis showed that, except for January, both volume and dry weight were significantly higher in February (Table 5.3).

Table 5.3 Tukey analysis of sample dates for shell volume and dry weight in *Perna canaliculus* from Cannon Hill, Port Ligur, 2004-2005. Each sample date is classified into groups of similar means, and each group is ranked from lowest to highest.

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Volume of Shell</th>
<th>Dry Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-APR-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>09-JUN-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>29-JUN-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>13-JUL-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>03-AUG-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>14-SEP-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>05-OCT-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>03-NOV-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>07-DEC-04</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>05-JAN-05</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>01-FEB-05</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Except for September, both dry weight and volume gradually increased over the sampling period as the mussels aged. In September there was a peak in dry weight and a correspondingly smaller peak in volume, but neither of these peaks appear to be statistically significant. In contrast, the condition index of mussels did not differ significantly between sample dates (p=0.081). However, there was a peak in September and, to a lesser extent, in February, corresponding with peaks in total Cd content (Figure 5.4).
Figure 5.4 a) Dry soft tissue weight of mussel (g), b) Volume of mussel shell (cm$^3$), (volume = length x width x height of shell), c) Condition index of mussel (g cm$^{-3}$), (Condition index = Dry tissue weight / volume of shell), of Perma canaliculus sampled from Cannon Hill, Port Ligar 2004 –2005. Error bars indicate standard deviation.
ANCOVA of the Cd concentration values showed that the condition index was not a significant covariate (p=0.943), but volume and dry weight were significant covariates (p=0.005 and p=0.026 respectively). Both volume and dry weight as covariates of Cd concentration reduced the difference between the sample dates (p=0.156 and p=0.113 respectively). Condition index and dry weight were significant covariates for total Cd content (p=0.002 and p=0.001 respectively). Both covariates removed any significant difference between the sample dates of total Cd content (p=0.058 for both). However, ANCOVA of Zn levels showed that dry weight and condition index were significant covariates (p=0.007 and p=0.001 respectively). Significant covariates for total Zn content were volume and dry weight (p<0.001 for both). The significant covariates, of Zn concentration and Zn total content, did not reduce the highly significant difference of Zn levels and total Zn between the samples dates (p<0.001).

Non-parametric analysis of the correlation between levels of Cd and Zn in mussels and the dry weight, shell volume and condition index of the mussels showed that the total Cd and Zn contents in soft tissues were positively correlated to all three of these factors. Zn concentration was negatively correlated to all factors, whilst Cd concentration was negatively correlated only with dry weight and shell volume (Table 5.4).

### Table 5.4 Spearman’s Rho correlations between mussel size and the trace metal content of the soft tissue of *Perna canaliculus*, sampled from Cannon Hill, Port Ligar, 2004-2005

<table>
<thead>
<tr>
<th></th>
<th>Dry Weight</th>
<th>Shell Volume</th>
<th>Condition Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cd] (μg/g)</td>
<td>-.308(*)</td>
<td>-.455(**)</td>
<td>.076(ns)</td>
</tr>
<tr>
<td>Total Cd (μg)</td>
<td>.670(**)</td>
<td>.422(**)</td>
<td>.632(**)</td>
</tr>
<tr>
<td>[Zn] (μg/g)</td>
<td>-.552(**)</td>
<td>-.412(**)</td>
<td>-.389(**)</td>
</tr>
<tr>
<td>Total Zn (μg)</td>
<td>.677(**)</td>
<td>.652(**)</td>
<td>.354(**)</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed).**

*Correlation is significant at the 0.05 level (2-tailed).**

ns Correlation is not significant

n=55
Natural log-transformed regression analysis of the trace metal data was performed on all of the above correlations, and the only significant regression was between total Zn and dry weight ($R^2 = 0.63$) (Figure 5.5).

![Graph showing correlation between LN Total Zinc and Dry weight (g)](image)

**Figure 5.5** Correlation of total zinc ($\mu$g) to dry weight (g) of *Perna canaliculus* collected from Cannon Hill, Port Ligar 2004–2005. LN Total Zinc = 4.15 + 0.23 dry weight ($R^2 = 0.63$)

**Discussion**

Analysis of seasonal variation of the concentration of Cd and Zn in mussel tissues over a single year is difficult. Variability in trace metal levels between individual mussels makes it difficult to detect a difference in trace metal levels between sampling dates. This difficulty has been compounded by the method of sampling. Mussels were sampled from the same mussel farm line, and so for each sampling period the mussels were progressively older and larger; therefore, the date of sampling was not an independent factor.
Whether age/size affects trace metals concentrations in mussels has been widely debated. Latouche and Mix (1982) reported that larger and, therefore, older *M. edulis* samples had significantly higher levels of Cd in somatic tissue and found no relationship between size and Zn concentration. Riget *et al.* (1996) also showed mussel size to be positively correlated with Cd levels but not those of Zn. On the other hand, Boyden (1977) showed that the Cd content of *M. edulis* was independent of size, and that Zn levels were negatively correlated with size, with higher concentrations of Zn in smaller animals. In addition to increase in age/size increases, seasonal changes in tissue weight have been identified as having a major influence on trace metal levels in mussels (Boalch 1991). The sampling method in this present study has incorporated both an increase in age and seasonal changes in tissue weight so that individual effects cannot be isolated.

However, if changes in age/size were the only factors affecting Cd and Zn levels in mussel tissue, one would expect a constant increase in total Cd and Zn over the sampling year. Although total Cd and Zn contents were positively correlated with shell volume, dry weight and condition index (Table 5.4), the increase over the year was not constant (Figures 5.2 and 5.3). There was a significant drop in total Cd for mussels sampled between September and November. However, accumulation of total Zn was consistent with soft tissue weight increase, illustrated by regression of the total Zn content against dry weight (Figure 5.5). The ANCOVA demonstrated the significant interactions between condition index, dry weight and shell volume with the total Zn content and Zn level in the mussels. However, both Cd and Zn levels were negatively correlated with shell volume and dry weight, indicating a decrease in Cd and Zn concentrations as the mussels age (Table 5.4). The negative correlation is also present for the Zn level and condition index, but this relationship did not exist for Cd. This implies that changes in the total Zn content in mussels were closely associated with mussel size and condition, indicating that *P. canaliculus* may be able to biologically regulate Zn, like *P. veridis* (Yap *et al.* 2003).

Both mussel shell volume and mussel tissue dry weight increased throughout the sampling period. However, the condition index, as a function of both volume and dry weight, fluctuated. Condition index decreased in June/July, corresponding to the period of mid-winter spawning of mussels in Marlborough Sounds (Patrick 2003). Mussels sampled in September exhibited the highest condition index, which was also when the highest Cd and lowest Zn
concentration in mussels occurred. This peak in condition of mussels does not correlate with any spawning event previously described or any increase in phytoplankton (Hickman et al. 1991). A larger sample size may have shown that the September peak in condition index was correlated with the level of Cd in mussel tissue, although no significant relationship was found in the present study.

Seasonal variation in reproductive cycle and condition of mussels can mask any significant seasonal variation in trace metal concentrations (Simpson 1979). Coimbra and Carraca (1990) were able to establish spawning maxima for both Cd and Zn levels in *M. edulis*. These results are not consistent with the present Cd levels measured in *P. canaliculus*, where, Zn peaks were observed in late June corresponding with a mid-winter spawning, while Cd levels peaked in September. Latouche and Mix (1981) suggest that when studying seasonal variation of trace metal levels in mussels, somatic and gonadal tissues should be analysed separately. Separate analysis of tissues in the present study may have been able to account for variations in the percentage contribution of the gonadal tissue with changes in reproductive state of the mussels. If the assumption is made that the peak in condition index in September is related to the peak of total Cd in September, then an increase in somatic tissue weight and Cd build up, prior to any shell growth, could have occurred.

**Conclusion**

This analysis illustrates the difficulty of a long-term biomonitoring programme for *P. canaliculus*, as physiological changes in mussels over time clearly influence the concentration of trace metals in tissue. In a normal population, a range of age classes exists to allow sampling of similar mussels at each sampling period. The aquaculture industry does not provide a normal population structure, with all mussels on a single line being of a similar age class. In the present study this population structure has led to compounding effects within analysis of seasonal variance. As a result, it is not possible to determine if changes in trace metal levels in mussels during the year were due to an environmental change in the level of available trace metals.

The total Zn content and Zn levels in mussels were found to vary significantly over the growing season, with increases in mussel size reflected in decreased Zn concentrations and an
increase in the total Zn content. In contrast, any significant seasonal variation in the level of Cd in mussels from Port Ligar was not detected. The variability of the Cd levels between the mussels was too high to detect any difference between sample dates. Although an increased sample size would have increased the sensitivity of the analysis, the residual variability in the level of Cd observed in the present study required a sample size of over 30 mussels, which was beyond the time constraints and feasibility of this study. However, analysis indicated that the total Cd content in soft tissue of mussels was significantly elevated in September. The detection of this rise in the total Cd content indicates that, with further sampling, Cd concentration in mussels may be shown to be significantly higher during September at least for this site in Port Ligar.
Chapter 6

Comparison of cadmium and zinc levels in

*Perna canaliculus* and *Mytilus galloprovincialis*

The worldwide mussel aquaculture industry is dominated by the genus *Mytilus*. Three of the top four mussel species by volume cultivated belong to this genus: the blue mussel, *M. edulis*, the Chilean mussel, *M. chilensis*, and the Mediterranean mussel *M. galloprovincialis*. During the early 1980s the *Mytilus* genus accounted for more than 90% of cultivated mussels (FAO 2002). By the early 1990s development of other mussel species in mariculture began to have an impact on mussel cultivation, leading to a rapid increase in size of mussel mariculture markets, which by 2002 was estimated to be worth around US$ 700 million (FAO 2002). However, over 60% of maricultured mussels were still of the genus *Mytilus*, with *M. edulis* alone accounting for 41% of worldwide mariculture in 2002 (Figure 6.1).

![Figure 6.1 Mussel sales worldwide in 2002, (FAO 2002)]
Although there is a large international market for blue mussels, it is considered a pest species by the NZ mussel industry, growing invasively on many *P. canaliculus* mussel farms. Blue mussels readily attach to mussel farm lines increasing weight burden and, in shallow waters, often out-competing green mussels. In areas where growth of blue mussels is a problem, mechanisms are put in place to reduce competition from blue mussels. One method is for mussel farm lines to be submerged by 1-2 m until green mussels are adequately established on the line, avoiding intense shallow-water competition from blue mussels. Though many such steps are taken to avoid growth of blue mussels, they are commonly found on mussel farm lines and have to be manually removed during harvesting of most farms within Pelorus Sound. Although the presence of blue mussels is undesirable, it does allow comparison of trace metal uptake between the two species growing in the same aquatic environment.

The worldwide distribution of *Mytilus* and consistent bioconcentration of trace metals and other pollutants, highlighted the potential of blue mussels as a biomonitor (Goldberg 1975). As a result, blue mussels have been used as the basis for the global environmental monitoring programme “Mussel Watch” (Goldberg 1986). Initially an extensive biomonitoring programme along the USA coast in 1976, the “Mussel Watch” programme rapidly spread as a cheap method of detecting changes in pollutant levels (Goldberg 1978). This programme in turn instigated a rapid increase in research into the dynamics of trace metal accumulation by *Mytilus*. As a result, most data currently available on bivalve trace metal accumulation have been collected for *M. edulis*.

In New Zealand, blue mussels were previously identified as *M. edulis*, but have since been reclassified as *M. galloprovincialis* (Koehn 1991; McDonald *et al.* 1991). Although different to the intended monitoring species in “Mussel Watch” monitoring programme, the difficulty in distinguishing between *Mytilus* species has resulted in the use of both *M. galloprovincialis* and *M. trossulus* in many of the Mussel Watch programmes worldwide (Lobel *et al.* 1990). Analysing the trace metal levels of *M. galloprovincialis* and *P. canaliculus* from the same location allows the comparison of any differences in the trace metal accumulation. The absence of significant variation in trace metal levels between the two species could indicate that trace metal uptake rate is similar in both species. In this case,
identifying significant factors that affect the uptake rate of trace metals in *Mytilus* spp. could potentially also apply to *P. canaliculus*.

**Methods**

Identification of *P. canaliculus* and *M. galloprovincialis* from cultivated lines is straightforward as they have an obvious colour difference. For wild mussels it can be difficult to differentiate species by colour alone, as the green colouration in wild *P. canaliculus* can be limited to the inner shell margin. Other methods to discriminate between the species include general differences in shell shape, with *Mytilus* sp. having a square end compared to *P. canaliculus*. However, a definitive method of distinguishing between the two species is to examine them internally. Where *Mytilus* sp. has an anterior adductor muscle and a single retractor scar, *Perna* sp. lack an anterior adductor muscle and has two retractor scars (Figure 6.2) (Siddal 1980).

![Muscle scar patterns on inside right valve of *P. canaliculus* and *M. edulis*. Note contrasting retractor muscle scars, *P. canaliculus* has a two-part scar where *M. edulis* has a single scar. Drawn ½ life size (adapted from Siddal 1980).](image)

Mussels of both species were collected from the same mussel line between 5 - 7 m by MSQP from site 12, Cannon Hill, Port Ligur, on 03/08/2004 (see Figure 5.1). The mussels were collected at random from the location rather than selected for similar size and so there is some size variation. Mussel were analysed for trace metal levels using methods described in
Chapter 3. The presence of any symbionts was recorded and, where possible, the symbiont was removed before trace metal analysis was undertaken. To account for discrepancies in weight wet between species, the concentrations of trace metal in relation to the volume ($\mu$g cm$^{-3}$) were calculated from the value for trace metal levels determined directly by analysis.

**Results**

By visual assessment alone, blue mussels seemed to be in poorer condition than green-lipped mussels, appearing watery with little flesh content. Every blue mussel sampled contained at least one type of symbiont: nine of the blue mussels had one or more pea crabs, *Pinnotheres novaezelandiae* (Figure 6.3); when two pea crabs occurred in the same mussel, both crabs were less than 5 mm carapace width; four blue mussels had a trematode infection, *Cercaria haswelli*, discourting the majority of vascular tissue bright orange/red (Jones 1975) (Figure 6.4). The presence of the trematode was so extensive that it was not possible to visually determine the sex of infected mussels. In contrast, no crabs were found in the sample of green mussels and only three *P. novaezelandiae* were found in all the green mussels investigated throughout this study.

![Figure 6.3](image)

*Figure 6.3* Two forms of *Pinnotheres novaezelandiae* found in *M. galloprovincialis* from Cannon Hill, Port Ligar Pelorus Sound. 1. Mature female 2. Stage I hard shell (may be male or female). Illustration three times life size (see also Scott 1961 and Jones 1975)
Figure 6.4 Trematode, *Cercaria haswelli*, infection of mussel tissue. Life size

A comparison of moisture content showed that the blue mussels had significantly higher mean moisture content of 85% compared to 80% for green mussels (p<0.001) (Figure 6.5). The wet weight of the mussels, however, showed both species were of similar average weight. In contrast, green mussels had a 34% higher dry weight, were significantly smaller in volume, and had significantly higher condition index than the blue mussels. However, all these physiological parameters were more variable in blue mussels (Table 6.1).

![Figure 6.5 Comparison of moisture content of blue and green mussels.](image)

*Figure 6.5* Comparison of moisture content of ■ *Mytilus galloprovincialis* and ◆ *Perna canaliculus* collected from Cannon Hill, Port Ligur, Marborough Sounds on 03/08/2004. *M. edulis* $y = 6.66 x (r^2=0.76)$ and *P. canaliculus* $y = 4.94 x (r^2=0.66)$
Table 6.1 Physiological comparison between *Mytilus galloprovincialis* and *Perna canaliculus* sampled from Cannon Hill, Port Ligar August 2004

<table>
<thead>
<tr>
<th>Species</th>
<th>Wet Weight (g)</th>
<th>Dry Weight (g)</th>
<th>Shell Volume (cm$^3$)</th>
<th>Condition Index (g cm$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Mytilus</em></td>
<td><em>Perna</em></td>
<td><em>Mytilus</em></td>
<td><em>Perna</em></td>
</tr>
<tr>
<td>Mean</td>
<td>12.44</td>
<td>12.58</td>
<td>1.81</td>
<td>2.53</td>
</tr>
<tr>
<td>Standard Error</td>
<td>1.51</td>
<td>0.65</td>
<td>0.25</td>
<td>0.13</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>4.77</td>
<td>2.07</td>
<td>0.80</td>
<td>0.42</td>
</tr>
<tr>
<td>p values <em>a</em></td>
<td>0.933</td>
<td>0.020</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

*p values for ANOVA analysis of the difference between the species*

Analysis of trace metal wet weight concentrations showed blue and green mussels were significantly different for Zn but not Cd (p=0.029, p=0.054 respectively). Analysis of dry weight trace metal concentrations showed blue mussels to be significantly higher for Zn and Cd (p=0.008 and p<0.001 respectively) (Figure 6.6).

Analysis of the total Cd and Zn content showed no significant difference between the two species of mussels for either metal (p=0.219 and p=0.073 respectively). A comparison of trace metal levels as a function of volume (μg cm$^{-3}$) also showed that neither metal differed significantly between the two mussel species (Cd, p=0.158 and Zn, p=0.476).
Figure 6.6 Comparison between trace metal concentration in wet weight and dry weight analysis for [Mytilus galloprovincialis] and [Perna canaliculus] sampled from Port Ligar August 2004. Error bars show standard deviation.

Discussion

Assimilation efficiencies (the percentage of a trace metal incorporated through gut lining and into an animal) of bivalves can vary widely between species (Reinfelder et al. 1997). To distinguish between trace metal levels in two species of mussels there has to be a
difference in the assimilation efficiency or ability to store trace metals once absorbed. The green and blue mussels tested showed no difference in wet weight concentration of Cd. However, results showed that the blue mussels had significantly higher levels of Cd in dry tissue (Figure 6.6). Zn levels were also significantly higher in blue mussels for both wet and dry concentrations. Although a difference between the species’ trace metal levels was apparent in dry weight analysis, it was not possible to determine if this difference was due to any variation in the uptake rates of the metals by the two mussel species.

A comparison of trace metal levels between the two species was made difficult by the presence of symbionts in all blue mussels. As noted earlier, the frequency of symbionts in blue mussels sampled was extremely high, 90% with pea crab, *Pinnothetes novaezelandiae*, and 40% with trematodes, *C. haswelli*. In comparison, green mussels showed a symbiont incidence rate of less than 1%, and no green mussel was found with a trematode infection. The high incidence of symbionts in blue mussels could be a result of susceptibility of the mussel species to infection or that host recognition by the symbionts is highly specific (Stevens 1990).

Results showed that condition of the blue mussels was significantly lower than that of the green mussels. If symbionts cause a reduction in condition of mussels, then a difference in trace metal concentrations may be a direct result of the presence of symbionts. Analysis of the incidence rate of *P. canaliculus* by pea crab, *Pinnothetes novaezelandiae*, showed no reduction in condition index of mussels as a result of pea crab infections (Hickman 1978). As all blue mussels sampled had some form of symbiont, the effect of symbionts on the condition index and trace metal levels of the blue mussels cannot be accounted for in the present study.

The significantly higher moisture content in the blue mussels (Figure 6.5), compounded with the drying of mussel tissue increasing the concentration of trace metals in dry weight proportionally to moisture content, would enhance any difference in trace metal levels between the two mussel species. This enhanced difference between trace metal content was evident in the results, especially for Cd (Figure 6.6). Analysis of dry tissue trace metal levels is normally used to avoid variations in moisture content as a result of rapid fluctuations in mussel condition and experimental error (Lucas & Beninger 1984; Hickman & Illingworth 1980). In the present study, the condition of the blue mussels was so poor that the green
mussels sampled had a significantly higher dry tissue weight although the shell volume was significantly lower (Table 6.1). The large difference in dry weights of animals tested confounds any comparisons between species. To ascertain if a difference in trace metal content is due to differences in uptake rate of trace metals between the species, one would need to determine if a difference in moisture content and dry weight is due to the presence of symbionts.

Low numbers of blue mussels at the sample site did not allow for a restricted size selection. The size of blue mussels analysed was significantly larger and more variable than that of green mussels. The higher level of variation in size and weight could be a result of self-seeding of mussels onto mussel farm lines, leading to a large discrepancy in age. A study comparing *M. edulis* and *M. trossulus* trace metal accumulation showed that the slower growing *M. trossulus* has proportionally higher levels of trace metals (Lobel *et al.* 1990). Variation in trace metal levels between the species in the study by the Lobel *et al.* (1990) was associated with differences in growth rates and age of samples rather than a difference in rate of bioaccumulation of trace metals. Therefore, any variation in the concentration of trace metal between blue and green mussels could be due to differences in age of samples as well as differences in the condition of the mussels.

The examination of trace metal levels as a function of weight of mussel emphasises significant difference in condition index. To avoid using the confounding factor of mussel dry weight in the analysis, total trace metal burden has been calculated. The total Cd and Zn contents in the soft tissue of mussels were not significantly different between the species, although blue mussels had significantly larger shells. A comparison of trace metal levels as a function of volume of shells (μg cm⁻³) also showed no significant difference between the mussel species. The lack of difference between the mussel species for both total trace metal content and shell volume trace metal levels, may be due to the variability of the size and condition of the blue mussels that were analysed. However, the analysis does indicate that if mussels of similar condition were tested, it is likely that the trace metal levels of the two species would be comparable.
Conclusion

Mussels are relatively inefficient at accumulating Zn and Cd compared to other bivalves, such as oysters and clams (Reinfelder et al. 1997), and yet it was still possible to detect a difference between the levels of the trace metals, Cd and Zn, for the two mussel species. What the present data did not show is whether a difference in trace metal levels was due to differences in uptake rate of trace metals or differences in physiological condition of the mussels.

From the present results it is possible to conclude that blue mussels grown in Port Ligar are more likely to contain trematodes and pea crabs than green mussels. Dry weight trace metal concentrations indicate that blue mussels with symbionts have significantly higher concentrations of Cd and Zn in dry weight analysis than green mussels. The presence of symbionts, however, makes it impossible to determine if there is a difference in uptake of trace metals between blue and green mussels or whether the difference in trace metal levels was due to lower condition of blue mussels caused by the presence of symbionts. Also the trace metal level of the symbionts, which were not determined in this study, could have had an effect on the level of trace metal found in the infected mussels. As C. haswelli could not be isolated from the blue mussels, the level of trace metal in the parasite would have been included in the trace metal level of the mussel.

The analysis was also hampered by variation in size and age of the blue mussels. In order to clarify if there is a difference in trace metal levels between the two species of mussels, analysis of similar age classes would be required. As green mussels were all sampled from a seeded mussel line one would expect them to be of similar age. Blue mussels that self-seed onto mussel farm lines do not have a similar age restriction.
Chapter 7

Sources of variation of cadmium and zinc levels in *Perna canaliculus*

Introduction

The concentration of Cd and Zn in mussels from Pelorus Sound is highly variable as outlined in earlier chapters. Some variation in Cd and Zn levels may be explained by factors that have been reported in the literature as having a significant effect on the uptake of trace metal in other species of bivalves. These include: distribution of Cd and Zn within the mussel tissue (Soto *et al.* 1996), genetic variability in uptake and excretion of trace metals (Lobel *et al.* 1989), location (Latouch & Mix 1981), and concentration of trace metal in the environment (Chan 1988). The degree to which each of the above factors could influence variation of trace metal levels in mussels in Pelorus Sound has been examined in small pilot studies undertaken in the present study.

The mussels were not depurated before analysis, and as a result trace metals in the gut may be a source of variability in trace metal levels (Phillips & Rainbow 1993). The location of trace metals stored within tissues of bivalves may indicate whether the gut contents could have been the cause of some variability in trace metal levels (Kennedy 1986). The mechanism of uptake and storage of a trace metal can be related to the distribution of the trace metal within the tissues of mussels (Soto *et al.* 1996). Cd has been reported to accumulate at different rates between the organs of other bivalve species, with the highest concentration commonly found in the gills or digestive tract (Segar *et al.* 1971; Romeo & Gnassia-Barelli 1995), which might be expected for a filter feeder. By analysing the variation of Cd levels within the different tissues of *P. canaliculus*, it may also be possible to determine if the mechanism(s) for uptake and storage are similar to those of other bivalve species.

Metallothioneins, proteins that forms complexes with heavy metals, have been associated with the uptake of Cd in the blue mussel *Mytilus galloprovincialis* (Viarengo *et al.* 1985; Bebianno & Langston 1992). Therefore, any variation in the expression of the
methallothionein genes between mussels may lead to some variation in the ability of the mussel to bioaccumulate trace metals. Breeding programmes have been introduced in oyster cultivation, and more recently in mussel cultivation, to improve the disease resistance, growth rates, meat quality, colour and taste of the shellfish (Ward and Thompson 2004). There appears to have been no research to date that has examined the genetic variability in the uptake of trace metals or expression of methalloneins within mussel populations. By comparing the variation between trace metal uptake of mussels from a breeding programme with mussels from wild collected spat, trace metal uptake may be shown to have a genetic link.

Cd levels in *P. canaliculus* grown in Pelorus Sound are much higher than those reported by Nielsen and Nathan (1975). Cadmium levels ranged from 0.10 to 1.0 ppm wet weight in mussels collected from around New Zealand, in comparison to the range of Cd levels of < 0.2 – 2 ppm wet weight observed in the historical data from Marlborough Sounds (Chapter 2). It is possible that changes have occurred in the anthropogenic input of Cd into the New Zealand marine environment, whether directly or indirectly through atmospheric pollution, and this might have increased in the 30 years since Nielsen and Nathan’s research. In which case, Cd levels in mussels from other locations in the South Island might also be higher than those previously reported by Nielsen and Nathan.

Water exchange in Pelorus Sound occurs over a period of 9 days (Heath 1982). The slow circulation within the sound may result in a Cd gradient in the water along Pelorus Sound. Determining the trace metal concentration of seawater without introducing contamination of the samples requires ultra-clean methods of trace metal analysis (Ahlers *et al.* 1990). However, phosphate has been found to be a good proxy for Cd (Hunter & Ho 1991), exhibiting similar trends with depth. It is found in much higher concentrations in the marine environment and hence it does not require ultra-clean methods for water sample collection, and instead the samples can be taken directly from an aluminium vessel. By analysing trends in the phosphate concentration within the waters of Pelorus Sound, it may be possible to determine if the pattern of Cd uptake by mussels is also reflected in the variation of phosphate levels in the water column.
The circulation within Pelorus Sound has been calculated using tidal cycles and the input of freshwater (Heath 1982). Along Pelorus Sound there are often salinity and temperature gradients as freshwater mixes with the seawater. The salinity and temperature gradients are seasonally variable in extent, and can form during periods of high rainfall (Heath 1982). As contamination of coastal waters is usually from land-based inputs, the high levels of freshwater input into Pelorus Sound could be a source of trace metals. The only published data on trace metal levels in rivers flowing into Pelorus Sound indicates they are low, with both Cd and Zn being below the study’s limits of detection (0.2 ppb and 5 ppb respectively) (cited in Smith & Williamson 1986). As access on foot to Pelorus River is possible, a sample of the river water was collected using ultra-clean trace metal techniques to determine if the river is a possible source of Cd in Pelorus Sound.

Methods

Distribution of Cd and Zn within P. canaliculus

To determine the distribution of Cd and Zn within mussel soft tissue, the tissues from three mussels were combined. As the tissue weight of gills from a single mussel was too low for precise FAAS analysis, combining the tissues enabled a more accurate analysis, and it also resulted in the determination of a mean value for different tissue types. The three mussels were randomly selected from the extra mussels in the sample collected from sample site 7, Grants Bay (Chapter 4), on 26 October 2004. The mussels were dissected and combined into the following tissue categories: foot, gonad, edge of mantle, gills, muscle and digestive tract including circulatory organs. Any remaining tissue was grouped into viscera, but byssus threads were not included (Figure 7.1). Each tissue group was weighed and its percentage contribution to total weight was calculated. Cd and Zn were analysed in tissue samples (see methods described in Chapter 3), and the percentage contribution of each tissue group to total trace metal content was also calculated.
Effect of genetic diversity on the variability of Cd and Zn levels in *P. canaliculus*

The Cawthron Institute has been breeding 60 families of *P. canaliculus*, to select for improved growth and survival characteristics. The trace metal levels of a mussel family from the Cawthron Institute were compared with those of normally seeded commercial mussels grown from wild-caught spat. Any difference in the variability of trace metal levels could be a result of the genetic purity of the Cawthron Institute-bred mussels. A sample of ten mussels from a single family line were collected by the Cawthron Institute's staff from Hallam Cove in January 2005 and another sample of ten mussels was collected from a commercial farm within 300 m of the Cawthron Institute's site by MSQP staff (Figure 7.2). Both samples were collected from 5 – 7 m depth, approximately mid-dropper, frozen and transported to Dunedin for analysis.
Levels of Cd and Zn in mussels from Stewart Island and Otago

Two locations were selected to compare the trace metal levels in mussels from South Island, New Zealand: Big Glory Bay, Stewart Island, and Portobello wharf on the Portobello Peninsula, Otago (Figure 7.2). Big Glory Bay was selected as it is the closest mussel farming area to Foveaux Strait, where Cd levels of over 30 ppm dry weight are commonly found in oysters (Nielsen 1975; Frew 1991). Portobello wharf was selected as it is situated in the middle of Otago Harbour, which has many potential sources of pollution, with two commercial ports, Ravensdown fertiliser plant, extensive road networks along the harbour edge and historical sewage disposal into the harbour.

A sample of ten mussels was collected from a farm in Big Glory Bay and another sample collected from the Portobello Marine Studies Centre wharf. The mussels were shucked with trace metal clean plastic knives and then frozen in individual plastic bags until analysis. Mussels were analysed using methods described in Chapter 3.
Figure 7.2 Location of mussels sampled in January 2005 for analysis of genetic diversity and spatial variation of trace metal levels in *Perna canaliculus*, NZ Map Grid 100,000.
Water Analysis of Pelorus Sound

During April 2004 sampling, CTD casts were taken to examine the extent of variation in the physical properties of the water along Pelorus Sound (sample sites 1 - 9). Water samples were collected using trace metal clean methods for phosphate analysis on 26 October 2004 from all bays that were sampled within the inner Pelorus Sound (sample sites 1 – 8, see Figure 4.1) and also from Cannon Hill, Port Ligar (see Figure 5.1). A fresh water sample was taken from Pelorus River mouth using ultra-clean trace metal methods. The river sample was analysed for total and dissolved Cd (Cd which passes through a 0.45 μm filter).

Phosphate water samples

When mussel samples were collected in October 2004, water samples were also taken for phosphate analysis. These samples were collected using a Niskin bottle from the upwind side of the vessel, on the opposite side to the mussel lines. Initially the Niskin bottle was lowered to 1 m for thirty seconds to allow sufficient time to rinse off any contamination arising from the surface water layer and previous sample sites. The Niskin bottle was then further lowered to 6 m and triggered by a messenger to collect a water sample.

On return to the surface, after initially rinsing the sample bottles with the collected water, water samples were transferred into 10% HCl acid-washed sample bottles. Water samples were kept cool until returning to shore, where they were frozen to -25°C within 7 hours of collection. The Niskin bottle was then drained and reprimed for the next sample site. During transit the Niskin bottle was sealed in a plastic bag to reduce contamination.

In the laboratory, the samples were thawed and brought to room temperature and analysed for phosphate using a method based on Murphy and Riley (1962) and with modifications by Strickland and Parsons (1972). A 40 ml sample of the seawater was combined with an acidic solution of ammonium molybdate, ascorbic acid and potassium antimonyl-tartrate. Any phosphate in the sample reacts stoichiometrically with the solution to produce a blue colour which could be measured spectrophotometrically in a 10 cm cell at a wavelength of 885 nm using distilled water as a reference. The concentration of the phosphate is determined using a standardized solution of anhydrous potassium dihydrogen
phosphate (KH$_2$PO$_4$) (3.0 μM). Blank distilled water samples were used to ensure there was no contamination of the reactive solutions. This method can be used to determine reactive phosphate concentrations in the range of 0.03 - 5 μg-atom P L$^{-1}$, (Strickland & Parsons 1972).

**Cadmium water samples**

A freshwater sample from the Pelorus River mouth was collected by hand using ultraclean trace metal methods (Ahlers et al. 1990). Sample bottles were ultra-cleaned trace metal, double bagged, and kept full of quartz-distilled 1% HCl until just prior to sampling. Using double gloves and avoiding any unnecessary touching of the sample bottle and bags, a water sample was collected upwind of recent cattle movements. To avoid any contamination from the surface water layer, the sample bottle was drained of acid and recapped until submerged 30 cm in mid-stream flow. A field blank was drained and exposed to the air for 30 seconds at the sample site. Both the sample and the field blank were then frozen to -25°C within 1 hour of collection. All samples were transported in a frozen state to Dunedin. Some samples, however, did thaw somewhat during transport but no sample had completely defrosted before being returned to a freezer at -15°C.

On return to the laboratory, the freshwater sample from the Pelorus River was analysed for Cd using anodic stripping voltametry (ASV) (Jagner et al. 1981). This is an electrochemical method in which metal ions are reduced from solution forming an amalgam with the electrode. The amalgam is then reoxidized, which generates a current signal proportional to the metal ions in solution. Measuring the amp output of the current at a specific voltage, -0.583 V for Cd, it is possible to determine the concentration of metal ions in solution (Jagner et al. 1981). The sensitivity of ASV increases as the time a sample is reduced increases, resulting in a lower limit of detection and improved accuracy.

The freshwater river sample was defrosted in the dark overnight and the empty field blank sample bottle was filled with Milli Q$^\circledR$ water. Both water samples were then acidified with 0.1% quartz-distilled HCl. A subsample of the acidified river water and field blank were filtered through 0.45 μm filter and a further subsample of the acidified river water was taken for total Cd analysis. In order to breakdown any organic molecules, all three subsamples were UV irradiated for seven hours. A 10ml aliquot of each subsample was processed using a 4000
second deposition time and the standard addition of 10 µL and 20 µL of 20 nM Cd to determine the concentration of any Cd peaks. All steps of this analysis require ultra-clean processes with all glassware being cleaned through multiple HCl quartz-distilled acid baths and Milli-Q® water rinses.

Results

Distribution of Cd and Zn within *P. canaliculus*

The different mussel tissue types varied with viscera accounting for the greatest proportion of soft tissue: viscera > edge of mantle > muscle > digestive tract > gills > gonad > foot (Table 7.1).

<table>
<thead>
<tr>
<th>Tissue Type</th>
<th>Weight (g)</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gills</td>
<td>1.09</td>
<td>8</td>
</tr>
<tr>
<td>Digestive tract</td>
<td>1.65</td>
<td>12</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.96</td>
<td>14</td>
</tr>
<tr>
<td>Viscera</td>
<td>4.91</td>
<td>35</td>
</tr>
<tr>
<td>Gonad</td>
<td>0.78</td>
<td>6</td>
</tr>
<tr>
<td>Foot</td>
<td>0.73</td>
<td>5</td>
</tr>
<tr>
<td>Edge of mantle</td>
<td>2.77</td>
<td>20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13.89</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

The concentration of Cd in tissues of mussels varied considerably (0.8 - 3.1 ppm dry weight). High Cd concentrations were found in the gills and digestive tract. The level of Cd in viscera, gonad, foot and edge of mantle was a third of the concentration of Cd found in the gills. The concentrations of Zn in mussel tissues were not as variable as those for Cd, although viscera, gonad and edge of mantle still had lower concentrations of Zn compared to the gills and digestive tract. However, unlike Cd, the highest concentration of Zn was found in the muscle tissue (Figure 7.3).
The variation in the total content of Cd and Zn between different mussel organs was different from that of concentration (Table 7.2). The highest percentage of both Cd and Zn was found in the viscera of the mussel. The percentage of Cd found in digestive tract and
gills (22% and 16% respectively), were higher than those for Zn (15% and 8% respectively). In contrast, double the percentage of Zn was found in the foot compared to Cd (6% and 3% respectively).

<table>
<thead>
<tr>
<th>Mussel Tissue</th>
<th>Cadmium Concentration (ppm)</th>
<th>Total (µg)</th>
<th>Fraction (%)</th>
<th>Zinc Concentration (ppm)</th>
<th>Total (µg)</th>
<th>Fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edge of mantle</td>
<td>0.84</td>
<td>0.77</td>
<td>11</td>
<td>28.6</td>
<td>26.4</td>
<td>17</td>
</tr>
<tr>
<td>Foot</td>
<td>0.91</td>
<td>0.22</td>
<td>3</td>
<td>36.8</td>
<td>8.9</td>
<td>6</td>
</tr>
<tr>
<td>Gonad</td>
<td>0.97</td>
<td>0.25</td>
<td>4</td>
<td>23.6</td>
<td>6.1</td>
<td>4</td>
</tr>
<tr>
<td>Viscera</td>
<td>1.12</td>
<td>1.83</td>
<td>27</td>
<td>27.4</td>
<td>44.9</td>
<td>30</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.64</td>
<td>1.08</td>
<td>16</td>
<td>44.0</td>
<td>28.8</td>
<td>19</td>
</tr>
<tr>
<td>Digestive Tract</td>
<td>2.73</td>
<td>1.50</td>
<td>22</td>
<td>42.0</td>
<td>23.0</td>
<td>15</td>
</tr>
<tr>
<td>Gills</td>
<td>3.06</td>
<td>1.11</td>
<td>16</td>
<td>35.3</td>
<td>12.8</td>
<td>8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>1.46</td>
<td>6.76</td>
<td>100</td>
<td>32.6</td>
<td>151.1</td>
<td>100</td>
</tr>
</tbody>
</table>

**Table 7.2** Distribution of total a) cadmium and b) zinc in the separated tissues of *Perna canaliculus* collected from Grants Bay October 2004.

**Effect of genetic diversity on Cd and Zn levels in mussels**

The formation of any mature reproductive tissues was not apparent in the Cawthron Institute’s samples, and so sexing mussels by colour differentiation of the gonad was not possible in these samples. A large brooding pea crab, *Pinnotheres novaezelandiae*, was found in one of the commercial mussels sampled. This mussel was of similar condition and weight to other mussels sampled, but had a high Zn concentration that was determined to be a statistical outlier and so was removed from subsequent analysis. Removing this outlier left a total of nine mussels for the statistical analysis of the commercial mussel sample.

The mussels from the Cawthron Institute’s breeding programme had significantly lower condition index and dry weight values (p<0.001 and p=0.001 respectively). The shell volume was not significantly different between the two mussel types (p=0.327) (Table 7.3).
Table 7.3 Analysis of the difference between the physical properties of Cawthron Institute pure bred and commercial *Perna canaliculus*, sampled from Hallam Cove January 2005.
Cawthron Institute sample: n=10, commercial sample: n=9

<table>
<thead>
<tr>
<th></th>
<th>Shell Volume (cm$^3$)</th>
<th>Condition Index (g cm$^{-3}$)</th>
<th>Dry Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cawthron</td>
<td>Commercial</td>
<td>Cawthron</td>
</tr>
<tr>
<td>Mean</td>
<td>84.0</td>
<td>93.1</td>
<td>27.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>69.2</td>
<td>57.8</td>
<td>19.7</td>
</tr>
<tr>
<td>Maximum</td>
<td>99.6</td>
<td>121.8</td>
<td>36.9</td>
</tr>
<tr>
<td>Range</td>
<td>30.5</td>
<td>63.9</td>
<td>17.2</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>8.8</td>
<td>22.7</td>
<td>4.5</td>
</tr>
</tbody>
</table>

After natural log transformation of both Cd and Zn concentrations, ANOVA showed a significant difference between the Cd and Zn levels in the two mussel groups. The Cawthron Institute’s mussel samples had significantly lower Cd and Zn levels in the soft tissue than commercial samples (p<0.001 and p=0.017 respectively). The degree of variability in Cd and Zn levels between the two mussel types was also different; the standard deviation was lower for Cd levels and higher for Zn levels in the Cawthron Institute’s mussels (Table 7.4).

Table 7.4 Analysis of the difference between the trace metal levels of Cawthron Institute pure bred and commercial *Perna canaliculus*, sampled from Hallam Cove, January 2005.
Cawthron Institute sample: n=10, commercial sample: n=9.

<table>
<thead>
<tr>
<th></th>
<th>Cadmium (ppm) dry weight</th>
<th>Zinc (ppm) dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cawthron</td>
<td>Commercial</td>
</tr>
<tr>
<td>Mean</td>
<td>1.46</td>
<td>2.46</td>
</tr>
<tr>
<td>Minimum</td>
<td>1.06</td>
<td>1.29</td>
</tr>
<tr>
<td>Maximum</td>
<td>2.06</td>
<td>3.67</td>
</tr>
<tr>
<td>Range</td>
<td>1.00</td>
<td>2.38</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.30</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Levels of Cd and Zn in mussels from Stewart Island and Otago

The levels of Cd in both Big Glory Bay and Portobello samples were lower than the levels found in most of Pelorus Sound mussels sampled in this study. Big Glory Bay mussels had a mean Cd level of 0.43 ± 0.11 ppm dry weight. Cd levels of Portobello mussels were at the limit of detection, with a mean Cd level of 0.25 ± 0.13 ppm dry weight. Zn concentrations were 59 ± 7 and 61 ± 6 ppm dry weight respectively.

Analysis of Water from Pelorus Sound

Physical Properties

The water along the length of Pelorus Sound was well mixed on 6 April 2004 as determined by a constant salinity of 33 psu and a temperature of 15.59 - 15.90 °C along the length of Pelorus Sound down to depth of 17.5 m (Appendix 2).

Reactive Phosphate Levels in Pelorus Sound

The concentration of dissolved reactive phosphate in all of the seawater samples was below the limit of detection for the method used at <0.9 ppb (<0.03 µg-atom P).
Cadmium in Pelorus River

Regression analysis of the standard additions to each of the samples allowed the calculation of the concentrations of Cd in the samples (Table 7.5). The filtered field blank sampled showed a low level of Cd contamination in the sampling method of 1.7 ppt (15 pM), the level of Cd in the filtered Pelorus River sample was 3.0 ppt (26.5 pM), which after correcting for contamination was 1.3 ppt dissolved Cd (11.5 pM). The unfiltered sample Cd level was 3.4 ppt (30 pM). As contamination of the blank sample may have occurred in the filtering process, the total Cd in Pelorus River mouth sample can only be recorded as being less than or equal to 3.4 ppt but greater than 1.7 ppt.

<table>
<thead>
<tr>
<th>Solution</th>
<th>Line</th>
<th>$R^2$</th>
<th>Cd content (pM) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sample</td>
<td>$y = 10.34 x + 0.3133$</td>
<td>0.997</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Filtered Sample</td>
<td>$y = 19.600 x + 0.520$</td>
<td>0.993</td>
<td>&lt;26.5</td>
</tr>
<tr>
<td>Filtered Blank</td>
<td>$y = 15.192 x + 0.2287$</td>
<td>0.989</td>
<td>&lt;15</td>
</tr>
</tbody>
</table>

$^a$Trace metal content = constant/slope of line

Discussion

Distribution of Cd and Zn within P. canaliculus

The distribution of Cd and Zn between the mussel’s organs was very different; Zn concentrations were relatively consistent between the mussel’s organs compared to Cd (Figure 7.3). This result is similar to the findings of Brooks and Rumsby (1965) who ascertained that Zn but not Cd was widely distributed among the different organs of scallops, Pecten novaeezelandiae, and oysters, Ostrea sinuata.
The three-fold difference in Cd concentration between the different mussel tissues suggests that there is some difference in sequestering of Cd between these organs. The gills of bivalves have been recognized as a major site for the concentration and uptake of Cd (Roesijadi & Klerks 1989). Therefore, it is no surprise that the highest concentration of Cd was found in the gills of *P. canaliculus*, as previously observed for other bivalve species such as *M. galloprovincialis* (Soto et al. 1996), scallops *Pecten novaezelandiae* and oysters *Ostrea sinuata* (Brooks & Rumsby 1965 and 67). However the scallop *Pecten maximus* and mussel *Modiolus modiolus* were found to have the highest concentration of Cd in the gut and digestive gland whereas only the gills were moderately enriched (Segar et al. 1971). The elevated concentration of Cd in gills and digestive tract determined in the present study indicate that, of all these bivalves including *P. canaliculus*, Cd could be partitioned into these organs to avoid the toxic effects of Cd disrupting the function of the rest of the animal (Viarengo et al. 1985). In scallops the concentration of Cd is typically so high in the gills and surrounding viscera that only the muscle and gonad tissues are sold for human consumption (Ashoka 1999). This tissue segregation is possible for scallops as the organs are easily separated but in mussels this type of separation is not possible.

Over a third of the total amount of Cd is found in the gills and digestive tract of *P. canaliculus*. A period of depuration of the mussels immediately after sampling may eliminate a large proportion of this Cd. Any potential to reduce the concentration of Cd in mussels by depuration relies, however, on the rate of dissemination of Cd into seawater. Compared to other trace metals, elimination of Cd is significantly slower; for example, the biological half-life of Cu in *M. galloprovincialis* has been reported to be 9 - 10 days, whereas Cd has a half-life of over 120 days (Viarengo et al. 1985). Even the Cd content of the gills of oysters, *Crassostrea virginica*, which would be expected to easily depurate, has an estimated half-life of approximately 35 days (Roesijardi & Klerks 1989). The strong binding of Cd by metallothioneins slows down and reduces any potential benefits of depuration (Viarengo et al 1985).

As the distribution of Zn is relatively constant throughout the tissues of *P. canaliculus*, it is unlikely that differences in the volume of the tissues could explain some of the present variation in the level of Zn between mussels. However, more research is required to determine if changes in the distribution of Cd throughout the tissues of *P. canaliculus* could
be a factor in the variation of Cd levels between mussels. The low concentration of Cd found in the gonad and viscera of *P. canaliculus* could indicate that changes in the reproductive cycle of the mussel would lead to a dilution of the Cd in the mussel, as the volume of low Cd concentration tissue increases, as suggested for *M. edulis* (Latouche & Mix 1981).

**Genetic variation between the mussels**

There was a large variation in size of the commercial mussels sampled. This variation in size could be due to the density of mussels grown on the culture lines, as a high growth density has been shown to lead to variation and reduction in the growth rate (Jenkins *et al.* 1985). Because of the variability in size of the mussels, it is more difficult to make a direct comparison between the trace metal levels in the mussels (Lobel *et al.* 1991a; Riget *et al.* 1996).

The genetically akin Cawthron Institute mussels were similar in size, weight, condition index and Cd levels. Whether the Cd levels are more alike due to a similarity in physical characteristics of the mussels or in the rate of Cd uptake cannot be established from the present results. If there was simply a genetic basis for trace metal uptake, one might expect that trace metal levels of genetically similar mussels would be less variable than those for wild samples. The present results show that a lower level of variability in Cawthron Institute mussels only occurred for Cd. The variability in Zn levels, though low for both samples, was lower in the commercial sample. This difference suggests that there is no genetically variable link to the uptake of Zn.

Dry weight and condition index of the Cawthron Institute mussels was significantly lower than for the commercial mussels. The poor condition index for the Cawthron Institute samples was apparent in the lack of sexually differentiated tissue, indicating that the reproductive cycles of mussels from the Cawthron Institute and commercial samples were not synchronised. Research has shown that trace metal levels in *M. edulis* can vary with reproductive cycle, with a peak in Cd levels during spawning where as Zn levels peak just prior to spawning (Coimbra & Carraca 1990). It is possible that the present differences in Cd and Zn levels between the Cawthron Institute and commercial mussel samples could be related to differences in reproductive cycle.
Levels of Cd and Zn in mussels from Stuart Island and Otago

The Cd and Zn levels in Big Glory Bay mussel sample (0.43 ± 0.11 and 59 ± 7 ppm dry weight respectively) were similar to the mussels sampled from Kenepuru Sound in this present study (see Chapter 4). However, the level of Cd found in the mussels from Big Glory Bay were significantly lower than reported levels of Cd in *M. edulis* from Stewart Island of 3.0-8.0 ppm (after converting to dry weight by a factor of 5), though similar levels of Zn were reported (43 – 55 ppm) except for Patersons Inlet (110 ppm dry weight) (Neilsen & Nathan 1975). The Cd and Zn levels from Portobello mussel samples (0.25 ± 0.13 and 61 ± 6 ppm dry weight respectively) were significantly lower than the Cd and Zn levels (0.97-2.08 and 71-124 ppm dry weight respectively) reported by Linwood (1993) for *P. canaliculus* on the outer Otago coastline. Considering the potentially higher levels of pollution in Otago Harbour, the low levels of Cd found in the mussels from this location is surprising. These results imply that any possible South Island-wide anthropogenic sources of trace metal cannot explain the high levels of Cd levels found in mussels within Pelorus Sound.

**Concentration of trace metals in Pelorus Sound water**

Cd concentration in the Pelorus River mouth sample (<30 pM) is significantly lower than the level of Cd found in oceanic water in the nearby Cook Strait area (170 pM) (Hunter & Ho 1991). Estuarine systems have been shown to have higher total Cd and reduced levels of dissolved Cd close to a river mouth compared to open ocean (Comans & van Dijk 1988). As water moves into the oceanic system, the bound Cd reacts with chloride ion from seawater to form complexes such as CdCl" and the fraction of dissolved Cd increases (Martin *et al.* 1993; Dai & Martin 1995). If the level of total Cd in Pelorus River had been high this mechanism of mussel Cd release might explain some of the present trends in Cd levels observed in the present study from Pelorus Sound.

However, as the Cd levels in Pelorus River are more than five times lower than those of the open ocean, it is unlikely that the river is a source of Cd for mussels in Pelorus Sound. Therefore, the most likely source of Cd to mussels growing in Pelorus Sound is seston (suspended organic and inorganic particulate material) or from the open ocean (in this case, Cook Strait). If Cd was found in sediment loading from terrestrial runoff, one would expect
an increase in Cd levels in mussels farmed directly below a logging area. However, Nikau Bay, an area that has been subject to high levels of recent logging (personal observation), exhibited low Cd levels in the mussels collected from this location (sample site 2, Chapter 4), suggesting that the source of Cd is unlikely to be from terrestrial runoff, at least in this area.

The low levels of phosphate in the water samples were below the previous levels reported for Pelorus Sound of 0.002 – 0.021 ppm (Gibbs et al. 1992 and 2002). Whether the phosphate levels in this present study were low due natural depletion of nutrients in the water or experimental error in sampling is not clear. The phosphate analysis methods recommends that samples are analysed within two hours of collection or rapidly frozen in a glycol bath (Strickland & Parsons 1972). As it was not possible to analyse samples in the field and for some samples freezing occurred over two hours after collection, it may be possible that biological activity in the sample depleted the dissolved reactive phosphate levels, although the samples that were frozen within one hour of collection still had low levels of phosphate. However, as the results for phosphate were below the limit of detection no conclusions as to the distribution of either phosphate or Cd in the water can be made, except to report that both are low. Gibbs et al. (1992) reported that the level of phosphate in the sound was continuously changing due to rainstorms, flood events, seasonal and tidal cycles, and nutrient limitation by phytoplankton growth. It is likely that all these events also result in fluctuations in the level of Cd in the water of Pelorus Sound, but the extent of these fluctuations cannot be determined from the present results.

Conclusion

The distribution of trace metals within the different organs of *P. canaliculus* was variable. The different distribution of Cd and Zn indicates that though chemically similar, the biological mechanisms controlling the concentrations of these metals in the tissues were different. As Zn had a relatively uniform distribution compared to Cd, it is unlikely that the distribution of Zn in the tissues of *P. canaliculus* caused any of the unexplained variability in Zn levels observed in mussels. However, the distribution of Cd, which concentrated in the gills and digestive tract of *P. canaliculus*, which would lead to some variation in the levels of Cd in the mussels, as the percentage of tissues vary with changes in reproductive cycle (Buchanan 2001).
A genetic link to the variation in trace metal levels in mussels is difficult to establish from the present results, as mussels bred by the Cawthron Institute were also more alike in size and weight. Selective sampling of commercially grown mussels may have produced the same reduction in variability of trace metal levels. However, because of the increased variability in size and weight of the commercial mussels, one might expect that all trace metal levels would be more variable, but this was not observed in the present study. The variability in Cd levels was higher between the commercial mussel samples, but Zn levels were more variable in the more uniform sized Cawthron Institute mussels, suggesting that there was a difference in the mechanism(s) of uptake of Cd and Zn. This could indicate that some of the unexplained variability in trace metal levels could be linked to genetic variability of mussel populations.

The level of Cd found in the mussels in Pelorus Sound cannot be explained by the variation in phosphate or the input of Cd from Pelorus River. Comparison of the present Cd levels with those for mussels collected from other locations in South Island showed that the variation in Cd levels and the Cd levels themselves were much higher in mussels from Pelorus Sound than from other South Island locations. Although in the latter case a much smaller sample size was collected from a much smaller area, which might distort this conclusion.
Chapter 8
Discussion and Conclusion

Due to the toxicity of Cd, limits to the Cd content of foodstuffs have been put in place to ensure that food is safe to eat. The Greenshell® mussel farming industry in New Zealand is bound by these Cd limits, yet very little was known until the present study about the levels of Cd and their variation between individual mussels. The aim of this study was to investigate the variation of Cd content in *P. canaliculus* cultivated in Pelorus Sound, Marlborough, and compare the results for mussel samples from other parts of the South Island. Additionally, the levels of the related element Zn were measured and compared to those of Cd to examine whether accumulation of the two trace metals was correlated.

Detailed analysis of historical data from the Marlborough Shellfish Quality Programme (MSQP) showed that trace metal levels in *P. canaliculus* were variable between sampling locations and over the time period of 1991 to 2002. Although some metals such as Hg, Cr, Pb, and Ni were often below the limit of detection, other metals such as As, Cd and Mn were extremely variable with five-fold differences in concentrations in mussels collected from different parts of Marlborough Sound. Over the 11-year sampling period from 1991 to 2002 most variability in trace metal levels, except for Cd, was seen between sampling years with the level of trace metals generally reducing from 1991 to 2002. The largest variation in trace metal levels in mussels from Marlborough Sound was for Cd, with a large significant variation detected between sampling years and locations. Cd levels tended to be low in mussels from inner Pelorus Sound and high in mussels from farms located close to and on the coast of Cook Strait.

The level of variation in Cd concentrations, detected from analysis of MSQP data, was found to be so high that mussels collected from some locations would potentially exceed the present European limit, of 1 ppm, for Cd in shellfish. This historical MSQP data involved analysis of homogenised samples of mussels and so although some comparison between locations could be made; it was not possible to determine the level of variation between individual mussels. A high level of variation between individual mussels increases the risk of exceeding any food safety limit.
Analysis of the variation of Cd and Zn between and within locations has shown that the
distribution of Cd in mussels from Pelorus Sound is similar to the between-farm variation
apparent from analysis of the MSQP historical data. However, the distribution of Zn in
mussels from Pelorus Sound was significantly more variable than that detected in analysis of
the MSQP data.

Most (80%) of the variation in Cd levels is explained by between-farm variation, with a
gradient of low Cd levels in mussels from Kenepuru Entrance to higher Cd levels in Beatrix
Bay and Grants Bay. Variation with depth (13%) occurred at most farms, with an increase in
Cd levels of mussels grown at depth, as reported previously by Neilsen (1974). Very little
variation in Cd levels could be accounted for by differences in sex, harvest date or spat.
However, a small but significant interaction between distance and spat was detected, even
with limited sample replication of this interaction. A larger sample size of the different spat
types between the locations may show the spat type to be a significant factor in the level of
Cd in mussels.

In comparison to Cd, the Zn levels showed more variation within the farms. However,
between-farm variation still explained a large proportion of variation in Zn levels (56%), but
depth, sex and harvest date also contributed to some extent (12%, 10% and 4% respectively).
In contrast to Cd, the highest levels of Zn were found in mussels from Kenepuru Entrance and
Beatrix Bay. A slight degree of negative correlation between Cd and Zn levels in mussels
could potentially relate to phytoplankton growth utilising Cd in Zn-limited water (Price &
Morel 1990).

Seasonal variation of Cd and Zn in mussels showed that both metals were accumulated
over the growing season, but with only correspondingly small fluctuations in the
concentration of the metals. The total Zn content of the mussels was significantly correlated
to the dry weight of the mussels, whereas total Cd content correlated with the Cd
concentration in the mussel. These correlations illustrate that the main causes of fluctuations
in the total Cd and Zn content were different. Mussel size was probably the most important
factor in the total Zn content, on the other hand the total Cd content of the mussels was
dependent on the concentration of Cd. The difference between Cd and Zn was also reflected
in the distribution of the metals in the tissues of the mussels. Whereas Zn was distributed
relatively uniformly throughout the mussel tissue, Cd accumulated in the digestive tract and gills. This difference in distribution indicates the presences of mechanisms to possibly sequester Cd, whereas Zn is potentially being utilised more uniformly throughout the tissue of the mussel.

Examining an interspecific genetic difference within *P. canaliculus* and an intraspecific difference between *P. canaliculus* and *M. edulis* was beyond the scope of this study. Whilst some differences in trace metal levels in spat type were found between locations, it was not possible to establish if differences were due to the mussels' size and condition index or to a true difference in trace metal levels in the different spat. The same problems occurred when analysing pure-bred *P. canaliculus* with commercial *P. canaliculus* and also *M. edulis* and *P. canaliculus*, although differences were seen in all comparisons of trace metal levels. A larger sample size would be required to eliminate effects of size and condition.

Identifying the source(s) of Cd and determining causes of variation in Cd levels in mussels between growing areas has also proved to difficult. From this present study there was no evidence of terrestrial input of Cd from geological or anthropogenic sources that could directly explain the gradient of Cd levels observed in the mussels from Pelorus Sound. As no data are currently available on the level of Cd in the seawater within Pelorus Sound, phosphate levels were analysed as a proxy to Cd levels in this study, but unfortunately the level of phosphate was below the limit of detection.

The circulation of water in Pelorus Sound is dominated by tidal currents with periods of high freshwater inflow forming a surface low salinity layer that quickly passes along the Sound. Pelorus Sound is such a restricted body of water that complete tidal flushing takes 18 tidal cycles (Heath 1982). Coastal seawater has a higher level of Cd than the Pelorus River (Chapter 7). As a result of slow tidal flushing, there is a potential to establish a Cd gradient within Pelorus Sound. This gradient could potentially explain the Cd gradient observed in mussels from Pelorus Sound.

A comparison of the average volume of water flowing into Pelorus Sound shows the freshwater input of 44 m s⁻¹ (Heath 1985) is less than 0.5 % of the tidal input of 535 x 10⁶ m³ (Heath 1985). Although during the present study the salinity was a constant 33 psu along the
length of inner Pelorus Sound (Chapter 7), the area often records lower salinities in the surface layer after periods of heavy rainfall. However, because of the hydrological dampening effect of Kenepuru Sound, salinity beyond Havelock arm rarely drops below 25 psu (Proctor & Hadfield 1998). Assuming the pattern of Cd mixing from fresh and seawater is consistent with the salinity, the maximum dilution factor most commonly seen at Kenepuru entrance is less than 1:3. The dilution in salinity is lower than the gradient observed in the Cd levels in mussels, 1:5 (Chapter 4). If the Cd level in the water column was directly related to the salinity, then one would expect mussels growing below the surface low salinity layer, that often forms in Kenepuru Entrance, to show a marked increase in Cd. Although an increase in Cd with depth was observed at Kenepuru Entrance, the extent of the difference is too small (less than 1:2). Therefore, an approximate estimate of the water and Cd input from river and tidal sources does not provide sufficient evidence to support a Cd gradient forming in the water along Pelorus Sound comparable to the gradient observed in the mussels.

Research has shown salinity to have an inverse relationship with the uptake of Cd in the bivalves _M. edulis_ (Wang et al. 1996), _Potamororbula amurensis_ and _Macoma balthica_ (Lee et al. 1998). This suggests that mussels growing in the low salinity water in an estuarine system would have a higher level of Cd, than mussels growing on the open coast. If the source of Cd was land-based, one might expect the mussels from Kenepuru Entrance in Pelorus Sound to have a high level of Cd compared to the other farms tested. The nature of the Cd level in the mussels from inner Pelorus Sound indicates that if the source of Cd was land-based, then it was in a form that was not readily bioaccumulated by the mussels. Previous studies have shown that in an estuarine environment the concentrations of dissolved Cd are dependant on the salinity and mixing of the water, with the formation of Cd chlorocomplexes increasing with distance from an estuary mouth (Comans & van Dijk 1988). If this situation is occurring in Pelorus Sound in such a way as to limit the ability of the mussels to bioaccumulate the Cd, then this might explain the trend of increasing Cd levels in mussels observed in the present study with increasing distance away from the Pelorus River mouth.

Another possible cause of the gradient in Cd levels in mussels along Pelorus Sound is the removal of Cd from the water by biota during the tidal cycle. Although calculating the amount of Cd removed by an unknown quantity of biota is not possible, an estimate of the Cd
uptake by the mussel farms provides a baseline to work from. Assuming the average uptake of Cd from the mussels in Pelorus Sound is 29 ng day\(^{-1}\) per mussel, the minimum uptake of Cd found between November and February in actively growing mussels from Port Ligur (Chapter 5) and the total number of mussels growing in Pelorus Sound is twice the harvest rate of 78 000 tonnes (NZ Mussel Industry Council 2005), the total uptake of Cd by mussels in Pelorus Sound would be at least 9 g day\(^{-1}\). However, the estimated daily tidal input of Cd into Pelorus Sound is significantly greater at approximately 27 kg Cd, calculated using a tidal cycle of approximately 535 x 106 m\(^3\) (Heath 1985) with an estimated Cd concentration in Cook Strait seawater of 0.23 nM kg\(^{-1}\) (Hunter & Ho 1991). This indicates that tidal Cd input is over 9000 times higher than the estimated uptake of Cd by mussels. Therefore, the Cd content in the water column is not likely to be limited by biota and to cause the gradient of Cd levels in mussels.

The gradient from mixing of freshwater with seawater and uptake of Cd by the mussels does not adequately explain the gradient in Cd levels observed among mussels collected along Pelorus Sound. The bioaccumulation of Cd by *M. edulis* has been calculated from an uptake model to be derived directly from the dissolved state (55%) and seston (45%) (Wang *et al.* 1996). If the likely variation of Cd in the dissolved state cannot explain the gradient of Cd in mussels along Pelorus Sound, then there must be some other interaction from food sources and other inorganic seston.

Phytoplankton has been implicated as a source of Cd to bivalves (Frew 1989), as some phytoplankton species are able to utilise Cd instead of Zn in the formation of some proteins (Lane *et al.* 2005). Unfortunately there appears to be no adequate, contamination-free methods to determine the Cd levels of phytoplankton separate from the particulate Cd content of the surrounding water. Without any such data on the trace metal levels in phytoplankton, it is not possible to determine if trace metal content of the mussels diet differs along Pelorus Sound. It is not just the concentration of trace metals in the phytoplankton that has the potential to affect the uptake rate of Cd, but also the availability of plankton (Wang *et al.* 1995). When food availability declines, the mussels increase the gut passage time of food, and as particles spend longer periods in the acidic environment of the gut, more ions are released. This is particularly true for elements such as Cd and Zn, which are strongly bound to the algal cell wall (Wang *et al.* 1995). Therefore, as a result of low food availability,
mussel assimilation efficiency of Cd from the phytoplankton increases. Hickman et al. (1991) found mussel condition index was closely linked to food availability; below a chlorophyll a threshold of 200 µg L\(^{-1}\) condition index declined significantly. From the findings of Hickman et al. (1991) it is possible to conclude that food availability is likely to be lower in areas with mussels that have lower a condition index. In this present study, the condition index was slightly positively correlated with Cd levels (Chapter 4), indicating that at least in 2004, food availability was not a factor in the Cd gradient observed for mussels sampled from Pelorus Sound.

The inorganic seston, which is usually processed and eliminated in pseudofaeces by *P. viridis* (Ke & Wang 2002), could potentially be a source of trace metals, especially in areas of recent deforestation (Johnston et al. 1981). High levels of Cd binding to hematite in seston have been shown to dramatically elevate the level of Cd in pearl oysters, *Pinctada carthariarium* (McConchie & Lawrance 1991). Water movement in Pelorus Sound acts as a double-ended sediment trap with most sediment deposited at the entrance and head of the Sound (Heath 1982). If inorganic seston had an effect on the trace metal levels of the mussels, one might expect there to be elevated levels where the majority of sediment is trapped, but this trend is not apparent in the present data.

Numerous hypotheses have been examined as to the probable cause of the Cd gradient in mussels from Pelorus Sound, although as yet there is insufficient evidence to indicate which ones might be correct. A comparison of Cd levels with Zn in the present study has illustrated how at least these two trace metal levels accumulate in mussels in different ways. Although the present research has not been able to isolate the source(s) of Cd and Zn, it is can be concluded that either the source(s) and/or the mechanism(s) of accumulation and depuration in the mussels of these two metals are different.

**Further Research**

The analysis of trace metal levels conducted in the present study has been limited by the incidence of too many significant variables such as size, condition and weight of the mussels.
As samples were taken from existing mussel farms, it was not possible to implement all the original experimental design planned for the study. Initial analysis of data indicated little variation with depth, which later proved to be a significant factor. As a result, the small sample size for different depths within a farm may have limited some of the analysis. Although size, condition and weight of the mussels did not prove to be significant covariates of Cd and Zn levels in this study, the significant variation of all these variables between locations would have masked any correlations. Laboratory analysis of the effect of size, condition and weight on the level of Cd and Zn in mussels would enable each variable to be isolated and possibly lead to the identification of further factors that could have a significant impact on the Cd and Zn levels in mussels from Pelorus Sound.

During the course of this study it was possible to detect a wide degree of variation in Cd and Zn levels in mussels from different locations and depth. One of the major assumptions of many biomonitoring programmes is that the trace metal content of the tested organism is correlated to that of the surrounding environment. If that assumption also holds for Pelorus Sound, then the level of Cd in the waters varies significantly along the Sound. It was hoped that during the course of the present study, it would be possible to identify the source(s) of Cd to the mussels in Pelorus Sound. The complex nature of trace metal analysis made the analysis of trace metal content of the seawater beyond the scope of the present study. Further research is required to see if the Cd gradient observed in the mussels is also apparent in the dissolved and/or particulate fraction of seawater in Pelorus Sound. With five-fold predicted differences in the level of Cd in the environment, as detected in the mussels form Pelorus Sound (Chapter 4), it may be possible to detect a difference in Cd levels in the water along Pelorus Sound with ultra-clean trace metal water analysis. Also, the observed increase in Cd and Zn levels in mussels with depth may, with further analysis, illustrate circulation patterns within Pelorus Sound that could explain some of the gradients in trace metal levels observed for the mussels.

The application of phosphate fertiliser to farmland has been implicated as a source of Cd to the marine environment (Buttler & Timperley 1996). As the phosphate levels in Pelorus Sound were too low to test in this study, further research is required to establish if a gradient of phosphate is evident in Pelorus Sound similar to the Cd gradient observed in the mussels. However, with a half-life of Cd of over 120 days in *M. galloprovincialis*, the
depuration of Cd in mussels can be considerably slower than the accumulation of Cd (Viarengo et al. 1985). As a result, any phosphate application that enhances Cd levels in mussels may be too episodic to detect.

It is unlikely that all the variation of Cd levels in the mussels will be explained by variation of Cd levels in the water. It is possible that the diet of the mussels varies along Pelorus Sound and that a difference in diet is having some impact on Cd levels in the mussels. Analysis of the distribution of food for mussels in Pelorus Sound generally uses chlorophyll a as a measure of phytoplankton abundance (Hickman et al. 1991). Recent studies in Beatrix Bay have found that picophytoplankton, (<5 µm), account for 40% on average of the biomass in the top 15 m of the water column (Safi & Gibbs 2003). Bivalves inefficiently consume small picoplankton, and so chlorophyll a analysis of water will over-estimate a mussel’s food availability (Safi & Gibbs 2003). Also, P. canaliculus is able to consume zooplankton, with evidence of consumption of adult copepods over 400 µm (Zeldis et al. 2004). More research is required into the diet of P. canaliculus and the trace metal content of plankton, in order to determine if variation in the type and level of food is contributing to the Cd gradient along Pelorus Sound.

With the implementation of breeding programmes for P. canaliculus, it would be beneficial to continue with the research started in the present study to determine if there is any genetic link with Cd and Zn uptake. Determining the mechanism for Cd uptake and isolating the proteins involved in sequestering and detoxifying Cd would potentially enable a breeding programme to be established which would reduce the level of Cd by possibly reducing the rate of Cd uptake and/or increasing the rate of Cd depuration.

Analysis of the variation of Cd and Zn levels between P. canaliculus and M. galloprovincialis was complicated due the presence of symbionts in the mussels. Initially it would be interesting to determine why M. galloprovincialis had such a high incidence of symbionts but also further research is required to determine whether symbionts affect trace metal levels in mussels. Once the cause of the high incidence of symbionts is known it may be possible to repeat the comparison of trace metal levels between the two mussel species without the confounding effect of the presence of symbionts. The potential role of symbionts in accumulating trace metal could also be investigated.
Due to the toxicity of Cd, limits on the level of this metal in food have been put in place to ensure that it is safe to eat. Current food safety limits have been calculated by assessing the concentration of trace metal in foodstuffs and the average contribution of each particular foodstuff to diet (JECFA 2003). Differences in the bioavailability of Cd in different foodstuffs are not considered. Studies of the bioavailability of Cd from different food groups show sunflower kernels and rice to be high in Cd (cited in Satarug et al. 2000). However, Cd from Bluff oysters, Ostrea lutaria, may have low bioavailability, as most of the Cd from oysters consumed by people was found to be lost in faeces (Mckenzie-Parnell et al. 1988). Also, detoxification by complexing of heavy metals in the oyster, Crassostrea gigas, has been shown to reduce the amount of bioavailable Cd to less than 25% (Boisson et al. 2003). Without knowing the level of bioavailability in foodstuffs, restrictions could be introduced in terms of maximum allowed levels of specific trace metals that would be unnecessarily restrictive. Further research is required to establish the bioavailability of Cd from molluscs, including mussels in an average human diet.
References


References


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References


References


Appendix 1

Drying of Mussel Tissue

The analysis of the trace metal levels in tissues is reported using dry weight concentrations. In order to ensure that a constant dry weight was achieved by the methods used in this study, a test of the drying procedure was carried out. Mussel tissue was dried in an 80°C fan oven over a period of 48 hours. The weight of the mussels was recorded after 24, 39, 46 and 48 hours of drying. The results show that all the mussels tested reached a constant weight after a period of 48 hours (Figure A1.1)

![Graph showing drying time vs dry weight percentage]

**Figure A1.1** Drying of mussel soft tissue in a fan assisted oven, assuming mussels reached final dry weight after 48 hours.
Appendix 2

Data

Data included on attached compact disc (see inside rear cover)

Folder 1  CTD Plots from Pelorus Sound 6 April 2004

Folder 2  Trace metal analysis of mussels in Pelorus Sound 2004 - 2005

Distance
Cadmium and zinc data in *P. canaliculus* from Pelorus Sound
6 April and 26 October 2004

Seasonal
Cadmium and zinc data in *P. canaliculus* from Port Ligar
April 2004 – February 2005

Species
Cadmium and zinc in *P. canaliculus* and *M. galloprovincialis*
from Port Ligar
3 August 2004

Tissue
Cadmium and zinc in separated tissues of *P. canaliculus* from
Grants Bay
26 October 2005

Genetics
Cadmium and zinc in *P. canaliculus* from pure bred and wild
mussel spat from Hallam Cove
January 2005